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# The World Journal of Biological Psychiatry


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**EDITORIAL**

# The World Journal of Biological Psychiatry

Dear Colleagues,

It is my great pleasure to present to you *the World Journal of Biological Psychiatry*, vol 10, issue 4, section 2 and 3.

As one of the leading journals in the field, *the World Journal of Biological Psychiatry (WJBP)* attracts a high number of articles. This is due to its high impact factor of 3,58 and also due to its high visibility within the field.

As official medium of the World Federation of Societies of Biological Psychiatry (WFSBP), it is a focus of *the WJBP* to cover also topics which are specifically of regional interest in a global world and which enhance education and knowledge in countries with limited continuing education possibilities. This is especially achieved through the WFSBP Treatment Guidelines which are published on a regular basis. With these comprehensive manuals which includes numerous recommendations the WFSBP aims to provide up-to-date treatment options and thus to further improve treatment for patients and to bring knowledge to a global standard.

Additionally, *the WJBP* gives authors and researches from regions with limited medical infrastructure the opportunity to publish their findings in a high quality international scientific journal.

It is because of this focus of *the WJBP* which is unique within the field of world-wide psychiatric journals that *the WJBP* attracted a high number of manuscripts which are of great interest to our readers.

With the presented special editions, these papers are now published. I am sure that you will find useful information and interesting updates for a constantly improving treatment of your patients and many inspirations for your research work.

Yours sincerely,

Siegfried Kasper, MD
Chief Editor

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**REVIEW ARTICLE**

# The effect of melatonergic and non-melatonergic antidepressants on sleep: weighing the alternatives

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**Abstract**

In DSM-IV the occurrence of disturbed sleep is one of the principal diagnostic criteria for major depressive disorder (MDD). Further, there is evidence of reciprocity between the two conditions such that, even in the absence of current depressive symptoms, disturbed sleep often predicts their development. The present review discusses the effects of antidepressants on sleep and evaluates the use of the recently developed melatonin agonist–selective serotonin antagonists on sleep and depression. Although many antidepressants such as the tricyclics, monoamine oxidase inhibitors, serotonin-norepinephrine reuptake inhibitors, several serotonin receptor antagonists and selective serotonin reuptake inhibitors (SSRIs) have all been found successful in treating depression, their use is often associated with a disruptive effect on sleep. SSRIs, currently the most widely prescribed of the antidepressants, are well known for their instigation or exacerbation of insomnia. The recently introduced novel melatonin agonist and selective serotonin antagonist antidepressant, agomelatine, which has melatonin $MT_1$ and $MT_2$ receptor agonist and 5-$HT_{2c}$ antagonist properties, has been useful in treating patients with MDD. Its rapid onset of action and effectiveness in improving the mood of depressed patients has been attributed to its ability to improve sleep quality. These properties underline the use of melatonin analogues as a promising alternative for the treatment of depression.

**Key words:** *Sleep, melatonin receptors, agomelatine, antidepressants, serotonin-2 receptor antagonists*

## Introduction

On a worldwide basis depressive disorders are a leading cause of disability, and are associated with high rates of morbidity, suicidal risks, and mortality. According to some estimates, major depressive disorders (MDDs) will be the second most prevalent cause for illness-induced disability by the year 2020 (Murray and Lopez 1997). Controversy still exists regarding the aetiological factors causing depression. Some studies suggest that nearly 40% to 50% of the risk for depression is genetic in nature. Depressive syndromes also occur due to stress or trauma (Fava and Kendler 2000). According to some investigators, depression is not a single disease, but rather a complex, heterogeneous syndrome consisting of numerous disease domains, each with its distinct cause and pathophysiology (Nestler et al. 2002; Berton and Nestler 2006). The existence of at least two kinds of depression, melancholia and non-melancholic mood disorder, is being increasingly recognized. The advances in our understanding of depression have thus led to the suggestion that the diagnostic category 'Major depression' should be eliminated (Shorter 2007).

Pharmacotherapies for the treatment of depression have been in use since 1950s. They include tricyclic

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antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), serotonin (5-HT)-norepinephrine (NE) reuptake inhibitors (SNRIs), and several serotonergic receptor blockers. They also include the recently introduced melatonin agonist and selective 5-HT antagonists (MASSAs) whose prototype is agomelatine.

Antidepressants are the third most widely prescribed class of therapeutic agents worldwide, with SSRIs accounting for 80% of the total market share (Celada et al. 2004). The present review is focused on the effects of antidepressants on sleep in depressive disorders. Additionally it highlights and evaluates the use of the recently developed MASSAs. In view of recent evidence that melatonin is implicated in the pathophysiology of depressive disorders (see Srinivasan et al. 2006 for a review), agomelatine could be an important new development for the treatment of these diseases.

## Sleep disturbances in depression

Sleep abnormalities constitute the most prevalent symptoms of illness, and particularly of mental illness (American Psychiatric Association 1994; Benca et al. 1992). It has been reported that patients with MDDs have nightmares at least twice a week and, compared to normals, have significantly higher scores on scales of suicidality (Agargun et al. 1998). Since the frequency of reported nightmares is related to the risk of suicide, such sleep disturbances are now used as predictors for suicidal behaviour (Agargun et al. 1998). Considerable controversy exists concerning the question of whether sleep disturbances in depression are a 'trait-like' feature (Berger and Riemann 1993). Some studies of patients with depression have shown changes in sleep architecture that persist even during the remission phase. Changes in sleep architecture often precede changes in patients' ongoing clinical state or can signal relapse (Kupfer et al. 1981).

Depressed patients experience difficulty falling asleep, difficulty staying asleep and early morning awakenings (Cajochen et al. 2000). Analysis of slow wave sleep activity (SWA) in NREM sleep has shown that delta wave counts in patients with MDD are decreased when compared to controls. Fast frequency beta activity and elevated alpha have been recorded during sleep in depressed patients, indicating that hyperarousal and increased sleep fragmentation are major characteristics of sleep in depression (Armitage 2007). The synchronization of slow or fast frequency electroencephalography (EEG) is lower in depressed adults and sleep microarchitectural abnormalities are present in non-medicated patients or in clinical remission suggesting that these are trait-like features of depressive illness (Armitage 2007). Disturbances in the organization of the sleep/wake cycle in MDD patients are thought to be due to abnormalities in the timing of the REM/NREM sleep cycle (Wirz-Justice 2006). The temporal distribution of REM sleep is also typically altered during overnight sleep in depressives (Schulz et al. 1979; Wehr et al. 1979; Cartwright et al. 2003). Decreased REM onset latency (REMOL; $<65$ min) has been shown to be a common occurence in severe or endogenous depression. It has been suggested that reductions in non-REM sleep, particularly slow-wave sleep (SWS), are the cause of associated reductions in REM latency (Lustberg and Reynolds 2000). For instance, Armitage found that reductions in the amount of time spent in SWS and an abnormal time course correlated with an increased risk for suicide as well as with a greater severity of depressive symptoms (Armitage 2007). Patients with least amounts of SWS also showed the greatest psychomotor retardation (Armitage 2007). These findings supported the conclusion that disruptions to sleep homeostasis are the main form of sleep disturbance in depression. Additionally, increases in REM sleep density have also been found to be specific to affective disorders (Wichniak et al. 2000) and are now thought to be a reliable sleep marker for depression (Lam 2006). Consistent with this view are findings that many antidepressants tend to reduce REM sleep as well as to increase REM latency.

## Effects of antidepressants on sleep

The close linkage between depression and sleep disturbance, as well as the increasing amount of evidence that their effects are reciprocal, underscore the importance of an understanding of how commonly used antidepressants can affect sleep (DeMartinis and Winokur 2007). Effects on sleep are particularly influenced by an antidepressant's to degree of inhibition of 5-HT or NE uptake, its effects on 5-$HT_{1A}$ or $5HT_2$ receptor sites or actions on $\alpha_1$- and $\alpha_2$-adrenoceptors or histamine $H_1$ receptor sites (Mayers and Baldwin 2005). While some antidepressants may improve sleep efficiency (SE) by ameliorating the depressive symptoms, others exert more rapid beneficial effects on initiation and maintenance of sleep (Sharpley and Cowen 1995; Tsuno et al. 2005; Thase 2006). The introduction of SSRIs and SNRIs (recent surveys indicate that the SNRI venlafaxine currently represents physicians' drug of choice) has drastically changed the strategies for the clinical treatment of MDD (Rosenzweig-Lipson et al. 2007; DeMartinis and Winokur 2007). Because the administration of SSRIs is commonly associated with insomnia (Anderson 2000), most pharmacoepidemiological surveys indicate that at

least one-third of patients taking SSRIs receive concomitant sedative-hypnotic medications (e.g., Thase 2006). These data support the suggestion that the effects of antidepressants on sleep should represent an important consideration in physicians' prescribing decisions for depressed patients. (Winokur et al. 2001; DeMartinis and Winokur 2007).

## Tricyclic antidepressants (TCAs) and their effects on sleep in depression

For over 30 years, from the 1960s to 1990, tricyclic drugs were the mainstay treatment for MDD. In addition to their applicability to depressive symptoms, all agents in this class are known to have nonspecific sedative hypnotic effects. TCAs are known to decrease sleep onset latency (SOL), improve sleep SE, and decrease wake time after sleep onset (WASO) (Ware et al. 1989). Because of their antihistaminergic properties these drugs also produce sedating effects during the day, and it has thus been suggested that their use should be avoided in depressed patients who are sensitive to these effects (Winokur et al. 2001).

With the exception of trimipramine all TCAs reduce or suppress REM sleep and increase REM latency (Vogel et al. 1990). It has been suggested that the REM sleep suppression which is associated with these drugs is an essential for their therapeutic action. NE- and 5-HT-containing neurons in the brain, which are directly affected by TCAs, are not only involved in the pathophysiology of affective disorders but also have a significant impact on sleep regulation. NE and 5-HT are known to inhibit electrical activity of the ventrolateral preoptic nucleus which contains a group of sleep active, $\gamma$-aminobutyric acid (GABA)-galanin producing neurons (Saper et al. 2005). It has also been suggested that blockade of histamine $H_1$ receptors or antagonism of $\alpha_1$-adrenoceptors is at the basis of their sleep-promoting effects (Mayers and Baldwin 2005). Polysomnographic (PSG) studies support the inference that TCAs generally cause sedative effects while clinical experience indicates that drugs such as amitriptyline and trimipramine shorten SOL and improve sleep continuity and efficiency (Winokur et al. 2001; DeMartinis and Winokur 2007). Table I summarizes the results of EEG studies on the effects of antidepressant drugs on sleep.

## Effects of monoamine oxidase inhibitors (MAOIs) on sleep in depression

The MAOIs tranylcypromine and phenelzine are associated with subjective complaints of insomnia, with polysomnography (PSG) studies confirming that prolonged SOL, impaired sleep continuity and increased WASO occur among patients who use these agents (Kupfer and Bowers 1972; Winokur et al. 2001). REM sleep suppression has also been noted in depressives who were taking phenelzine and tranylcypromine. This effect occurs soon after initiation of treatment and persists for months during continuation of therapy. However use of reversible monoamine oxidase A inhibitors such as moclobemide produces less pronounced REM sleep suppression (Monti 1989).

## Serotonin-norepinephrine reuptake inhibitors (SNRIs)

Venlafaxine, duloxetine and milnacipran belong to the SNRI category of drugs that inhibit presynaptic uptake of both 5-HT and NE (Stahl et al. 2005). In a double-blind placebo controlled study, administration of venlafaxine, at doses ranging from 75 to 225 mg/day, induced increases in WASO after 1 month of treatment. REM sleep latency increased significantly while the total amount of time spent in REM sleep diminished (Salin-Pascual et al. 1997; Winokur et al. 2001; Argyropoulos and Wilson 2005). Yang et al. (2005) reported that venlafaxine was associated with REM sleep suppression and increased in REM sleep latency, although, in contrast to previous studies, no differences in SOL nor SE were observed between depressed patients and control subjects. Venlafaxine was also found to increase the frequency of periodic leg movements in sleep (PLMS). These repetitive and highly stereotyped limb movements, which can occur during sleep and/or the waking state, are the result of EEG arousals or awakenings and might cause difficulties in initiating and maintaining sleep. The PLMS movements, presumably the result of enhanced serotonergic availability and secondarily decreased of dopamine (DA) effects caused by the drug (Yang et al. 2005).

## Effects of selective serotonin reuptake inhibitors (SSRIs) on sleep in depression

The selective serotonin reuptake inhibitors (SSRIs), the most commonly used antidepressants, have been considered a major treatment breakthrough ever since the importance of serotonin in mood regulation was recognized. SSRIs now constitute 80% of prescriptions of all antidepressants on the market (Celada et al. 2004). These drugs block the presynaptic uptake of 5-HT and enhance the activation of the postsynaptic receptors, thus prolonging the interaction of 5-HT with the multiple serotonin receptor subtypes. However, SSRIs also have a number of side effects, the most prominent being

Table I. Effects of antidepressants on EEG sleep parameters.

| Drug | Sleep efficiency | Slow wave sleep | REM sleep | Sedative effect | Key references |
|---|---|---|---|---|---|
| *Tricyclics* | | | | | |
| Amitriptyline | Increased (S) | Slight increase | Decreased (S) | Increased (S) | (Kupfer 1982; Winokur et al. 2001; DeMartinis and Winokur 2007) |
| Doxepin | Increased (S) | Slight increase | Decreased (M) | Increased (S) | |
| Imipramine | Slight increase | Slight increase | Decreased (M) | Increased | |
| Nortriptyline | Slight increase | Slight increase | Decreased (M) | Increased slightly | |
| Desipramine | No effect | Slight increase | Decreased (M) | Increased slightly | |
| *MAOIs* | | | | | |
| Phenelzine | Slight decrease | No effect | Decreased (S) | No effect | (Kupfer and Bowers 1972; Winokur et al. 2001) |
| Tranylcypromine | Decreased (M) | No effect | Decreased (S) | No effect | |
| *SSRIs* | | | | | |
| Fluoxetine | Decreased (S) | Decreased | Decreased or no effect | Slight or no effect | (Armitage et al. 1994; Sharpley et al. 1996; Winokur et al. 2001) |
| Paroxetine | Decreased (S) | Decreased or no effect | Decreased (M) | Not studied | |
| *SNRIs* | | | | | |
| Venlafaxine | No observation | No observation | Decreased (M) | Increased (M) | (Luthringer et al. 1996; Winokur et al. 2000) |
| *5-HT receptor antagonists* | | | | | |
| Trazodone | Increased (S); maintains sleep continuity | No effect | Decreased slightly | Increased slightly | (Stahl 1996; Rush et al. 1998; Winokur et al. 2000, 2001; Millan 2006; DeMartinis and Winokur 2007) |
| Nefazodone | Increased slightly | No effect | Increased slightly | No effect | |
| *Serotonergic-noradrenergic antidepressants* | | | | | |
| Mirtazapine | Increased (S) | Increased in normal subjects; no study in depressives | No effect | Increased (S) | (Winokur et al. 2000, 2001; Shen et al. 2006) |
| *MASSAs* | | | | | |
| Agomelatine | Increased (S) | Increased (S) | No effect | No effect | (Lopes et al. 2005; Guilleminault 2005; Pjrek et al. 2007; Salva et al. 2007) |

S, significant; M, moderate.

their effects on sleep and sexual function (Moltzen and Bang-Andersen 2006). In an early study, sertraline (with maximum doses up to 200 mg/day achieved within a 10-day period) significantly prolonged SOL and reduced TST 14 days after treatment (Winokur et al. 2001). However, there was neither a reduction in SE nor an increase in WASO. It has been reported that nearly 25% of depressed patients treated with SSRIs have subjective complaints of insomnia (Armitage 2007). Fluoxetine administration has been shown to cause disruptions in sleep continuity, reductions in SE and increases in WASO (Winokur et al. 2001). In a group of patients with major depression, fluoxetine at doses of 20 mg/day for 4 weeks caused significant decreases in SE, a finding that correlated well with the fluoxetine levels in plasma (Armitage et al. 1997). Reductions in sleep efficiency following fluoxetine use were also observed by Trivedi et al. (1999). REM sleep suppression has also been a consistent finding in depressed patients who are being treated with fluoxetine (Armitage 2007). Paroxetine is also a drug that similarly reduces SE in depressed subjects, with an increased number of awakenings being observed after 4 weeks of treatment. It has not, however, been found to influence TST nor SOL (Staner et al. 1995). Similar effects have also been noted in normal healthy subjects receiving paroxetine (20 mg/day). Compared to placebo paroxetine was found to produce significant reductions in SE, as well as increases in WASO. Further, REM sleep minutes were reduced and REM latency was significantly prolonged (Sharpley et al. 1996). Yang et al. (2005) used PSG measurements to study 274 patients who had been receiving SSRIs. The investigators found an association between the use of the drugs and suppression of REM sleep as well as increases in REM sleep latency. There were no differences between control and study subjects in terms of SOL or SE. Similar to findings associated with venlafaxine, subjects given SSRIs showed significant increases in PLMS. Inasmuch as PLMS can contribute significantly to difficulties in initiating and maintaining sleep, it is suggested that caution should be exercised in choosing antidepressants for depressed patients who have pronounced sleep complaints (Yang et al. 2005).

### Effects of serotonin-2 receptor antagonist/serotonin reuptake inhibitors (SARIs) on sleep in depressed patients

Trazodone and nefazodone are the two drugs that belong to the SARIs category, their main action being the inhibition of 5-$HT_2$ receptors, which are also involved in the regulation of sleep (Millan 2006). Trazodone also inhibits the $\alpha_1$-adrenergic and the histamine $H_1$ receptors (Stahl et al. 2003). Nefazodone inhibits $\alpha_1$ adrenergic receptors and inhibits NE uptake but has weak actions on histamine receptors. Because of its effects on $H_1$ receptors trazodone administration produces sedating effects and causes daytime somnolence (Winokur et al. 2001). Administration of trazodone to depressed patients has been found to increase TST, reduce SOL, reduce the number of awakenings and arousals, reduce total REM sleep time, and prolong REM latency (Winokur et al. 2001). In an 8-week study of six depressed patients who also had symptoms of insomnia, trazodone treatment resulted in a 44% improvement in SOL, a 14% improvement in TST, and noteworthy improvements in SE (Scharf and Sachais 1990). The administration of nefazodone administration to depressed patients has been found to preserve sleep continuity and to decrease the number of awakenings (Rush et al. 1998). Evidence of its effects on sleep efficiency however has been less consistent, with either increases or no effects being reported (Armitage 2007).

### Serotonergic-noradrenergic antidepressant drugs and sleep in depression

Drugs belonging to the serotonergic-noradrenergic antidepressant category, e.g., mirtazapine and mianserin, have a dual-action profile, combining the enhancement of the noradrenergic neurotransmitter system with specific actions on particular serotonergic receptor subtypes. They exert a potent antagonism of presynaptic $\alpha_2$-heteroceptors and $\alpha_2$-autoreceptors that results in an increased release of both 5-HT and NE (Stahl 1996; Wilson and Argyropoulos 2005). They also act as 5-$HT_2$ and 5-$HT_1$ serotonin receptor antagonists, thus contributing to their overall anxiolytic and soporific actions (Haddjeri et al. 1995; de Boer 1996; Shen et al. 2006). Mirtazapine also exhibits potent antihistaminergic ($H_1$) receptor activity. The use of mirtazepine in patients with major depression has been shown to significantly reduce sleep disturbances (Winokur et al. 2000; Shen et al. 2006). In a study of six adult patients with MDD, the administration of mirtazapine in doses of 15 mg/day for 1 week followed by increased doses of 30 mg/day for an additional week caused significant reductions in SOL as well as significant increases in TST as compared to baseline (Winokur et al. 2000). In a comparison of with other antidepressants, mirtazepine use was associated with better SE than fluoxetine (Winokur et al. 2003) or paroxetine (Ridout et al. 2003). Mirtazapine was also

shown to be superior to venlafaxine (Guelfi et al. 2001) or paroxetine (Schatzberg et al. 2002) in terms of improving sleep scores as measured by the Hamilton Depression Rating Scale (HAMD). In the earliest PSG study conducted on sleep architecture in normal volunteers, mirtazapine (30 mg/day) decreased SOL, WASO and stage-1 sleep and increased SWS (Ruigt et al. 1990). A similar effect was found in another study conducted on healthy volunteers in whom the administration of mirtazapine (30 mg/day) caused a significant improvement in SE with reductions in nocturnal disturbances as compared to placebo (Aslan et al. 2002). Although similar in efficacy to the SSRIs, mirtazapine's side effects, which include increased appetite, weight gain and excessive daytime sedation (mediated by $H_1$ blockade), have prevented its acceptance as a first-line medication (Thase 2006; Papakostas et al. 2007).

Mianserin has sleep promotion properties, possibly through inhibition of histamine ($H_1$) receptors (Sharpley and Cowen 1995; Mayers and Baldwin 2005). Earlier studies conducted with depressed patients revealed that, among depressed women with cancer, mianserin (10–20 mg/day) was superior to placebo for improving HAM-D sleep scores (Costa et al. 1985).

## Use of sedative-hypnotic medications on sleep in depressives

Although originally developed as anxiolytics benzodiazepines (BZDs) are widely used for the treatment of insomnia. BZDs have been found effective for reducing SOL and increasing sleep time. Wider use of this class of drugs has been limited however by the finding of undesirable side effects following long term use. These include the development of tolerance, rebound insomnia and cognitive deficits (Jindal and Thase 2004; Thase 2006). In one of the placebo-controlled long-term trials in patients with MDD, it was found that the beneficial effects of clonazepam on patient's sleep complaints were limited to the first three weeks of therapy (Smith et al. 2002).

Despite their clinical use for nearly 20 years, there has been a lack of controlled studies using PSG for assessing the effectiveness of BZDs as an add-on therapy with either SSRIs or SNRI in MDD (Thase 2006). Moreover, an APA Task Force which evaluated the tendency of BZDs to cause dependance, toxicity, and abuse has recommended that BZDs not be used for treating insomniacs, particularly elderly patients (Jindal and Thase 2004).

## Other hypnotic medications with antidepressants on sleep

Inasmuch as sleep disturbances, particularly insomnia, are often found with antidepressant medications, the use of hypnotic drugs has often been resorted to for dealing with the associated sleep problems. A novel atypical antipsychotic, risperidone, is effective for reducing sleep disturbances in patients with treatment resistent depression (Ostroff and Nelson 1999). Similarly, in a study of SSRI-treated depressed patients, it was found that those receiving daily doses of fluoxetine (<40 mg), sertraline (<100 mg) or paroxetine (<40 mg) reported significant insomnia. These patients were then entered into a double-blind phase where they were assigned randomly to zolpidem (10 mg) or placebo for 4 weeks followed by single-blind placebo for 1 week. Those depressed patients who received zolpidem demonstrated significant improvements in sleep with longer TST, better sleep quality and reduced WASO (Asnis et al. 1999).

## Need for an innovative antidepressant that improves sleep

The evidence reviewed above demonstrates that while some antidepressants promote sleep initiation and maintenance (5-HT receptor antagonists and TCAs), many antidepressants, particularly SSRIs such as fluoxetine, or the SNRI venlafaxine, exert adverse effects on sleep. As such, the sleep-promoting benefits of these antidepressants are either limited or nonexistent (Lam 2006). While all of these drugs exert their antidepressant effects mainly through alterations of NE and/or 5-HT or both, and are generally considered safe and effective, they are far from ideal. Inasmuch as many of them can exacerbate insomnia, concomitant administration of either BZDs or highly specific $GABA_A/\alpha_1$ receptor ligands such as zolpidem, have been proposed as combined treatment strategies (Thase 2006). Although these sedative-hypnotics reduce SOL and prolong TST, they also cause unwanted effects such as dependence. These findings have thus led to calls for newer antidepressants with different modes of action.

Since disturbed sleep is a hallmark symptom in depression, it has been suggested that an ideal antidepressant medication should address sleep disturbances from the outset of treatment (Rouillon 2006). Further, the evidence that depressed patients also suffer from circadian rhythm disturbances has reinforced the view that these disturbances may underlie the development of MDD (Jindal and Thase 2004).

## Antidepressant medication and the role of melatonin

Although both decreased and increased levels of melatonin have been reported in depression, antidepressant medications have been shown to increase melatonin levels, mainly by increasing NE levels that could stimulate pineal β-adrenoceptors (Venkoba rao et al. 1983; Thompson et al. 1985; Sack and Lewy 1986; Golden et al. 1988; Srinivasan 1989; Borjigin et al. 1999; Szymanska et al. 2001). The CNS distribution of melatonin receptor mRNA has been shown to be significantly modified by prolonged treatment with antidepressants such as desipramine, clomipramine, and fluoxetine. With the exception of fluoxetine, these drugs were found to increase the amount of mRNA for $MT_1$ receptors in the hippocampus but to decrease the amount of $MT_2$ mRNA (Larson et al. 2006; Hirsch-Rodriguez et al. 2006) Based on these findings it was hypothesized that endogenous levels of melatonin could contribute to antidepressant effects depending upon the pattern of melatonin receptor expression in the brain.

It has been suggested that diminished melatonin secretion is at least partially responsible for the deterioration of sleep maintenance that is seen in insomniacs. In a study undertaken in 382 postmenopausal women with a family history of depression, a delay in urinary 6-sulfatoxymelatonin (a6MTs) excretion was found (Tuunainen et al. 2002). Other studies in aging women have documented that reductions in circulating melatonin levels accompany menopause, and as a consequence programs of melatonin replacement therapy have been proposed (Bellipanni et al. 2001, 2005). In a study conducted on 10 patients with MDD, slow release melatonin tablets in doses of 5 mg/day (which was slowly raised to 10 mg/day at the end of 2 weeks) were administered for 4 weeks along with fluoxetine (20 mg/day) (Dolberg et al. 1998). Melatonin treatment promoted a significant improvement in sleep quality, as evidenced from scores on the Pittsburgh Sleep Quality Index (PSQI). As reported earlier (Fainstein et al. 1997), despite melatonin-induced enhancements of sleep quality, no improvements were found in the clinical status of the depressed patients (Dolberg et al. 1998). In another study of patients suffering from both delayed sleep phase syndrome and depression, melatonin treatment not only significantly improved the TST but also significantly reduced psychometric scores for depression (Kayumov et al. 2001). In two studies of combination therapy in patients with MDD or treatment-resistant depression, the combination of melatonin (slow release formulation) plus fluoxetine or other (unspecified) antidepressant medication was found to improve the sleep quality of the patients. The results did not show however that melatonin had an additive effect on the depressive symptoms (Dolberg et al. 1998; Dalton et al. 2000). Other evidence that antidepressant treatment can promote favourable melatonin receptor expression has led to the suggestion that combination therapy using an antidepressant plus a melatonergic agent may be an effective strategy for treating sleep disorders in the context of depression (Hirsch-Rodriguez et al. 2006; Larson et al. 2006).

## Melatonin agonists and selective serotonin antagonists (MASSAs) such as agomelatine for sleep disturbance and depression

In developing an effective treatment strategy for sleep problems associated with depression, prescribing decisions usually represent a compromise between what is ideal and what is realistically possible. The first step in the decision making process is to specify the desired characteristics of an ideal drug. Kupfer (2006) has suggested that when considering sleep disturbances in depression an antidepressant should satisfy several requirements. The ideal antidepressant should: (1) decrease SOL; (2) decrease WASO; (3) promote a feeling of being refreshed after a night's sleep; and (4) maintain alertness during the day. The widely used SSRIs generally fail these criteria because they produce insomnia, fragmentation of SWS, and disturbances to REM sleep, and consequently they are not ideal drugs for sleep disturbance in depression. Unfortunately, as reviewed above, most non-SSRI antidepressants are also less than ideal for managing sleep disturbances and in fact often exacerbate them. There is thus a clear need for an antidepressant agent which addresses these deficiencies.

One such antidepressant is the newly developed agent agomelatine (Valdoxan®, Servier). As the first member of MASSAs, agomelatine is a melatonin $MT_1$ and $MT_2$ receptor agonist with 5-$HT_{2c}$ antagonist properties and has been found to be beneficial in treating patients with MDD (Loo et al. 2002; Kennedy and Emsley 2006; Kupfer 2006; Montgomery 2006; Pandi-Perumal et al. 2006). Agomelatine is a naphthalenic compound with a overall selectivity ($>100$ fold) for $MT_1$ and $MT_2$ receptors but has no significant affinities to muscarinic, histaminergic, adrenergic or dopaminergic receptor subtypes (Rouillon 2006). The proven chronobiotic (i.e. substance that adjusts the timing or reinforces oscillations of the central biological clock) action of agomelatine is due to its agonist activity on melatonin receptors $MT_1$ and $MT_2$ in the suprachiasmatic nucleus (SCN) (Redman and Francis 1998; Weibel

et al. 2000; Tuma et al. 2001; Van Reeth et al. 2001). Inasmuch as disruptions in circadian rhythms are linked to depressive states, agomelatine's effectiveness in treating these symptoms support the conclusion that it has a broader range of effect than other antidepressants, and thus may more effectively address the complexities of depressive illness.

## Agomelatine in depression

Agomelatine is a potent agonist at melatonin receptors in the SCN and an antagonist at 5-$HT_{2C}$ receptors. This inhibition enhances NE and DA release in the frontal cortex and is thought to contribute substantially to agomelatine's antidepressant action (Millan et al. 2003, 2005; Fuchs et al. 2006; Hamon and Bourgoin 2006; Montgomery 2006; Pandi-Perumal et al. 2006).

Agomelatine has demonstrated its efficacy in reducing depressive symptoms in patients with MDD in three multicentre, multinational studies carried out mainly in Europe. In a multicentre multinational placebo-controlled study involving 711 patients from 102 centres located in Belgium, UK, and France, agomelatine (25 mg/day) was significantly better than paroxetine or placebo in reducing depressive symptoms as measured by the HAM-D rating scale (Loo et al. 2002). Additionally, in a severely depressed patient subgroup, agomelatine was significantly better than placebo in improving HAM-D scores (Loo et al. 2002). In another multicentre study of 212 depressed patients in 21 centres across Finland, Canada, and South Africa, agomelatine administration for the duration of 6 weeks similarly produced significant reductions in HAM-D scores in both depressed and severely depressed patients (Kennedy and Emsley 2006). The effectiveness of agomelatine in severely depressed patients is particularly significant inasmuch as this patient group is resistant to drug therapy involving either SSRIs or SNRIs (Clerc 2001). Agomelatine represents an innovation in the treatment of depression inasmuch as it exhibits antidepressant efficacy at a dose of 25 mg/day, has few adverse effects, and is associated with early resolution of depressive symptoms (den Boer et al. 2006).

## Agomelatine's effects on sleep in normal subjects and depressive patients

In a crossover design study, PSG was used to evaluate the effect of agomelatine (5 or 100 mg before bedtime) on sleep architecture in eight healthy young men (Cajochen et al. 1997). Agomelatine increased REM sleep significantly without affecting other stages of sleep. In a similar study of non-depressed healthy elderly men (older than 60 years), administration of agomelatine for 15 days did not affect the total sleep time and sleep stages but phase advanced core body temperature by 2 h and cortisol secretion by 1.5–2.0 h (Leproult et al. 2005).

In depressed patients, agomelatine has been found effective in reducing sleep complaints, increasing the duration of SWS, and normalizing sleep architecture. For example in a PSG study of 6 weeks of agomelatine treatment the duration of SWS increased without affecting REM sleep duration, thus producing, at the study's conclusion, an overall improvement in both sleep quality and continuity (Quera-Salva et al. 2005). These effects were evident even during the first week of treatment with agomelatine.

In a 6-week double-blind study, agomelatine and venlafaxine were compared in their effects on subjective sleep quality (Guilleminault 2005). Agomelatine at doses of 25 mg/day was given to 165 patients and venlafaxine at 150 mg/day to 167 patients. Compared to venlafaxine, agomelatine promoted earlier and greater improvements on the criteria of 'Getting to Sleep' (GTS) and 'Quality of Sleep' as assessed by the Leeds Sleep Evaluation Questionnaire (LSEQ). These changes, which were noted from the first week of treatment with agomelatine, did not occur with venlafaxine (Guilleminault 2005).

The effect of agomelatine on the cyclic alternating pattern of sleep (CAPS) was evaluated in NREM sleep by using PSG (Lopes et al. 2005). After 7 and 42 days of treatment, a significant decrease in CAPS time and CAPS cycles was seen when compared with baseline data. The findings thus demonstrated that agomelatine normalizes NREM sleep in depressed patients (Lopes et al. 2005). The changes in NREM sleep variables preceded the improvements seen in HAM-D scores. Inasmuch as there was a complete elimination of NREM sleep disruption which preceded the improvement in subject mood, it may be possible to infer that part of agomelatine′s antidepressant effect is mediated through its ability to improve sleep architecture (Zupancic and Guilleminault 2006).

Agomelatine is thus a dual-action drug, which can improve sleep quality in depressed patients and additionally can produce rapid antidepressant action (Zupancic and Guilleminault 2006). This is particularly relevant clinically inasmuch as improvements in sleep quality among depressed patients are associated with a reduced rate of recurrence of depressive symptoms, and, conversely, that complaints of poor sleep among depressed patients tend

to be associated with a poor response to subsequent antidepressant treatment (Kupfer 2006). Agomelatine thus fulfils many of the criteria in the definition of an ideal antidepressant drug since its ability to improve sleep architecture addresses the interrelated symptoms of depressive illness, and it appears to do this better than most of the available antidepressant agents. In a recent study of 37 acutely depressed outpatients with seasonal affective disorder (SAD), the administration of agomelatine (25 mg/day) in the evening for a period of 14 weeks significantly decreased SIGH-SAD (structural Interview Guide for the HAM-D rating Scale) and CGI-S and -I scores (Clinical Global Impression of severity and Improvement) from week 2 onward ($P<0.001$) (Pjrek et al. 2007). The Circascreen (a self rating scale for the assessment of sleep and circadian rhythm disorders) also improved during the study ($P<0.001$). The response rate was found to be 75.7% while the remission rate was 70.3%. The significant reduction in the patient-rated Circascreen score occuring after 6 and 10 weeks demonstrated that agomelatine significantly improved sleep disturbances and daytime fatigue (Pjrek et al. 2007). In this study it was also noted that agomelatine displayed excellent tolerability at a daily dose of 25 mg and was virtually devoid of any of the side effects typical of other antidepressants such as SSRIs. This study, the longest that has ever been undertaken with agomelatine, is significant since it found no side effects following 14 weeks of administration of the drug (Pjrek et al. 2007). Moreover this is the first study demonstrating the clinical efficacy of agomelatine in patients with SAD.

Recently EEG analysis of the effects of agomelatine on sleep in MDD patients has been reported (Quera-Salva et al. 2007). PSG results revealed that SE increased and intra-sleep awakening decreased progressively from day 7 on, the differences from baseline being close to significance at day 14 ($P=0.068$ and $P=0.076$) and significant at the last evaluation (42 days after treatment) ($P=0.05$ for the increase in SE and $P=0.04$ for the decrease of intra-sleep awakening). SWS duration and percentage of sleep period time on SWS (stages 3 and 4) increased significantly after agomelatine treatment (Salva et al. 2007). Agomelatine did not influence REMOL nor the amount of REM sleep (Table I). From these observations it was concluded that agomelatine, by increasing SWS and activity during the first sleep cycle, corrected the abnormalities of sleep regulation that is very much impaired in depression (Borbely et al. 1984).

The available evidence therefore suggests that agomelatine may have considerable promise as an antidepressant that can address the sleep abnormalities that are found in mood disorders. Further studies are necessary however to support definitive conclusions regarding agomelatine's efficacy and long-term effects.

## Ramelteon in sleep disorders

Ramelteon (Rozerem®, Takeda Pharmaceutical Company Ltd), is a tricyclic synthetic analog of melatonin that was approved by the USA's Food and Drug Administration for the treatment of insomnia in 2005. Ramelteon is a melatonin agonist which acts, like endogenous melatonin itself, acts via $MT_1$ and $MT_2$ receptors. In three large clinical trials ramelteon has been found effective in reducing SOL, and increasing total duration of sleep in insomniacs (Roth et al. 2005, 2006; Erman et al. 2006). In all doses that have been tested (ranging from 8 to 64 mg/day) ramelteon's safety has been found to be similar to that of placebo (Cajochen 2005; Erman et al. 2006). Since ramelteon has a greater affinity for $MT_1$ and $MT_2$ receptors, as well as a longer duration of sleep promoting action, it has greater overall efficacy than exogenously administered melatonin for treating sleep disorders (Pandi-Perumal et al. 2007). These characteristics suggest that ramelteon drug may have significant value for treating the sleep problems associated with depressive illness. As in the case of agomelatine, long-term studies with ramelteon have yet to be performed.

## Conclusions

Insomnia is experienced as a major stressor by affected patients who also have MDD. This is not always recognized in clinical practice. Inasmuch as depressive disorders are linked to high suicide rates, traditional approaches to acute antidepressant therapy have tended to focus only on the most overt symptoms of these disorders. By contrast, the associated disruptions to sleep in depressed patients have been viewed as allied symptoms but not as contributors to the clinical picture in a primary sense. In general most of the currently used antidepressants, and especially the more recently introduced SNRIs, SSRIs and SARIs, have served well the clinician's objective to reduce MDD patients' self-destructive tendencies. Although these drugs have a demonstrated efficacy for acutely treating depressive symptoms, certain of them possess considerable adverse effects due to their antinoradrenergic or antihistaminergic properties. Further, a growing body of evidence points to the importance of disruptions to circadian rhythms as possible contributors to overall depressive symptomatology

(Jindal and Thase 2004; Zupancic and Guilleminault 2006; McClung 2007).

It is also clear that currently used antidepressant agents tend to disrupt sleep architecture, induce insomnia, as in the case of SSRIs, or produce daytime drowsiness. Agomelatine, a novel antidepressant, has been found successful in treating MDD patients. Because of its dual mechanism of action on $MT_1$ and $MT_2$ melatonin receptors in the SCN and its 5-$HT_{2c}$ antagonist properties, agomelatine has been effective in improving both quality of sleep and mood in depressed patients. These effects, which include its normalization of sleep architecture, contrast greatly with the sleep impairments produced by almost all of the currently used antidepressants. Moreover, agomelatine's sleep enhancement effects have been documented to occur from the very beginning of treatment, presumably mediated by melatonin receptors, and indirectly indicate that circadian rhythm disruption is an important but underappreciated mechanism in the development of depressive illness. Agomelatine may be an antidepressant of choice for treating patients with MDD. Ramelteon, a new synthetic analog of melatonin with greater affinity for $MT_1$ and $MT_2$ receptors than melatonin, also holds considerable promise for treating sleep disorders associated with depressive illness. The long term efficacy and acceptability of either agomelatine or ramelteon remains to be studied.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**REVIEW ARTICLE**

# Spectroscopic findings in attention-deficit/hyperactivity disorder: Review and meta-analysis

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**Abstract**

*Objectives.* The last decade has seen an increasing interest in the method of magnet resonance spectroscopy (MRS) since this is the only research tool that allows a non-invasive in vivo assessment of neurochemical aspects of ADHD without employing ionising radiation. In this paper we review published MRS results with respect to childhood, adolescence and adult ADHD. *Method.* We searched the Medline (Pub Med) database using the key words ADHD, attention-deficit/hyperactivity disorder, magnet resonance spectroscopy, MRS and spectroscopy. Citations of identified articles were also searched for relevant studies. Meta-analyses were performed for the measured metabolites and regions of assessment. *Results.* Sixteen studies could be identified that used MRS to investigate the neurobiology of ADHD. Two regions could be identified as the focus of spectroscopic investigations – the frontal lobe including anterior cingulate cortex and parts of prefrontal cortex and the basal ganglia, mostly striatum, alongside the fronto-striato-thalamo-frontal circuits. As for metabolites, in the majority of studies the ratios to creatine and not absolute concentrations of metabolites were estimated. Choline compounds, *N*-acetyl-aspartate and glutamate/glutamine (to creatine ratios) could be identified as being altered in several studies in ADHD. The meta-analysis showed increased choline compounds in several researched regions. *Discussion.* MRS is a promising tool for the non-invasive in vivo assessment of the cerebral neurochemistry in ADHD. More regions of interest (ROI) like amygdala, hippocampus, thalamus and cerebellum should be assessed in future studies. Further methodological improvements of MRS are desirable in order to assess the absolute metabolite concentration of several ROIs at the same time. Such developments will open novel perspectives in spectroscopic investigations of ADHD.

**Key words:** *ADHD, magnetic resonance spectroscopy, choline, glutamate,* N-*acetyl-aspartate*

## Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a serious mental disorder, which begins in childhood and may persist into adult life in a substantial subgroup of patients (Biederman et al. 2005). Patients with ADHD experience significant impairments of social adaptation in various fields, e.g., professional and social life, as a consequence of inattentiveness, impulsivity and hyperactivity. The prevalence rate of ADHD in children ranges from 3 to 12%, depending on the sample selection and diagnostic criteria utilized (Biederman et al. 2005; Moore et al. 2006; Tzavara et al. 2006). In 40–60% of the afflicted children ADHD symptoms persist into adulthood (Gurvitis et al. 1996; Natsume et al. 1997) including cases with partial remission. The prevalence rate of adults meeting full criteria for ADHD (DSM-IV) is estimated to range from 1 to 2% (Moore et al. 2006). Since the hyperactivity tends to decline more than inattentiveness, ADHD in adulthood is less obvious and thus is often overlooked (Biederman et al. 2000). However, patients with persistent ADHD frequently experience significant dysfunctions in occupational and vocational performance, continuous social impairments, low self esteem, higher rates of motor vehicle accidents and higher risks of substance abuse and other psychiatric conditions (Spencer et al. 2002). The high prevalence, the global impairment and the

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chronicity of this disorder led the Center for Disease Control and Prevention to identify ADHD as a serious public health problem in the year 2002 (Spencer et al. 2002).

Several brain structures have been identified to be abnormal in the context of ADHD. The most common findings in neuroimaging studies are smaller volumes of the total brain, the right frontal cortex and white matter, the cerebellum and striatal subcortical structures (Castellanos et al. 1996, 2002; Schweitzer et al. 2000; Lenartowicz et al. 2005; Nebel et al. 2005; Konrad et al. 2006). In their current review of functional neuroimaging studies in ADHD, Bush et al. (2005) identify the following regions as being reported to be abnormal: the prefrontal cortex (PFC), including dorsolateral PFC (DLPFC) and ventrolateral PFC (VLPFC), the dorsal anterior cingulate cortex (dACC) and the striatum, the parietal cortex, the superior temporal sulcus, thalamus, the brain stem reticular activating system and the cerebellum.

Neurochemically, dopamine plays the central role in most pathogenetic models of ADHD (Biederman et al. 2005). The nigrostriatal and mesolimbic subdivisions of the dopaminergic system modulate motor as well as affective, cognitive and attentional faculties. A subtle cross-regulation and interaction of dopaminergic and glutamatergic neurotransmission in prefrontal, striatal and midbrain sites are the prerequisite for these diverse functions (Sesack et al. 2003).

Magnetic resonance spectroscopy (MRS) is a reliable, non-invasive method for in vivo detection of neurometabolites. Glutamate (Glu), glutamine (Gln) often taken together as Glx, *N*-acetylaspartate and *N*-acetylaspartylglutamate (NAA), myo-inositol (mIns), choline-containing compounds (Cho), γ-aminobutyric acid (GABA) and creatine (Cr) can be detected as ratios relative to Cr or in absolute figures with $^{1}$H-MRS (see Figure 1 for typical spectra). $^{31}$P-MRS provides information on membrane phospholipids (MPL) and on the metabolism of high-energy phosphate by assessing phosphomonoesters (PME) and phosphodiesters (PDE), phosphocreatine (PCr), andenosine triphosphate (ATP) and inorganic orthophosphate ($P_i$), respectively. There is a methodological characteristic within the various MRS methods. In 2D chemical shift imaging (CSI) a slice has to be selected and in single voxel spectroscopy a voxel has to be selected prior measurement. While CSI allows the specification of regions of interest (ROI) within the selected slice later during post processing, single voxel spectroscopy is restricted to the originally selected voxel. CSI further allows the comparison of ROIs, if they are within the selected slice. On the other hand, detection of absolute metabolite concentrations is well established for single voxel spectroscopy while it is very difficult for CSI. That is the reason why almost all reviewed CSI studies only calculated metabolite ratios.

$^{31}$P-MRS allows the detection of phosphorus-containing metabolites concentrations. These methods should be treated separately due to methodical differences (Ross et al. 2001). Even though there are many spectroscopic studies in psychiatric disorders like major depression, schizophrenia or bipolar disorder (Olbrich et al. 2008; Scherk et al. 2007), there are only few papers about spectroscopic findings in ADHD. At the same time MRS seems to be a promising tool in ADHD research, since the assessed neurochemical brain properties and dopaminergic

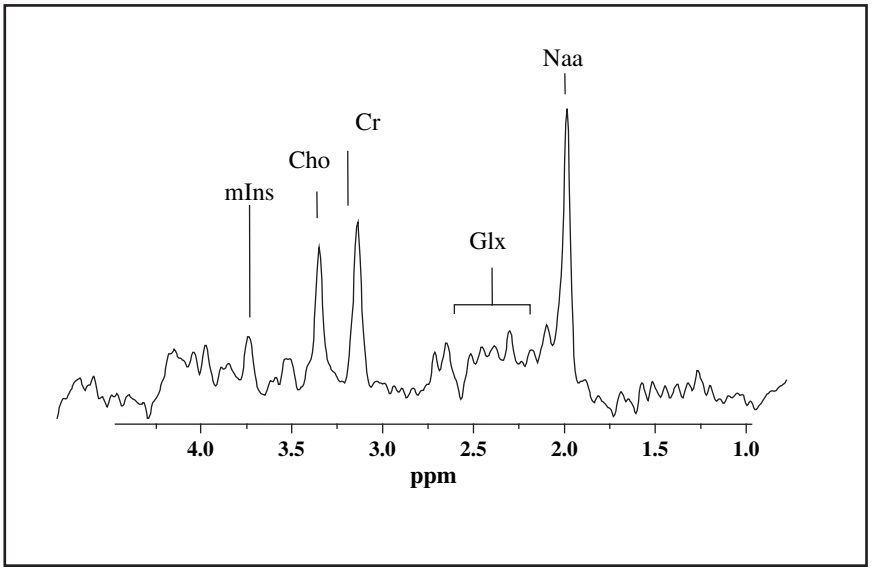


Figure 1. Typical spectra of $^{1}$H-MRS.

and glutamatergic dysfunctions are known to be of importance in the pathogenesis of ADHD.

This review was made in order to systematize current findings and to identify perspectives for further investigations. To our knowledge this is the first systematic review of spectroscopic findings in ADHD.

## Methods

We searched the Medline (Pub Med) database using the key words ADHD, attention-deficit/hyperactivity disorder, magnet resonance spectroscopy, MRS and spectroscopy. Citations of identified articles were also searched for relevant studies. All identified papers which employed MRS in ADHD were included into this review. No limits were placed in respect to year of publication or the age of ADHD population. The endpoint for the literature search was end of September 2007. In this paper we describe and summarize the different MRS methods that have been used and the respective results of the different studies.

Additionally we included the identified studies in our meta-analyses. Meta-analyses were performed separated by metabolite and region of assessment, if at least two controlled studies contributed applicable data. Relational values (ratios to creatine) were pooled with absolute values using standardized means. In the case of high heterogeneity (with $P$ for $I^2 \leq 0.10$), random effect methods were applied. For this purpose we used the statistical software ReviewManager 4.2.8.

One controlled trial (Fayed and Modrego 2005) was excluded from meta-analyses, because variances were not presented and a normal distribution could not be assumed. Another two controlled studies were not comparable to the remaining trials, because different regions (Hesslinger et al. 2001) and outcome variable (Stanley et al. 2006) were examined.

## Results

Sixteen published articles using different MRS methods in ADHD research could be identified.

### *MRS methods used in ADHD*

*Single voxel spectroscopy.* This is the most used localization method of MRS in psychiatric research, which allows obtaining both ratios and absolute figures of neurometabolites in defined region of interest (voxel). The voxel size, localization and number of acquisitions are crucial for the quality of signal (signal-to-noise ratio). The voxel has to be placed precisely into the region of interest. Since typical voxel size should be at least 4 (or better 8 ml), there are some difficulties in measuring small structures of the brain. Another limitation is the quality of spectra if the voxel is positioned in the proximity of ventricles or blood vessels (which is of particular relevance when measuring the basal ganglia, amygdala or striatal structures) (Barker et al. 2001).

*Chemical shift imaging.* This allows the precise definition of extended regions of interest. Post processing algorithms allow the comparative measurement of neurochemical signals within a slice of the brain, the mapping of the respective metabolite concentrations and a visual differentiation between grey and white matter voxels (see Figure 2) (Brown 1992). The cardinal limitation of this method is a methodological difficulty in absolute quantification of metabolites.

The employed methods of all included studies are summarized in Table I. Only two of the 16 included studies employed CSI while all others used single voxel spectroscopy (SVS). All studies in children used SVS. The majority of studies employed Press sequences, among them all studies with adults.

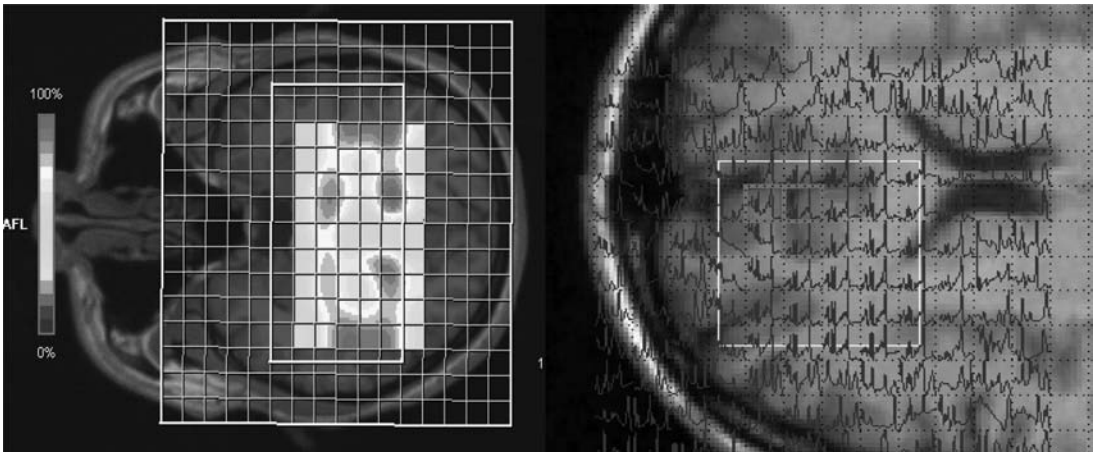


Figure 2. CSI. Metabolic map and definition of prefrontal grey matter in post processing.

Table I. Studies employing MRS in ADHD research.

| Study and Year | Sample: Patients | Sample: Controls | Method | Regions | Findings |
|---|---|---|---|---|---|
| *Studies with children* | | | | | |
| 1. Jin et al. 2001 | $n=12$ | $n=10$ | Single voxel $^1$H-MRS PRESS; TE 35.5 ms; TR 1500 ms | Striatum bilateral | NAA/Cr↓; Cho/Cr right↑ |
| 2. Carrey et al. 2002 | $n=4$ | – | single voxel $^1$H-MRS, before and after medicaltion | Left striatum | Glx/Cr ↓ |
| | | | PRESS; TE 135 ms; TR 1500 ms | Right PFC | → |
| 3. MacMaster et al. 2003 | $n=9$ | $n=9$ | Single voxel $^1$H-MRS | Left striatum | Glx/Cr↑ (n.s.) |
| | | | PRESS; TE 135 ms; TR 1500 ms | Right PFC | Glx/Cr↑ |
| 4. Carrey et al. 2003 | $n=14$ | – | Single voxel $^1$H-MRS, before and after medicaltion | Left striatum | Glx/Cr↓ |
| | | | PRESS; TE 135 ms; TR 1500 ms | Right PFC | → |
| 5. Sparks et al. 2004 | $n=8$ | $n=6$ | Single voxel $^1$H-MRS, | Left striatum | → |
| | | | PRESS; TE 135 ms; TR 1500 ms | Right PFC | → |
| 6. Carrey et al. 2007 | $n=13$ | $n=10$ | Single voxel $^1$H-MRS, absolute quantification | Left striatum | Glx, Cr↑; NAA, Cho, mIns→ |
| | | | PRESS; TE 30 ms; TR 2000 ms | Right PFC | Glx, Cr, NAA, Cho, mIns→ |
| 7. Yeo et al. 2003 | $n=23$ | $n=24$ | single voxel $^1$H-MRS STEAM; TE 30 ms; TR 2000 ms | Right frontal lobe | NAA, Cho, Cr→ |
| 8. Courvoisie et al. 2004 | $n=8$ | $n=8$ | Single voxel $^1$H-MRS STEAM; TE 20 ms; TR 1600 ms | Right frontal lobe<br>Left frontal lobe | Glu/Cr, NAA/Cr, Cho/Cr↑<br>Glu/Cr↑ |
| 9. Sun et al. 2005 | $n=20$ (10+10)* | $n=10$ | Single voxel $^1$H-MRS PRESS; TE 35.5 ms; TR 1500 ms | Right lenticular nucleus<br>Left lenticular nucleus | NAA/Cr↓; Cho/Cr, mIns/Cr, Glx/Cr→ |
| 10. Fayed and Modrego 2005 | $n=8$ | $n=12$ | Single voxel $^1$H-MRS PRESS; TE 30 ms; TR 2500 | Left centrum semiovale (white matter) | NAA/Cr↑; Cho/Cr, mIns/Cr→ |
| 11. Fayed et al. 2007 | $n=22$ | $n=8$ | Single voxel $^1$H-MRS PRESS; TE 30 ms; TR 2500 | Left centrum semiovale (white matter) and prefrotal right (grey and whit matter) | NAA/Cr↑; Cho/Cr, mIns/Cr→ |
| 12. Moore et al. 2006 | $n=15$ (+8)** | $n=7$ | Single voxel $^1$H-MRS STEAM; TE 30 ms; TR 2000 ms | ACC (both side in one voxel) | Glx/Cr, Glx/mIns↑; mIns/Cr→ |
| 13. Stanley et al. 2006 | $n=10$ | $n=15$ | Multiple voxel 31-P-MRS | PFC left/right (r/l) | Free-PME r/l, free-PME/free-PDE↓ |
| | | | | Basal ganlia l/r | Free-PME r/l, free-PME/free-PDE↓ |
| | | | | Superior temporal region l/r | → |
| *Studies with adults* | | | | | |
| 14. Hesslinger et al. 2001 | $n=10$ (5+5)* | $n=5$ | Single voxel $^1$H-MRS absolute quantification | Left dorsolateral PFC | NAA↓; Cho, Cr, mIns, Glx, GABA→ |
| | | | PRESS; TE 30 ms; TR 3000 ms | Left striatum | → |
| 15. Perlov et al. 2007 | $n=28$ | $n=28$ | $^1$H-MRS CSI | Right ACC | Glx/Cr↓; Cho/Cr, mIns/Cr, NAA/Cr→ |
| | | | PRESS; TE 30 ms; TR 1500 ms | Left ACC | → |
| 16. Colla et al. 2008 | $n=15$ | $n=10$ | $^1$H-MRS CSI | Right ACC | Cho↑; NAA, Cr→ |
| | | | PRESS; TE 135 ms; TR 1500 ms | Left ACC | Cho↑; NAA, Cr→ |

*Combined and inattentive subtypes of ADHD; **children with ADHD and bipolar disorder; ↑ higher; ↓ lower concentration in ADHD group; → no metabolite differences between groups.

Regarding the used echo times (TE), the distribution of short TE (~<30 ms) and long TE (135 ms) shows no clear prevalence. The same is true for the chosen repetition times (TR), where slightly more studies chose a short TR of 1500 ms in comparison to longer TRs in the second subgroup. Longer TRs result in a better signal-to-noise ratio; however, this increases acquisition time. Only two studies, one in children and one in adults, used absolute quantification algorithms.

*$^{31}$P-MRS.* $^{31}$P-MRS is based on the same physical principles as $^1$H-MRS but uses the resonances of phosphor instead of proton ions. Since their resonance frequencies are markedly different standard clinical MR scanners need additional costly

equipment to carry out $^{31}$P-MRS. Such additional $^{31}$P-MRS options are not offered for any scanner model. $^{31}$P-MRS allows the measurement of high-energy phosphate (important for assessment of energy metabolism) or phospholipids which can be stated as a marker for neuronal degeneration or proliferation and (de novo) connectivity (Vance 1988; Geddes et al. 1997; Stanley et al. 2006). Due to the low phosphor concentration in the human brain this method meets a high specificity at the cost of a lower sensitivity. Only one study was identified which used $^{31}$P-MRS, which reflects among others the limited availability and technical challenge of this method.

### *Spectroscopic findings in ADHD*

$^{1}$H-MRS research has only recently been applied to ADHD. Nevertheless, several research groups have investigated neurometabolic differences between ADHD patients and healthy controls using this non invasive in vivo method. Table I summarizes the findings of the reviewed studies. In this paper we describe the respective focusing on the different investigated regions of interest separately for children and adults. The single $^{31}$P-MRS studies are dealt with separately due to the important methodical differences.

### *Findings in pediatric ADHD*

Jin et al. (2001) reported significant bilateral decreases of NAA/Cr ratios in the striatum of 12 boys (mean 13 years) with ADHD compared to 10 healthy controls (mean age 13 years). They also found a significant increase of Cho/Cr ratio in right striatum. An increased Cho/Cr ratio in the left striatum did not reach level of significance. Furthermore, the authors studied ADHD children after one oral dose of 10 mg methylphenidate. No medication effect was observed on measured metabolic concentrations. Carrey et al. (2002) showed a dramatic decrease of Glx/Cr ratio in the left striatum after 14–18 weeks of treatment with methylphenidate or atomoxetine in their case series of 4 ADHD patients (two boys, aged 7–12 years). They also observed a decrease of Glx/Cr ratio in the right prefrontal cortex (PFC) in two patients treated with atomoxetine, but not in patients treated with methylphenidate. MacMaster et al. (2003) studied nine ADHD patients (six male, 7–16 years) and nine age- and gender-matched control subjects in the same regions. In the left striatum, there was a trend towards increased Glx/Cr ratios in the ADHD group. Significantly higher ratios of Glx/Cr were found in the right PFC of ADHD patients as opposed to control subjects. The patient and control group did not differ with respect to other metabolites measured in this study. This research group also observed a significantly decreased Glx/Cr ratio in the left striatum of 14 paediatric patients (11 male, 7–13 years) following pharmacological treatment with stimulants (methylphenidate, dexedrine or atomoxetine). No other effects of pharmacological treatment on the ratios of NAA/Cr or Cho/Cr were found. Likewise, the prefrontal Glx/Cr ratio did not change with treatment (Carrey et al. 2003). Later they reported unchanged levels of NAA/Cr, Glx/Cr and Cho/Cr in the left striatum and right PFC following medication of three pediatric ADHD patients ($n=8$, six males, 7–11 years) and healthy controls ($n=6$, four males, age-matched) (Sparkes et al. 2004). In their latest article the authors reported significantly increased Glx and Cr concentrations (not as a ratio but using absolute quantification algorithms) in the left striatal region of ADHD patients ($n=13$, only male, 6–11 years) as compared to healthy controls ($n=10$, only male, age-matched). No other metabolite abnormalities in the striatum (NAA, Cho, mIns) or any other measured region (PFC and occipital lobe) was found. Pharmacological treatment with methylphenidate resulted in reduction of striatal Cr concentration only. No significant differences were noted for the remaining striatal metabolite concentrations (Carrey et al. 2007).

The right frontal lobe was investigated by Yeo et al. (2003) – predominantly in white matter – in children with ADHD ($n=23$, 17 boys, mean age 9.47) and healthy controls ($n=24$, 17 boys, mean age 9.40) to show no significant differences in concentration of most metabolites (NAA, Cho, Cr). However, the concentrations of Glx or other glutamate compounds were not been measured in this study. The ADHD group contained patients with varying medication regimes, possibly hampering the statistical power of the study. Another research group (Courvoisie et al. 2004) found significantly higher levels of Glu/Cr ratios in both right and left frontal lobe as well as significant higher levels of NAA/Cr and Cho/Cr ratios in the right frontal lobe in eight ADHD children (seven boys, mean age 8.93) compared to eight matched healthy controls. No significant differences for mIns/Cr ratios were found in either left and right frontal lobe when compared to healthy controls.

Sun et al. (2005) observed significant decreased NAA/Cr ratios in the right and left lenticular nuclei in 10 boys with combined ADHD subtype compared to 10 age-matched controls and 10 patients with inattentive subtype of ADHD. No significant differences between groups were found for Cho/Cr, mIns/Cr and Glx/Cr ratios.

Fayed and Modrego (2005) demonstrated significantly increased NAA/Cr ratios in the white matter of the left centrum semiovale in ADHD children (three male, five female) compared to age-matched healthy controls (five male, seven female) and autistic children (18 male, three female). No significant differences were reported for Cho/Cr and mIns/Cr. In their latest study Fayed et al. (2007) investigated 22 adolescences with ADHD (18 male, four female) and eight controls (four male, four female) measuring the left centrum semiovale and the right corticosubcortical prefrontal region. They found significantly elevated NAA/Cr ratios and no other alterations in both regions of the patient group.

Moore et al. (2006) acquired the MRS data from the anterior cingulate cortex (ACC) of children aged 6–13 years. The authors compared 15 ADHD and eight ADHD plus bipolar disorder children with seven healthy controls (among them four siblings of ADHD children). Three children with ADHD and two with ADHD plus bipolar disorder were medicated (miscellaneous substances) at the time of investigation. The authors used both the x-to-Cr and x-to-mIns ratios for their calculations. Significantly higher Glx/Cr and Glx/mIns ratios were detected in the ADHD group compared to both control and ADHD plus bipolar disorder groups. The level of mIns/Cr ratio did not differ between groups. The ratios also did not differ in patients compared to their siblings.

Using a multi-voxel $^{31}$P-MRS approach Stanley et al. (2006) studied 10 ADHD children (nine boys, age range 7.0–11.9 years) and 15 healthy comparison subjects (13 boys, age range 6.6–12.6 years). The regions of interest were defined in the PFC (ACC, superior frontal gyrus and prefrontal white matter), the basal ganglia (head of caudate, putamen, anterior portion of the thalamus, anterior horn of the lateral ventricle, and the white matter tracts around this structures) and the superior temporal region (superior temporal gyrus, and pre- and post-central gyrus). Irrespective of hemispheres, free-phosphomonoesters (free-PME) levels were significantly lower in the PFC and the basal ganglia in ADHD children compared to healthy controls. The equilibrium of MPL turnover (free-PME/free-PDE-ratio) was significantly lower in the basal ganglia and tended to be lower in the PFC of ADHD patients. There were no significant group differences for the free-PDE, PCr, $P_i$, ATP and broad-PDE levels, and $PCr/P_i$ ratios in PFC, basal ganglia or superior temporal region.

*Findings in adult ADHD*

The left dorsolateral PFC (DLPFC) and left striatum were investigated by Hesslinger et al. (2001). They found significant lower concentrations of NAA in the left DLPFC in five unmedicated male ADHD (hyperactive-impulsive subtype) patients (mean age $27.2 \pm 3.27$ years) compared to five male ADD (inattentive subtype) patients (mean age $27.8 \pm 3.03$ years) and five male healthy controls (mean age $27.0 \pm 2.92$ years). The authors reported no differences in other metabolite concentrations (Cho, Glx, Cr, mIns) in the left DLPFC or any differences in above-mentioned metabolite concentrations in the left striatum between groups.

The region of ACC was investigated in two studies. Our research group showed significantly decreased ratios of Glx/Cr in the right ACC (28 adult ADHD patients – combined subtype, mean age $30.5 \pm 7.7$ years compared to 28 gender- and age-matched healthy controls). No further metabolite differences between groups were found (Perlov et al. 2007). Colla et al. (2008) reported significantly increased signals of choline compounds in the ACC in adult ADHD patients which correlated with reduced hit reaction time in continuous performance task. Fifteen ADHD patients (eight male, seven female) and 10 healthy controls (four male, six female) were studied. No differences between groups were reported concerning the signals of NAA or Cr.

## Results of meta-analyses

The results of meta-analyses are summarized in Table II. In spite of the low number of MRS studies we detected a rather stable increase of the signal of the choline compounds in different regions of interest. In children the meta-analysis revealed significant changes of choline compounds in left striatum and right frontal lobe. In adults the differences in choline compounds were significant in the right and left ACC (for effect sizes and methodical description see Table II). No other significant metabolite differences were detected in the meta-analysis.

## Discussion

In this systematic review we took care to identify all MRS studies in the context of ADHD. The strength of MRS is to detect metabolite signals in the human brain in vivo and non-invasively. Beside postmortem studies and studies using radioactive tracers (PET and SPECT) MRS is the only way to study

Table II. Results of meta-analysis.

| Population and variable | No. of studies | No. of participants | Statistical method | Heterogeneity $I^2$ | Effect size (95% CI) | *P* |
|---|---|---|---|---|---|---|
| Children | | | | | | |
| *Left striatum* | | | | | | |
| NAA [1,2] | 2 | 45 | SMD (random) | 93%+ | −1.05 [−3.86, 1.76] | 0.46 |
| Inositol [1,2] | 2 | 45 | SMD (fixed) | 0% | −0.17 [−0.76, 0.42] | 0.56 |
| Glutamix [1,2,3] | 3 | 61 | SMD (random) | 73%+ | 0.70 [−0.36, 1.75] | 0.20 |
| Choline [1,2] | 2 | 45 | SMD (fixed) | 0% | 0.66 [0.05, 1.27] | **0.03** |
| *Right frontal lobe* | | | | | | |
| NAA [4,5,6] | 3 | 93 | SMD (random) | 70%+ | 0.74 [−0.13, 1.61] | 0.10 |
| Inositol [4,5] | 2 | 46 | WMD (fixed) | 0% | 0.01 [−0.04, 0.07] | 0.62 |
| Choline [4,5,6] | 3 | 93 | SMD (fixed) | 31% | 0.52 [0.08, 0.95] | 0.02 |
| *Right PFC* | | | | | | |
| Glutamix [2,3] | 2 | 41 | SMD (random) | 76% | 0.76 [−0.40, 1.92] | 0.20 |
| Adults | | | | | | |
| *Right ACC* | | | | | | |
| NAA [7,8] | 2 | 80 | SMD (fixed) | 0% | −0.22 [−0.66, 0.22] | 0.33 |
| Choline [7,8] | 2 | 80 | SMD (fixed) | 0% | 0.72 [0.26, 1.18] | 0.002 |
| *Left ACC* | | | | | | |
| NAA [7,8] | 2 | 80 | SMD (fixed) | 0% | 0.08 [−0.36, 0.53] | 0.72 |
| Choline [7,8] | 2 | 80 | SMD (fixed) | 0% | 0.54 [0.09, 0.99] | 0.02 |

$P \leq 0.10$; SMD, standardized mean difference, WMD, weighted mean difference, NAA, *N*-acetyl-aspartate; PFC, prefrontal cortex; ACC, anterior cingulate cortex.
[1]Jin et al. 2001; [2]Carry et al. 2007; [3]MacMaster et al. 2003; [4]Courvoisie et al. 2004; [5]Fayed et al. 2007; [6]Yeo et al. 2003; [7]Colla et al. 2008; [8]Perlov et al. 2007.

pathobiochemical processes causing ADHD. The aim of this review was to summarize systematically the known MRS data in ADHD and to analyze further ways of progress with this method in ADHD research. The focus of our review was on published results rather than on methodological details.

Our main positive finding is that of increased signals for the choline compound in children as well as adults across different cerebral regions of interest.

Before discussing our results, some limitations of this review have to be mentioned. First, the precise MRS methodology used was rather heterogeneous. Not surprising the frequency of use of certain methods or features seems to depend on its availability in clinical research, costs and technical challenges. This is reflected in the low proportion of studies employing absolute quantification, CSI or $^{31}$P-MRS, even though each of them could provide specific information not accessible with standard protocols. Further technical developments and improvements of these alternative techniques will allow a more wide-spread application of them in the future.

The small number of identified studies using the MRS methods in ADHD research is another more important limitation. However, still we could identify and examine 16 studies. The methodical differences of the identified studies and differences in measured regions of interest further contributed to the limitations of the meta-analyses. Finally, most identified studies investigated children with ADHD of variable age and only three studies dealt with adult ADHD.

We could identify some regions which were the focus of interest in the last decade. The most frequently measured region is the frontal lobe including the prefrontal cortex with DLPFC and ACC (eight studies in paediatric and two in adult ADHD) followed by the striatum (four studies in pediatric and one in adult ADHD). Regions like the lenticular nucleus, the white matter of the centrum semiovale, the basal ganglia (excluding the striatum) and different parts of cerebellum have all been measured in one study only.

Most MRS studies in ADHD focused on pediatric patients. Presently, there are only three studies investigating adult ADHD patients. Only one of sixteen spectroscopy studies used $^{31}$P-MRS.

In $^{1}$H-MRS studies NAA concentration or NAA/Cr ratios were found to be decreased in the striatum (Jin et al. 2001), the lenticular nuclei (Sun et al. 2005) in paediatric and in left DLPFC in adult ADHD (Hesslinger et al. 2001). Increased signals were found in the right frontal lobe (Courvoisie et al. 2004) and in the white matter of the left centrum semiovale (Fayed and Modrego 2005; Fayed et al. 2007) in paediatric patients. Decreased NAA/Cr ratios in the ventral striatum and the lenticular nuclei as a part of the basal ganglia of ADHD children are in line with previously reported volume abnormalities in these regions (Aylward et al. 1996;

Mataro et al. 1997; Semrud-Clikeman et al. 2000). The decreased NAA signal in the DLPFC of adult ADHD could be interpreted as a sign of neuronal dysfunction and does not necessarily indicate cell death (Dautry et al. 2000). This interpretation is in line with findings of Stanley et al. (2006) of decreased free-PME in prefrontal regions and basal ganglia. However, the increased NAA/Cr ratios in right frontal lobe and left white matter found for example by Courvoisie et al. (2004) do not support this assumption, even though increased NAA/Cr ratios could also be caused in terms of reduced Cr-signals and do not necessarily reflect an essentially increased NAA-signal (Tebartz van Elst et al. 2001).

Glx concentrations or Glx/Cr ratios have been reported to be increased in left striatum, right PFC and ACC in pediatric ADHD (MacMaster et al. 2003; Carrey et al. 2007; Moore et al. 2006). In adult ADHD, one study reported decreased Glx/Cr ratios (Perlov et al. 2007). The glutamate in frontal circuits is supposed to be an important regulator of dopamine, which is thought to be primarily affected in ADHD (Carlsson et al. 1999a, 1999b; Krause et al. 2006). Via feedback mechanism, dopamine concentration could possibly influence the concentration of glutamate (Tebartz van Elst et al. 2005; Olbrich et al. 2008). Following this assumption, the hyperactivity in ADHD children (which is generally not observed in adults) could be understood as an attempt to stimulate a hypofunctional dopaminergic system accompanied by higher glutamate concentrations in pediatric ADHD patients. This hypothesis could be supported by the observation of decreased Glx/Cr ratios in the left striatum of children after the medication with methylphenidate and accordant loss of hyperactivity (Carrey et al. 2002, 2003). Another hypothesis explaining the different Glx/Cr ratios in ADHD and healthy controls could be an altered energy metabolism in ADHD patients. Alterations in energy metabolism of monoamines have been suggested as a core deficit in ADHD (Todd et al. 2001). Following synaptic release glutamate is absorbed by adjacent astroglia and converted to glutamine (Gln). Gln then forms the substrate of mitochondrial Glu synthesis. Glu–Gln cycling is closely coupled to glial glucose utilization and lactate production and is energy-dependent. Russell et al. (2006) hypothesised an insufficient astrocyte function in terms of formation and energy exchange in ADHD. Following this hypothesis, on one side the insufficient lactate supply in oligodendrocytes impairs the myelination of axons during development and on the other side the deficiency in ATP production in neurons disturbs neuronal functioning.

The findings of lower free-PME levels in the basal ganglia and PFC bilaterally (Stanley et al. 2006) are consistent with these energy hypotheses. Moreover, Cr as an important energy metabolite in the human brain was used as a reference substance in most identified studies and for that reason could not be evaluated to be changed in ADHD. The usage of an absolute quantification method is essential for the investigation of Cr concentrations in ADHD.

Concerning mIns or its ratios to Cr, no differences in concentration were found in any spectroscopic study of ADHD patients. Only in the study of Moore et al. (2006) was significantly increased ratios of Glx/mIns found in ADHD; however, the Glx/Cr ratio was also increased which might imply that changes are rather caused by increased Glx than by mIns.

The best studied regions in ADHD research i.e. the prefrontal cortex and the striatum are part of prefronto-striato-thalamo-frontal circuits that are known to be of critical importance for the modulation of higher cognitive, emotional and attentional processes (Mega et al. 1994). Another important relay station within these circuits which has been ignored in spectroscopic ADHD research so far is the thalamus. This highly interesting region has been previously found abnormal or altered after therapy with methylphenidate in two PET studies (Zametkin et al. 1993; Schweitzer et al. 2004) and one SPECT study (Kim et al. 2001).

Since ADHD is not only characterized by the symptoms of hyperactivity and attentional deficiency but also by impulsivity and emotional dysregulation the amygdala and related limbic structures seem to be one of the most interesting regions for further spectroscopic ADHD investigations. Subtle amygdala and hippocampal abnormalities have already been reported in volumetric ADHD studies (Plessen et al. 2006); however, we were unable to replicate this finding in adults (Perlov et al. 2008). Furthermore, the amygdala has been shown to be altered in some studies with borderline patients – a syndrome with a high psychopathological similarity and comorbidity with ADHD – and in dysthymia and depression, which are well known to be frequent comorbidities in ADHD (Tebartz et al. 1999, 2000; Rusch et al. 2003; Tebartz van Elst 2005, 2007; Zetzsche et al. 2006).

The hippocampus is known to be involved in attentional processes such as visuospatial working memory (Bedard et al. 2004) and in modulating executive functions (Sergeant et al. 2002). Disturbances in these functional domains belong to the core symptoms of ADHD. Therefore, hippocampal spectroscopy could be a further region of interest in spectroscopic ADHD studies.

As for the cerebellum, Bush et al. (2005) identified it already as an interesting region for further spectroscopic investigations. The cerebellum has

been recently recognized as modulating cognitive functions beyond its role in adjusting motor output. The role of the cerebellum in a wide range of cognitive and affective functions has been demonstrated in lesion studies (Townsend et al. 1999; Schmahmann 2004; Golla et al. 2005) and using functional MRI (Desmond et al. 1998).

Interestingly, our meta-analyses revealed a significant difference for the choline compounds. Cho was increased in all regions for which more than one study was performed, even though this metabolite was only significantly altered in two of the original studies in children (in the striatum (Jin et al. 2001) and in the right frontal lobe (Courvoisie et al. 2004)) and in only one study in adults (Colla et al. (2008) reported significantly increased signal of Cho in both left and right ACC). These findings tie in nicely with findings of altered membrane phospholipids in a $^{31}$P-MRS study (Stanley et al. 2006); however, further studies dealing with choline compounds are necessary to confirm these alterations.

Generally the choline signal is regarded as reflecting the turn-over of cell membranes (Gujar et al. 2005). However, some authors also discuss a possible role of choline-containing substances in the energy metabolism (Purdon et al. 2002) or a possible relationship to cholinergic neurotransmission (Freeman et al. 1976). Significant increases in the Cho resonance are commonly observed in neurodegenerative disorders such as Alzheimer's disease and multiple sclerosis (MS), as well as cases of ischaemia and head trauma, presumably due to the release of Cho-containing compounds during membrane breakdown. Increased Cho signals can also be associated with cancer, most likely due to the increased cellular density found in tumours (Stork et al. 2005). In summary, the issue of the precise meaning of altered Cho-signals in MRS is not yet resolved and further basic research is needed to produce more clarity in this matter.

In summary little is done on the field of MRS research in ADHD, with only 16 studies being published so far and all findings being still preliminary. In particularly, in adult ADHD there are only three studies. Most studies measured relative signals, i.e. metabolite ratios and not absolute metabolite signals. Cho, NAA and Glx could be identified as the most important altered metabolites in spectroscopic ADHD research. The choline signals in particular turned out to be altered across different brain regions in childhood and adult ADHD. New methodological developments offer the opportunity of absolute metabolite quantification in larger cerebral areas and thereby possibly open new perspectives in studying ADHD pathophysiology.

Two regions could be identified to be actually in focus of spectroscopic investigations: the frontal lobe including ACC and parts of PFC and the basal ganglia, in particular the striatum.

Further studies should focus on further critical brain areas such as the cerebellum or limbic structures like amygdala or hippocampus known to be abnormal from morphological ADHD studies. The thalamus, as crossing point of several fronto-striato-thalamo-frontal brain circuits should also attract more attention for a better understanding of the neurochemical mechanisms implicated in the pathophysiology of ADHD.

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## Statement of interest

No author has a conflict of interest concerning this manuscript.

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**REVIEW ARTICLE**

# From psychosurgery to neuromodulation: Deep brain stimulation for intractable Tourette syndrome

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**Abstract**
Tourette syndrome is a neuropsychiatric disorder characterized by motor and vocal tics. It is often associated with depression, obsessive-compulsive symptoms, self-injurious behaviour and attention deficit-hyperactivity disorder (ADHD). In intractable patients, neuromodulation using deep brain stimulation (DBS) has widely replaced psychosurgery. Three different key structures are defined for DBS, the medial portion of the thalamus, the globus pallidus internus and the anterior limb of the internal capsule/nucleus accumbens. This is a comprehensive overview on the effect of DBS on motor and non-motor symptoms using different case series and two larger studies.

**Key words:** *Deep brain stimulation, Tourette syndrome, thalamus, globus pallidus internus, nucleus accumbens/anterior limb of internal capsule*

## Tourette syndrome: Clinical symptoms, pathophysiology, treatment options

Tourette syndrome is a chronic neuropsychiatric disorder characterized by motor and vocal tics (Swain et al. 2007). The onset is usually in childhood with aggravation of tics in early adolescence. Most patients improve by their late teens or early adulthood (Leckman et al. 2001; Singer 2005). The severity of tics waxes and wanes over time. Teasing on the street and in school by other students increases tic severity and intensity while focused activity such as reading reduces tics (Leckman et al. 1989; Leckman et al. 1997; Leckman et al. 2001). Tourette syndrome is often accompanied with comorbidities such as obsessive-compulsive disorder (OCD), depression and attention-deficit-hyperactivity-disorder (ADHD). OCD symptoms vary in degree and especially light forms are very often associated with Tourette syndrome (Singer 2005; Swain et al. 2007).

Pathomechanisms include a strong genetic component. However, research has failed to define a single gene locus for the disorder (Grados et al. 2008; Laurin et al. 2008). Neuroanatomically, a dysfunction of the cortico-striato-thalamo-cortical circuit has been described to be responsible for the cardinal symptoms of the disorder (Peterson et al. 2003; Singer et al. 2005). Interaction between basal ganglia and forebrain circuits as well as the limbic system modulate tic severity and frequency (Peterson et al. 1998; Stern et al. 2008). Structural neuroimaging studies show volume changes in the basal ganglia (caudate nucleus, globus pallidus, putamen) and the amygdala (Peterson et al. 1994, 2003; Amunts et al. 2007; Peterson 2007). Functional neuroimaging studies highlight the role of the anterior cingulate cortex in tic suppression (Bohlhalter et al. 2006; Kawohl et al. 2008). The pharmacological treatment is enriched by the use of atypical neuroleptics and SSRI (Peterson et al. 1998; Scahill et al. 2003; Kawohl et al. 2007a,b; Seo et al. 2008). Cognitive-behavioural therapy (CBT) techniques such as habit reversal training have been proven to be effective, but improvements do not sustain over time (Carr et al. 1996; Marsh et al. 2004; Carr et al. 2005; Marsh et al. 2007).

A certain population of patients, however, neither responds to a sophisticated pharmacological treatment regimen nor to CBT interventions. These patients are usually severely affected and often suffer from comorbidities such as obsessive-compulsive

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disorder (OCD) and frequent self-injurious behaviour (SIB). For these groups of patients, an alternative therapy regimen is needed. Since 1955, various attempts have been undertaken to reduce symptoms in these patients through neurosurgical procedures (for a review see Temel and Visser-Vandewalle 2004; Babel et al. 2001). Different target regions have been defined such as frontal lobes (prefrontal lobotomy, bimedial frontal leucotomy), limbic system (limbic leucotomy and anterior cingulotomy), thalamus and cerebellum. An amalgamation of different neurosurgical procedures has been used, e.g., anterior cingulotomies in combination with intrathalamic lesions. However, the outcome has often been unsatisfactory regarding the improvement of tics and self-injurious behaviour, accompanied by severe side effects such as hemiplegia or dystonia (for a review see Temel and Visser-Vandewalle 2004; Temel et al. 2004; Babel et al. 2001).

Benabid and co-workers (Benabid et al. 1987; Benabid 2007) laid the groundwork for today's deep brain stimulation through their intraoperative observation that high-frequency stimulation (>100 Hz) produced clinical benefits similar to subsequent ablation. Based on the lesioning approaches of thalamic nuclei by Hassler and Dieckmann (1970), Visser-Vandewalle and colleagues applied high-frequency stimulation instead of lesioning in three treatment-resistant Tourette patients and led the way towards neuromodulation instead of lesioning in the treatment of Tourette syndrome (Vandewalle et al. 1999; Visser-Vandewalle et al. 2003).

## Target structures and stimulation parameters for DBS in Tourette syndrome

To date (based on pubmed July 2008), several case reports and two studies, including five and 18 Tourette patients, report on the outcome of deep brain stimulation in Tourette syndrome. Five target points in three different structures have been used: (1) thalamus ((a) medial portion of the thalamus (cross point of the centromedian nucleus, substantia periventricularis and nucleus ventrooralis internus), (b) medial portion of the thalamus (centromedian nucleus, parafascicular nucleus); (2) globus pallidus internus ((a) posteroventrolateral part, (b) anteromedial part), and (3) the nucleus accumbens/anterior limb of the internal capsule (Hardesty et al. 2007; Larson et al. 2008).

The outcome of stimulation and side effects depend on the chosen target structures as well as on the stimulation parameters. After surgery many programmers wait up to 4 weeks to allow for resorption of oedema near the lead, which may cause transient improvement (i.e. "lesional effect"). The initial programming needs – independently of the target structure – fine-tuning in most cases. Basic principles for DBS programming are the use of another contact when side effects and therapeutic response co-occur. Another contact may replace the stimulator case as the anode resulting in a more focal stimulation. If efficacy is lacking, increasing the intensity and modifying the pulse width will improve the outcome (Larson 2008). Side effects such as sedation or vertigo are reported to respond to changes in the stimulus intensity (Welter et al. 2008). The reviewed studies for Tourette patients use variable stimulation intensities between 1.5 and 6 V across the different targets; the pulse frequency is set between 60 and 185 Hz with a pulse width varying from 60 to 210 μs. The individual settings are displayed for each study in the corresponding tables. There is no distinguishable pattern in the stimulation setting for each target.

A systematic assessment of stimulation parameters such as Moreau et al. (2008) performed in Parkinson patients and Kuncel et al. (2007) in patients suffering from essential tremor is not yet available in Tourette patients. To date, the most often used target points for deep brain stimulation in Tourette patients lie in the thalamus.

### Medial portion of the thalamus

The first case of deep brain stimulation in Tourette syndrome was reported by Vandewalle et al. (1999). In a male 42-year-old patient DBS eliminated the tics from 38 to 0 tics per minute over a follow-up period of 12 months. Another report including three male Tourette patients by the same group showed a reduction of tics following stimulation varying from 53% (one week) up to 90.1% (follow-up period, 5 years). Two of the three reported patients had suffered from self injurious behaviour and obsessive-compulsive disorder symptoms (Visser-Vandewalle et al. 2003). The authors report that the SIB as well as OCD/OCB disappeared completely. As side effects the authors reported a sedative effect when the stimulator was programmed to the level best to reduce the tics and changes in sexual behaviour in two out of three patients (Visser-Vandewalle et al. 2003). Sexual behaviour was affected differently in the two male patients. One reported increased sexual drive, the other erectile dysfunction (Temel et al. 2004).

Maciunas et al. (2007) used the anterior extent of the centromedian–parafascicular complex as a target point for DBS in their study. In this group, three out of five Tourette patients responded with a reduction of tics in average by 50%. The follow-up period was

3 months. Measures of anxiety, depression and OCD showed a trend towards improvement. SIB was not assessed systematically before and after surgery. The score of the Yale Brown Obsessive Compulsive Scale (YBOCS) (Goodman et al. 1989a,b) was reduced from 12.6 to 7.0 points. As a side effect of DBS in one patient, a psychotic episode occurred. No biomarker or clinical salient feature was evident to distinguish the responders from non-responders. Bajwa et al. (2007) report on one 50-year-old male patient with OCD and SIB as comorbidities. Stimulation resulted in an improvement of 66% on the Yale Global Tic Severity Scale (YGTSS) and of 76% on the YBOCS (follow-up period, 24 months).

In a larger study by Servello et al. (2008) including 18 patients, bilateral thalamus stimulation resulted in an improvement varying between 24 und 79% in 15 of 18 patients. The follow-up period ranged from 3 to 18 months. Nine patients were reported to suffer from self-injurious behaviour, nine from obsessive-compulsive behaviour/disorder. The authors report that the comorbid symptoms decreased after DBS, although they do not give a detailed description of the comorbidities and their course after implementation of DBS.

In summary (according to pubmed July 2008), 28 Tourette patients (three female, 25 male) underwent surgery for DBS with thalamic nuclei as targets. Over 50% of the patients had suffered from comorbidities, especially OCD and SIB. Twenty-five of the 28 Tourette patients responded to DBS concerning their motor symptoms. The outcome on tics was a reduction of approximately 49%, with a follow-up period ranging from 3 months to 5 years. The assessment of improvement of comorbidities is difficult due to the limited data given. Table I reports the details as far as assessed in each study.

### Globus pallidus internus

Ackermans et al. (2006) raised the question whether DBS in Tourette syndrome should focus on two targets rather than one. Based on the previous studies/protocols of globus pallidus internus (GPi) stimulation in dystonia, different groups applied DBS in the GPi in Tourette syndrome patients. Case reports choosing the internal part of the globus pallidus as a target are shown in Table II.

Four different case reports/case series describe patients with four implanted electrodes: two in thalamic target structures and two in the globus pallidus internus (van der Linden et al. 2002; Houeto et al. 2005; Ackermans et al. 2006; Welter et al. 2008).

In the study by Ackermans and co-workers the stimulation results at either site were comparable (tic reduction between 85 and 92% over a follow-up period of 12 months) so the authors decided to externalize the pallidal electrodes. They reported that compulsions resolved in both patients of which one was stimulated in the thalamus and one in the GPi.

After separate and combined measurements, Houeto et al. (2005) reached the same conclusion and externalized the pallidal electrodes in their patient (tic reduction 70%, follow-up period, 24 months). Both stimulation sites abolished SIB.

A case report of a 27-year-old man described by van der Linden and colleagues is also in accordance to these results. These authors decided to externalize the pallidal electrodes (tic reduction up to 95%, follow-up period, 6 months). Pallidal stimulation induced a higher tic reduction in comparison to thalamic simulation at lower stimulation intensities (van der Linden et al. 2002) which prolongs the battery life. As comorbidity, a mild depression is reported but no follow-up data regarding the mood after DBS are available.

In a double-blind randomized study reported by Welter et al. (2008), three patients underwent bilateral stimulation in the thalamus and the GPi. Stimulation of the GPi alone resulted in improvement between 65 and 96%. Bilateral stimulation of the thalamus alone yielded an improvement of 30–64%, simultaneous stimulation, however, did not further reduce tic severity (43–76%). Depressive mood, emotional hypersensitivity, anxiety and impulsiveness tended to decrease with thalamic or simultaneous thalamic and pallidal stimulation but not with pallidal stimulation alone. The follow-up period varied between 20 and 60 months. Accidental failure of pallidal stimulation led to reoccurrence of SIB in one patient.

To the best of our knowledge, Shaded et al. (2007) report the only case of an adolescent aged 16 years in whom DBS in the GPi was performed. He reported a reduction of YGTSS scale from 90 to 14 for a follow-up period of 6 months. The boy suffered from severe SIB and OCD in addition to the Tourette syndrome. The Child Yale-Brown Obsessive Compulsive Scale (CYBOCS) was reduced from a score of 16 to 5 points. However, the patient was required to wear a body shield after surgery to prevent him from harming the stimulator system.

Another single case report on electrode implantation in the globus pallidus internus in a 27-year-old man is reported by Diederich et al. (2005). In the follow-up period of 14 months, the YGTSS total score decreased from 83 to 44. The obsessive-compulsive behaviour described as mild remained

Table I. Overview for DBS in thalamic structures.

| Authors | Site of stimulation Stimulation parameter | N | Follow-up period | Comorbidity | Outcome measure YGTSS or Videotape based | Medication | Special remarks |
|---|---|---|---|---|---|---|---|
| Vandewalle et al. 1999 | Bilateral thalamus (centromedianer nucleus, substantia periventricularis, nucleus ventrooralis internus) 4 V,130 Hz, 450 μs | $N=1$, male, 42 years old | 12 months | NA | YGTSS NA, videotape 100%, r | NA | |
| Visser-Vandewalle et al. 2003, | Bilateral thalamus (centromedianer nucleus, substantia periventricularis, nucleus ventrooralis internus) 3–6 V, 125 Hz, 210 μs) | $N=3$, males, 28, 42, 45 years old | Case 1: 5 years, case 2: 1 year, case 3: 8 months | 3 OCB/OCD 2 SIB | YGTSS NA, % reduction of tics videotape-based: case 1, 82.5% 1 week, 90.1% 5 years; case 2, 60.5% 1 week, 72.2% 1 year; case 3, 53.6% 1 week, 82.6% 8 months | NA | Sedative effect that accompanies the positive effects on the tics; effects on sexual behaviour in 2/3 patients; OCD/OCB, SIB disappeared completely |
| Maciunas et al. 2007 | Bilateral thalamus (anterior extent of the centromedian-parafascicular complex) 3.5–3.6 V, 90–210 μs, 90–210 Hz | $N=5$, 5 male, 18–34, mean 28.2 years old | 3 months | 4 OCD 5 Depression 3 ADHD | YGTSS improvement 66% | Idem pre- and post-surgery except one patient due to psychotic episode | BDI-2 10.6± 7.6 vs. 4.2 ±5.4 HAM-D 13.6± 1.5 vs. 9.6±6.5 HAM-A 16.4± 4.2 vs. 8.0±7.0 YBOCS 12.6±10.7vs. 7.0±4.2 SIB NA Responders: 3/5 |
| Bajwa et al. 2007 | Bilateral thalamus (centromedianer nucleus, substantia periventricularis, nucleus ventrooralis internus) 2.0 V, 130 Hz, 90 μs | $N=1$, male, 50 years old | 24 months | SIB, OCD | YGTSS improvement 66% | Haloperidol reduced from 6 to 1mg | YBOCS 29 vs. 7 SIB improved, not abolished |
| Servello et al. 2008 | Bilateral thalamus (centromedian-parafascicular, ventral oral complex) 2.5–4 V, 130 Hz, 60–120 μs | $N=18$, 3 female, 15 male, aged 17–45 years, mean 28.4 years old | 3–18 months | 9 SIB 9 OCB 2 Depression 1 ADHD | YGTSS improvement varies between 24 and 79%, mean 64% | 3 no medication necessary, 15 reduced medication | The comorbid symptoms of OCB, OCD, anxiety, self-injurious behaviours and premonitory sensations decreased after DBS. |

Table II. Overview for DBS in the globus pallidus internus structures.

| Authors | Site of stimulation | N | Follow-up period | Comorbidity | Outcome measure YGTSS or video-tape based | Medication | Special remarks |
|---|---|---|---|---|---|---|---|
| Van der Linden et al. 2002 | Implantation of four electrodes, two globus pallidus internus and two medial thalamus (stimulation parameter not reported) | $N=1$, male, 27 years old | 6 months | Mild depression | YGTSS NA, bilateral thalamus 80% with high stimulus intensities 7 days after surgery, bilateral GPi with 95% with low intensities 7 days after surgery and 6 months | NA | Externalization of pallidal electrodes |
| Welter et al. 2008 | Implantation of four electrodes; bilateral thalamus (centromedian, parafascicular complex) Bilateral Gpi (ventromedial part) 1.5 V, 130 Hz, 60 μs | $N=3$, 2 female 36 and 30 years old, 1 male, aged 30 years old | Patient 1: 60 months, patient 2 33 months, patient 3 20 months | (1) SIB, anxiety, depression, symptoms suggestive of borderline personality (2) SIB, mental counting (3) generalized anxiety disorder | YGTSS and video assessment Bilateral stimulation of the GPi resulted in improvement between 65 and 96% Bilateral stimulation of the thalamus 30–64%, simultaneous stimulation did not further reduce tic severity (43–76%) | Patient 1: dopamine antagonist medication discontinued. Patient 2: dopamine antagonist reduced by 66% | Depressive mood, emotional hypersensitivity, anxiety and impulsiveness tended to decrease with thalamic or simultaneous thalamic and pallidal stimulation but not with pallidal stimulation alone. Failure of pallidal stimulation led to reoccurrence of SIB in patient 1; adaptation of stimulation in patient 2 proved difficult; best stimulation was pallidal stimulation 20 h on 4 h off per day |
| Houeto et al. 2005 | Implantation of four electrodes; bilateral thalamus (centromedian, parafascicular complex) Bilateral Gpi (ventromedial part) 1.5 V, 130 Hz, 60 μs | $N=1$, female, 36 years old | 24 months | SIB, anxiety, depression, symptoms suggestive of borderline personality | Bilateral thalamus or bilateral GPi or simultaneous stimulation of thalamic and GPi electrodes reduced YGTSS 70% | Neuroleptics successfully withdrawn | Both targets eliminated SIB and ameliorated coprolalia; no potentiation effect |
| Diederich et al. 2005 | Bilateral globus pallidus internus, 2 V, 185 Hz, 60 μs | $N=1$, male, 27 years old | 14 months | Mild OCB | YGTTS improvement 47% | NA | Pronation/supination bradykinesia left extremities, haematoma at right electrode tip; OCB unchanged |

Table II (*Continued*)

| Authors | Site of stimulation | N | Follow-up period | Comorbidity | Outcome measure YGTSS or video-tape based | Medication | Special remarks |
|---|---|---|---|---|---|---|---|
| Ackermans et al. 2006 | Case 1 bilateral thalamus, nucleus ventrooralis intrenus, centromedian nucleus, substantia periventricularis, 130 Hz, 120 μs, 6.4 V Case 2 Implantation of four electrodes, two bilateral thalamic as in case 1, two bilateral posteroventrolateral GPi, 3.1 V, 170 Hz, 210 μs | $N=2$, 2 male, 27, 45 years old | 12 months | 2 OCB, OCD, SIB case 1 breaking glass | YGTTS NA, videotape based; case 1, 85%; case 2, 92% reduction | NA | In case 2 pallidal electrodes were adapted to stimulator, case 2 turning on of the stimulation results in sudden dystonic jerk of the whole body , Compulsions resolved in both patients, sexual functions altered case 1 |
| Shahed et al. 2007 | Bilateral Gpi Right: 5 V, 160 Hz, 90 μs; left: 5 V, 145 Hz, 90 μs | $N=1$ male, 16 years old | 6 months | OCD, SIB, depression, ADHD | YGTSS 84% | NA | CYBOCS 16 vs. 5 After implantation body shield was required to prevent harm of the system by SIB |
| Dehning et al. 2007 | Bilateral Gpi 4.2 V, 145 Hz, 210 μs | $N=1$, female, 44 years | 12 months | OCD, SIB, | YGTTS 88% | NA | No details given about SIB and OCD. |

unchanged. As a side effect, the patient suffered a small haematoma around the tip of the right electrode resulting in pronation/supination deficits of the left extremities.

Bilateral GPi stimulation in one patient is also reported by Dehning et al. (2007). In a 44-year-old woman with SIB and OCD, the YGTSS improved from a score of 83 to 10 in a follow-up period of 12 months. The influence on SIB and OCD is not reported.

Stimulation in the internal part of the globus pallidus in a total of 10 Tourette patients (three female, seven male) led to an improvement of approximately 76% of the tics. Comorbidities such as OCD and SIB improved in four out of seven, but no details are given. The follow-up period varied from 6 to 60 months.

### Anterior limb of the internal capsule/nucleus accumbens

Focusing on the comorbidities such as OCD and SIB, the question of a third target for DBS in Tourette syndrome arises. The rationale is based on two observations.

One is the modulation of tic severity and frequency via interaction of the basal ganglia, forebrain circuits and the limbic system (Rauch et al. 1995; Peterson et al. 1998; Graybiel et al. 2000; Jankovic et al. 2001; Rauch et al. 2003; Stern et al. 2008). Neuroimaging studies show volume changes in the basal ganglia (caudate nucleus, globus pallidus, putamen), the amygdala (Peterson et al. 2001; Peterson 2007) and the anterior limb of the internal capsule (Neuner et al. under review).

The second observation is the positive outcome of nucleus accumbens stimulation for therapy-resistant OCD (Nuttin et al. 2003; Sturm et al. 2003).

Flaherty et al. (2005) reported a reduction of 25% in the YGTSS score and a marked improvement of SIB after bilateral implantation of electrodes in the anterior limb of the internal capsule/ventral striatum in a 37-year-old female patient. The follow-up period was 18 months. Noteworthy is the described change of mood in dependence of the chosen contact for stimulation. The authors describe that high voltage stimulation of the ventral most contacts in the vicinity of the nucleus accumbens induced mild apathy and depression, whereas with high voltage stimulation at the dorsal most contacts, in the body of the capsule, produced hypomania. Middle contact stimulation which helped tics the most was also accompanied by mood stability. Kuhn et al. (2007) report on a 26-year-old male patient who underwent implantation of bilateral electrodes in the ventral capsule internal/nucleus accumbens. This patient suffered from severe Tourette syndrome with comorbid SIB and OCD. Because of the severity, the patient had to be restrained continuously in a nursing home. After implantation of the electrodes, the YGTSS score was reduced from 90 to 53 and the YBOCS score from 25 to 9. SIB improved drastically and the patient did not have to be restrained anymore (Kuhn et al. 2007). The follow-up period was 30 months. We observed a comparable positive effect on tics and SIB/OCD in a 36-year-old male patient who had electrodes implanted bilaterally in the nucleus accumbens. Most impressive is the successful treatment of SIB and the urge to destroy glass (Neuner et al. 2005, 2007, submitted). The reduction of tics and SIB was stable for 38 months. The case reports for stimulation of the ventral striatum/nucleus accumbens are summarized in Table III.

Stimulation in the anterior limb of the internal capsule/nucleus accumbens is reported in three Tourette patients (three male). All patients suffered from SIB and two from symptoms of OCD. The stimulation resulted in a tic reduction of approximately 43%, the follow-up period varied from 18 to 38 months. In one patient (Neuner et al. 2008), SIB stopped. In the other two it was significantly reduced. YBOCS score, reported in two out of three patients, improved by approximately 50%.

## Conclusions

Three different target regions have been chosen for the treatment with DBS in intractable Tourette syndrome. Overall, 40 patients have been treated with deep brain stimulation which reduces the tics in average by approximately 60% within a broad range in responders (37 out of 40) from 24 to 100%.

The most severe reported surgical complication was a small haematoma around the tip of one electrode in two patients (Diederich et al. 2005; Ackermans et al. 2008). Ackermans et al. (2008) report vertical gaze palsy in a 39-year-old Tourette patient after implantation of DBS in the medial portion of the thalamus. After the follow-up period of 6 months, upward saccadic velocities were reduced by 20–25°. For this case, no outcome measures regarding the tics are reported. Servello et al. (2008) report as minor surgical complications wound healing problems in one patient and an abdominal wall haematoma in another. Side effects of stimulation with thalamic stimulation are sedation – depending on stimulation intensity – and changes in sexual behaviour such as erectile dysfunction (Temel and Visser-Vandewalle 2004; Temel et al. 2004). For pallidal stimulation, dystonic jerks were described in one patient when stimulation was

Table III. Overview for DBS in in the anterior limb of the internal capsule/nucleus accumbens.

| Authors | Site of stimulation | N | Follow-up period | Comorbidity | Outcome measure YGTSS or Videotape based | Medication | Special remarks |
|---|---|---|---|---|---|---|---|
| Flaherty et al. 2005 | Anterior limb of the internal capsule/ventral striatum 4.1 V, 185 Hz, 210 μs | $N=1$, female, 37 years old | 18 months | SIB | YGTSS improvement 25% | NA | SIB improved, different electrode settings altered her mood profoundly; high voltage stimulation of the ventral most contacts in the vicinity of the nucleus accumbens produced mild apathy and depression, whereas high-voltage stimulation at the dorsal-most contacts, in the body of the capsule produced hypomania. Middle contact stimulation, which helped tics the most, generated a stable euthymic state. Unplanned Off situations due to malfunction of the DBS system led to worsening of the clinical symptoms. |
| Neuner et al. 2006 | Bilateral nucleus accumbens/ventral capsula interna 6.0 V, 130 Hz, 90 μs | $N=1$, male, 36 years old | 12 months | SIB, OCD | YGTSS improvement 44% | None | SIB abolished |
| Kuhn et al. 2007 | Bilateral nucleus accumbens/ventral capsula interna 7 V, 130 Hz, 90 μs | $N=1$, male, 26 years old | 30 months | OCD, SIB | YGTSS improvement 41% | NA | SIB reduced, YBOCS 25 to 9 YGTSS total reduction of 41%, Rush Video Scale 50% |

ADHD, attention deficit-hyperactivity disorder; BDI, Beck Depression Inventory; CYBOCS, Children Yale Brown Obsessive Compulsive Scale; DBS, deep brain stimulation; GPi, globus pallidus internus; HAM-A, Hamilton Anxiety Scale; HAM-D, Hamilton Depression Scale; NA, not available; OCD, obsessive-compulsive disorder; OCB, obsessive-compulsive behaviour; SIB, self injurious behaviour; YGTSS, Yale Global Tic Severity Scale; YBOCS, Yale Brown Obsessive-Compulsive Scale.

switched on. Welter et al. (2008) reported on nausea and vertigo dependent on the intensity of pallidal stimulation sensations in two out of three patients.

Concerning the effect on comorbidities, the interpretation of outcome is even more difficult due to lack of a standard in assessment and reporting. Based on the limited data reported, the targets in the GPi and anterior limb of the internal capsule /nucleus accumbens seem to have a greater influence on SIB/OCD than stimulation in thalamic structures. From preliminary results, the need for a large multi-centred study tackling the question of primary target location arises. Based on the limited results, one possible approach is to assign patients to one of the three emerging targets based on their comorbidity. From our point of view, to date, the available data do not yet allow one to make a recommendation for one specific target structure. Okun et al. (2008) point out the need to carefully discuss the potential benefit of the DBS on motor and non-motor features. As Mink and colleagues indicate, the selection and inclusion of patients requires a multidisciplinary team to ensure a comprehensive assessment of a patient suffering from intractable Tourette syndrome (Mink et al. 2006; Okun et al. 2008). Outcome parameters should be reported for tics based on the Rush Video Scale, the YGTSS and comorbidities such as depression on the Hamilton Depression Scale, anxiety on the Hamilton Anxiety Scale and obsessive-compulsive symptoms on the YBOCS (Hamilton, 1959; Hamilton, 1967; Goodman, 1989a,b; Leckman et al. 1989; Goetz et al. 1999; Pappert et al. 2003). Self-injurious behaviour should be specified and reported in frequency and intensity.

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## Statement of interest

All authors report that they have no conflict of interest.

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**ORIGINAL INVESTIGATION**

# Vision in depressive disorder

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**Abstract**

*Background.* Reduced dopaminergic transmission has been implicated in the pathophysiology of major depression. Furthermore, dopaminergic neurotransmission plays an important role in the physiology of visual contrast sensitivity (CS). To test the hypothesis that altered dopaminergic neurotransmission plays a role in major depression we measured contrast sensitivity in patients with major depression and in healthy control subjects. *Methods.* Twenty-eight patients diagnosed with major depressive disorder were compared to 21 age-matched control subjects on their ability to detect a Gabor target with slightly elevated luminance contrast embedded in seven equi-contrast distracters. *Results.* Contrast discrimination thresholds were significantly elevated in unmedicated and medicated patients with major depression compared to control subjects, at all pedestal contrast levels tested. *Conclusions.* Contrast discrimination performance is reduced in depressive patients and might reflect a state of altered dopaminergic neurotransmission.

**Key words:** *Depressive disorder, contrast sensitivity, dopamine, schizophrenia*

## Introduction

In the last decades there has been on-going controversy over the aetiology of major depression. Currently an explanation based on an imbalance in neurotransmission is widely accepted. Several candidate systems have been discussed including the adrenergic and serotonergic system. Recently a number of authors raised the point of a special relevance of the dopaminergic system in the pathophysiology of major depression (Willner 1995; Ebert and Lammers 1997; Ebert and Berger 1998; Zhou et al. 2005).

### *Depression and dopamine*

As many as 40–50% of patients with Parkinson's disease, a disorder characterized foremost by a nigro-striatal dopamine (DA) deficiency, show depressive symptoms, even before the onset of the classical motor symptoms (Yamamoto 2001; Brandstadter and Oertel 2003). Drugs decreasing the dopaminergic function often provoke depressive symptoms (for example, reserpine neuroleptic drugs or α-methyldopa, Willner 1983), whereas antidepressants have the opposite effect (Wells and Marken 1989; Zhou et al. 2005). Various classes of antidepressants share the neurophysiological mechanism of sensitizing dopamine receptors and increasing the binding and amount of the dopamine receptors, the latter determined by the mRNA activity (Mai 1990; Ainsworth et al. 1998; Di Matteo et al. 2000; Lambert et al. 2000; Lammers et al. 2000; Larisch et al. 1997). The turnover of dopamine has been associated with the amount and severity of depressive symptoms (Lambert et al. 2000). Animal studies indicate that a genetically determined susceptibility to stress by the mesocortical DA system may facilitate the development of depressive behavioural symptoms via inhibition of subcortical DA transmission (Ventura et al. 2002). The dopamine hypothesis has developed from a theory of single dopamine deficiency towards a model of complex alteration of the dopaminergic system (Delgado 2000). At this point the pathology is thought to occur at the level of the dopamine receptor or beyond (i.e., the second messenger systems) including the interaction between other neurotransmitter systems (Randrup et al. 1975; Mai

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1990; Ainsworth et al. 1998; Ebert and Berger 1998; Lambert et al. 2000; Lammers et al. 2000; Zhou et al. 2005).

*Depression and vision*

Several studies addressing the issue of depression and visual deficits could produce preliminary evidence for such an inherent link (Leinhaas and Hedstrom 1994). However, so far there is but one study published that has explored visual symptoms in patients with major depression, indicating subjective visual impairment in depressed patients (Friberg and Borrero 2000). Although based solely on questionnaire data, and as such of limited validity (e.g., it was an open study without a control group and the measured items – i.e., subjective impression of luminance – were not well operationally defined), the results suggest that visual disturbances are related to depressive disorder.

*Dopamine in the visual system*

It is well established that dopamine deficiency syndromes, e.g., Parkinson's disease, are accompanied by an impaired function (Bodis-Wollner, 1990). The retina is the first stage in visual signal processing in which dopamine plays a role (Bodis-Wollner et al. 1987; Ghilardi et al. 1989; Langheinrich et al. 2000). Dopamine is found in both amacrine and the interplexiform cells (Frederick et al. 1982). Functions of the visual system which are influenced by the dopaminergic systems include both the size of the receptive field and the contrast sensitivity (CS) of the individual neurons. Previous electrophysiological, pharmacological and clinical studies have indicated that the impairment in CS is evoked by a decrease in dopamine activity of the retina (Bodis-Wollner et al. 1987; Mestre et al. 1990; Masson et al. 1993; Tebartz van Elst et al. 1997; Langheinrich et al. 2000). Recently a study analyzing CS in schizophrenia showed that unmedicated patients displayed contrast detection thresholds significantly below those of healthy subjects. In contrast, patients treated with atypical antipsychotic drugs showed unimpaired visual contrast detection; and those given typical antipsychotic drugs exhibited higher visual contrast detection thresholds (Chen et al. 2003).

*Assessment of dopaminergic dysfunction*

Detecting dopaminergic alterations directly in depression is difficult. Different techniques like single photon emission tomography (SPECT) and positron emission tomography (PET) techniques are still very crude and, in practice, do not allow for the quantification of subtle mesolimbic dopaminergic activity, but only indicate vast nigrostriatal neurotransmission (Scheibel 1997).

One approach to assess visual processing is to determine the effect of stimulus contrast in visual search tasks for a suprathreshold contrast target among distractors with a specific pedestal contrast level. Indeed visual search performance has been shown to be impaired in patients with Parkinson's disease (Lieb et al. 1999).

*Working hypothesis*

Based on the theoretical considerations summarized above in this study we asked whether depressive patients suffer from a reduced visual contrast discrimination, which might represent an impaired dopaminergic neurotransmission.

## Patients and methods

*Patients*

Patients were identified at the Department of Psychiatry of the University Hospital of Freiburg following approval by the local ethics committee. Twenty-eight patients (mean age = 37.8 years; SD = 9.5) were included after giving their informed consent. Inclusion criteria were the current diagnosis of a major depressive episode according to DSM-IV criteria classified as either major depressive disorder (MD) single episode (DSM-IV: 296.2) MD recurrent episode (DSM-IV: 296.3) or bipolar disorder (BD) the most recent episode being depressed (DSM-IV: 296.5). Exclusion criteria were as follows: (1) any other DSM-IV first axis psychiatric disorder except for anxiety disorders, (2) any general neurological or medical condition, and (3) any eye diseases except for corrected hyperopia or myopia. The psychiatric diagnosis was established by senior consultant psychiatrists based on a semi-structured clinical interview following DSM-IV criteria (Table I). Ten of the 28 patients included suffered from MD (single episode; DSM-IV: 296.2); 12 from MD (recurrent depressive disorder; DSM-IV: 296.3) and six from BD the most recent episode being depressed (DSM-IV: 296.5). The present level of depression was measured using the Beck Depression Inventory (BDI). None of the patients had a history of psychotic symptoms. The control group consisted of 21 age- and gender-matched healthy subjects without a history of neurological or mental disorders all of whom scored in the normal range of the BDI. They were selected from the general population of the city of Freiburg and had a mean age of 33.1 years (SD = 10 years).

Table I. Diagnoses comorbidity and medication of patients.

| Patient no. | Age (years) | Sex | Diagnosis [DSM-IV] | Comorbidity [DSM-IV] | Medication* |
|---|---|---|---|---|---|
| 1 | 42 | F | 3 | | D |
| 2 | 51 | M | 3 | | B |
| 3 | 27 | F | 2 | | A |
| 4 | 40 | M | 3 | | A |
| 5 | 47 | M | 3 | | B |
| 6 | 47 | M | 3 | | D |
| 7 | 19 | F | 3 | | B |
| 8 | 29 | F | 1 | | C |
| 9 | 31 | M | 2 | | A |
| 10 | 28 | F | 1 | | B |
| 11 | 48 | M | 2 | DSM-IV 300.02 DSM-IV 300.4 | A |
| 12 | 34 | F | 2 | | D |
| 13 | 46 | M | 1 | | C |
| 14 | 50 | M | 2 | | B |
| 15 | 34 | F | 2 | | D |
| 16 | 24 | F | 2 | DSM-IV 300.21 | B |
| 17 | 43 | M | 2 | | A |
| 18 | 22 | F | 1 | | A |
| 19 | 31 | F | 2 | | C |
| 20 | 49 | M | 2 | | A |
| 21 | 40 | M | 1 | | A |
| 22 | 36 | F | 3 | | B |
| 23 | 56 | F | 3 | | D |
| 24 | 32 | F | 3 | DSM-IV 300.23 | B |
| 25 | 36 | M | 1 | | A |
| 26 | 39 | M | 3 | | A |
| 27 | 31 | F | 3 | | D |
| 28 | 47 | F | 3 | | B |

1 =DSM IV 296.5; 2 =DSM IV 296.2; 3 =DSM IV 296.3; A, no medication; B, SSRI; C, other monotherapy (tricyclic medication or mirtazapin); D, combination.

*Contrast discrimination test procedure*

Subjects were seated at a 114-cm distance from the screen. Eight Gabor stimuli (circular patches of sine wave gratings) with constant orientation (45°) and spatial frequency of 2 c/deg were presented simultaneously (see Figure 1).

In every run, 80 trials were shown: 40 contained a target in which higher contrast led the target stimulus to 'pop out' against the others and 40 without an increment (all stimuli had the same contrast). In a forced-choice algorithm subjects had to decide whether or not one grating popped out against the others (i.e., one stimulus with higher contrast).

The contrast increment threshold was determined by the Best-Pest procedure for all trials with a contrast increment (best parameter estimate by sequential testing). The target contrast was adjusted by the Best-Pest in each target trial according to the subject's previous response. Therefore, the present task was a 2AFC task, even though eight stimuli were used.

Although the guessing rate was relatively high (i.e., 50%) we believe that the threshold measurements obtained were reliable, since we could collapse our measurements over all target locations (see below), thereby increasing the number of observations for each pedestal contrast level to 80 per subject (Treutwein 1995). These data were used to determine the underlying psychometric function (increment threshold, Green and Swets 1966; Liebermann and Pentland 2003). Trials without a target stimulus were used to identify the response bias (Wickens 2001). In separate conditions we varied the Michelson contrast (describing the luminance difference between the light and dark bars of the distractors) along seven contrast levels (1, 3, 10, 20, 30, 40, and 50%).

*Data analysis*

The experimental hypotheses were tested for statistical significance with respect to the effect of relative pedestal contrasts using ANOVA with the following main effects: group (patients versus controls) pedestal contrast level (repeated factor). Post-hoc analysis was performed to test the effects of type of diagnosis medication and the score on the BDI. In addition we tested for possible differences on reaction time, false alarms, and hit rates between patients and controls.

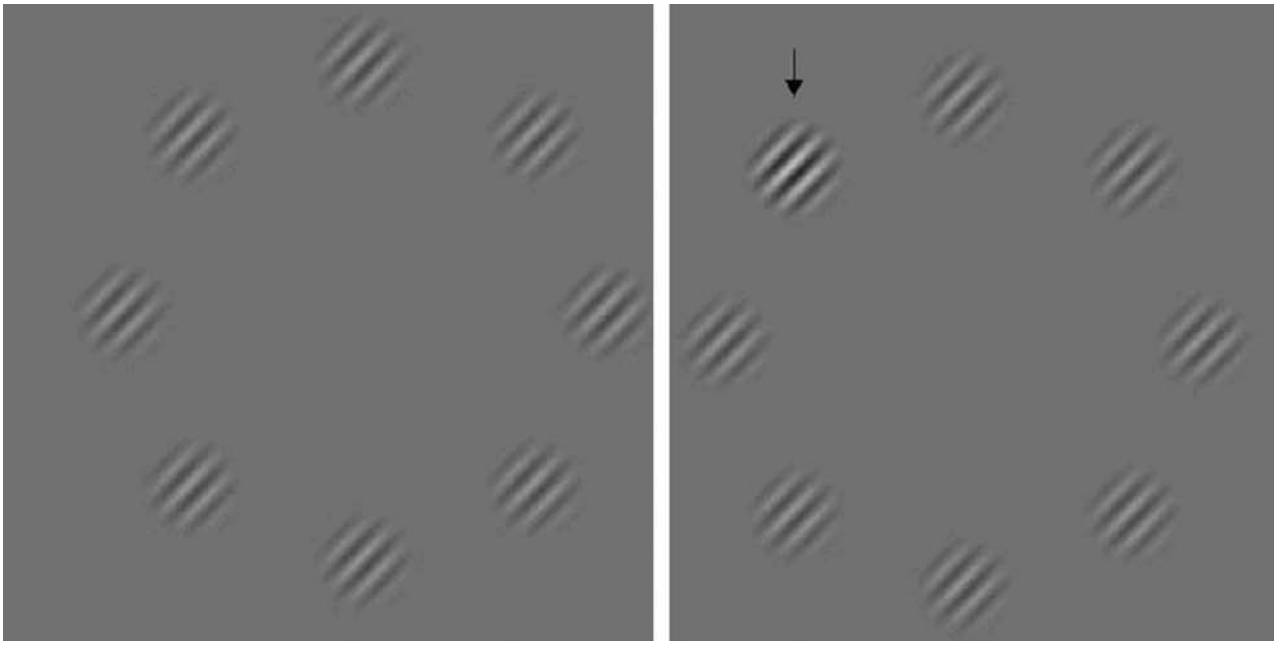

Figure 1. Example of Gabor stimuli used in the experimental set-up. The panel on the left illustrates a trial with no target stimulus (i.e., all stimuli have the same contrast). The panel on the right shows a trial with a high contrast target at the '10:30' position (arrow not present in experiments).

## Results

The results with respect to the relative contrast increment thresholds are shown in Figure 2. The seven pedestal contrast levels ($c = 0.01$ corresponding to 1% Michelson contrast to $c = 0.5$ corresponding to 50%) are plotted against the log relative contrast increment threshold log ($C_{target}/C_{reference}$).

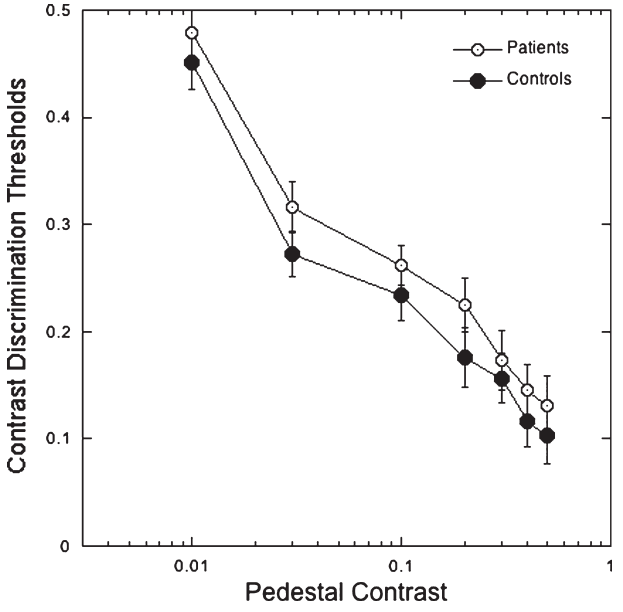


Figure 2. Log contrast increment thresholds as a function of reference contrast: the filled circles give the mean values for the control group the open circles present the mean values for the patient group. Error bars show $\pm 1$ standard error of the mean.

Overall there was no significant effect of target position so the data for each target location were pooled for the statistical analyses.

There was no interaction between group and contrast ($F_{6,329} = 0.46$; n.s.) suggesting that the effect of pedestal contrast on the relative contrast increment threshold is similar for both groups.

The patient–control group comparison revealed a highly significant difference between these groups with respect to contrast increment threshold ($F_{1,6} = 24.9$; $P = 0.0001$). Patients' thresholds were elevated for all pedestal contrast levels (Figure 2).

The factor subdiagnosis (first episode, recurrent depression or bipolar disorder) did not interact significantly with contrast increment thresholds ($F_{2,25} = 0.3$; n.s.). In addition there were no significant differences in response bias. There were no significant differences in false alarm rate. The false alarm rate increased with increasing distracter contrast, but the results from the patients is comparable to those of the controls. The pedestal contrast level had no effect on the false alarm rate see below.

### *CS and medication effects*

The medication taken by the patients at the time of study varied. A one-way ANOVA with the main effect antidepressive medication as a four-level variable (A = no medication, B = only SSRI, C = other monotherapy (one patient with tricyclic antidepressant and two patients with mirtazapin), D = combination of different antidepressant medications)

revealed a significant effect ($F_{3,24} = 4.04$, $P = 0.019$) of medication on contrast sensitivity. This effect was attributable to the small group of three patients receiving monotherapy other than SSRIs (two patients with mirtazapin and one patient with a tricyclic agent) which displayed particularly high pedestal contrasts.

Patients with MD not receiving any medication ($n = 10$) compared with the same number of randomly matched subjects from the control group also showed significantly increased contrast increment thresholds ($F_{1,19} = 4.7$, $P = 0.05$). When the 10 unmedicated patients were compared to the whole subgroup of control subjects this difference in thresholds was even more pronounced.

*Reaction time*

Figure 3 illustrates the relationship between log reaction time and the pedestal contrast level. No significant effect of pedestal contrast was found. However a significant difference between patients and controls could be noted ($F_{1,6} = 14.2$; $P = 0.0002$). The patients required approximately 60 ms longer to respond to the contrast stimuli. This effect was evident at low and high pedestal contrast levels.

*Reaction time and BDI.* An analysis of the relationship between reaction time and BDI did not reveal a significant association ($F_{3,15} = .26$; n.s.).

*False alarm rate*

There were no significant differences in false alarm rate between the patient and control groups. The false alarm rate increased with increasing distracter contrast, but the results from the patients is comparable to those of the controls (Figure 4).

*Effect of age on contrast increment thresholds*

Linear regression analysis between age and contrast increment threshold and between reaction time within the control group did not reveal any correlation between the subjects' age and these dependent variables (age and log reaction time: $R^2 = 0.20$ n.s.; age and contrast increment threshold: $R = 0.16$ n.s.).

## Discussion

In this study we analyzed possible differences in contrast discrimination thresholds between patients with major depressive disorder and healthy control subjects. The main finding is a highly significant increase in discrimination thresholds in patients with depression. The elevation in contrast discrimination thresholds corresponds to a 15% increase over the mean thresholds of the control group. This effect was apparent over all contrast levels tested and all depressive subsyndromes. Unmedicated patients also displayed significantly increased contrast discrimination thresholds.

Before embarking on a discussion of these findings we first discuss the possible limitations of this study.

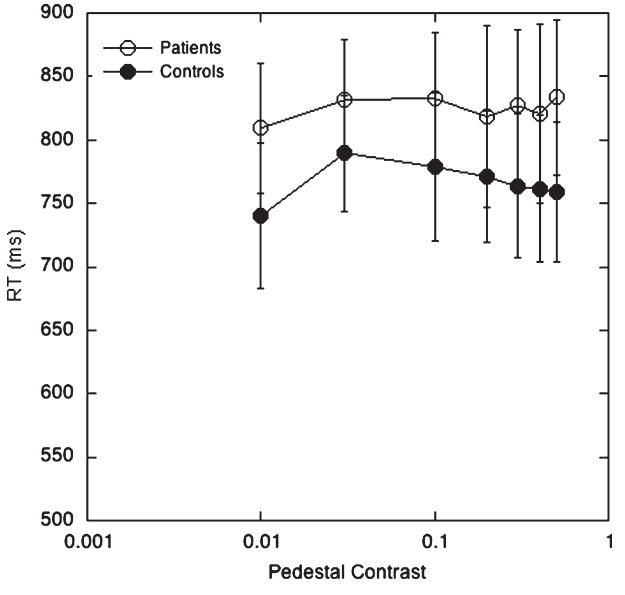


Figure 3. Comparison of reaction time between patients and controls. The symbols and error bars are as in Figure 2.

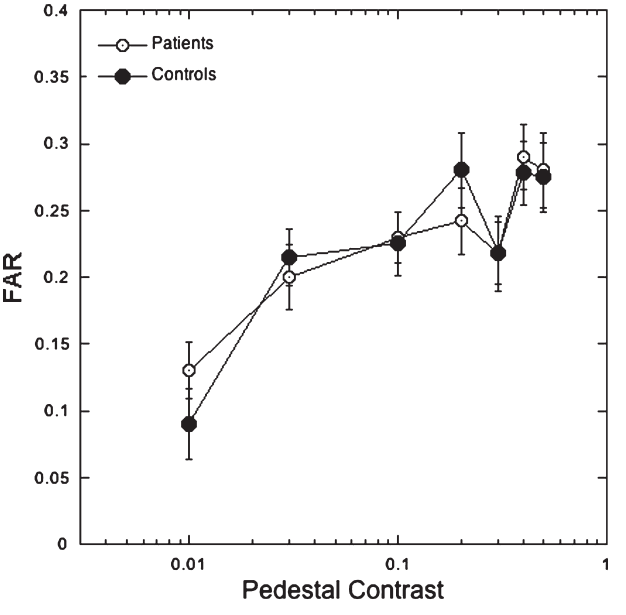


Figure 4. Comparison of false alarm rate (FAR) between patients and controls. The symbols and error bars are as in Figure 2.

*Attentional effects and the role of working memory*

Patients with depression often suffer from memory, attention, and motor disturbances (Ravnkilde et al. 2002). This explanation for our results seems unlikely since both patients and controls showed a similar dependence on pedestal contrast. The patients showed the same effect for higher distracter contrast as did the control group suggesting that fatigue or lack of alertness cannot explain our findings. Working memory is very robust for simple visual targets like those used in the present study (Greenlee and Magnussen 1998; Lee and Harris 1996; Magnussen and Greenlee 1999). Using preattentive vision in visual search minimizes memory influence on the task, since the search task requires the simple detection of the target presented among distracters (Palmer 1990). No explicit memory demands were placed on the subjects in this form of visual search. Finally the Best-Pest algorithm used in this study is especially constructed to minimize a response bias based on impaired attention or working memory by 'zooming in' into the critical contrast increment threshold.

*Response bias (false alarm rate)*

Because the false alarm rate of patients does not differ significantly from that of controls we can rule out that the differences in discrimination thresholds are related to differences in response bias.

*Medication effects*

Since both unmedicated and medicated patients displayed a significantly increased contrast increment threshold we can exclude that the observed effect is just a consequence of medication. Instead it is probably related to a not yet specified independent factor associated with depression. On the other hand increased discrimination thresholds in the group of three patients with mirtazapine and tricyclic medication might hint to a differential effect of different psychotropic medications on contrast perception. This notion is supported by the report by Chen and co-workers demonstrating a differential modulation of contrast sensitivity depending on neuroleptic medication (Chen et al. 2003). However one should be cautious with the interpretation of this observation since the subgroup is very small.

*Reduced CS in depressive disorder*

The results illustrated in Figure 2 indicate that the relative pedestal contrasts decrease with increasing pedestal contrast. This effect is evident in both groups and is characteristic for contrast increment threshold functions (Foley and Legge 1981). Compared to controls the patients require more relative contrast to detect the presence of a target. This effect is apparent over all contrast levels tested suggesting that the alteration in contrast discrimination performance is not restricted to low near-threshold contrast levels.

*Severity of depression.* Contrast increment thresholds were independent of the present severity of depression in the patient group. This suggests we might have a ceiling effect here.

*Reaction time.* As can be seen in Figure 3, the differences in RT between both groups are striking. However the methodology chosen for this pilot study ensures that performance was not affected by altered RT (i.e., all participants were given enough time to respond). Thus this observation has to be regarded as an independent component and cannot explain the increased thresholds in depressive patients, in the sense of a speed–accuracy tradeoff. Furthermore there were no significant correlations between RT on the one hand and contrast increment threshold or magnitude of depression as measured with the BDI on the other hand.

Reaction time is a cross-modal function between visual and motor systems (Lemke 1999). Motor symptoms are common in depression. Homovanillic acid (HVA) the metabolite of DA is decreased in patients with motor system impairment whereas agitated patients show an elevated HVA level (Praag et al. 1975; Northoff et al. 1996). One possible conclusion of these observations is that motor system impairment goes along with reduced DA turnover. Whether the reverse is also true remains unknown.

*Elevated visual contrast increment threshold as indicator of disturbed dopaminergic neurotransmission?*

In Parkinson's disease, dopaminergic disturbance in the visual system is just one aspect of a more widely distributed dopaminergic pathology predominantly affecting the nigrostriatal system. In accordance with the studies mentioned above, the disturbance of dopaminergic neurotransmission in the visual system of patients with depressive disorder might represent just one aspect of a more widely distributed pathology affecting the mesolimbic and nirgrostriatal dopaminergic system (Willner 1983; Bodis-Wollner 1997; Tebartz van Elst et al. 1997; Chen et al. 2003).

Further studies using longitudinal and cross-modal designs are needed to assess the validity,

specificity and sensitivity of this promising method determining diagnostic contrast increment thresholds in depression and other psychiatric disorders like schizophrenia.

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**Statement of interest**

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# Non-fatal overdose of duloxetine in combination with other antidepressants and benzodiazepines

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**Abstract**

The pharmaco-toxicological profile of duloxetine, a novel SNRI antidepressant, is still not completely known; in particular, intoxication cases have been scarcely studied. Here a duloxetine overdose case, in combination with other antidepressants and benzodiazepines, is reported and the chemical–clinical correlations discussed; this is probably the first detailed report of such a case. The patient referred to have ingested nine tablets of Cymbalta® (more than 500 mg of duloxetine) and high amounts of four other drugs (venlafaxine, trazodone, sertraline and clonazepam). The patient was dozy and confused and some electrolyte imbalances were found. After gastrolavage, toxicological analyses revealed high plasma levels of duloxetine (384 ng/ml) and low levels of the other supposedly involved drugs. The overdose resulted to be not fatal and the outcome was relatively benign, also thanks to the fast emergency assistance. This case suggests that clinicians should be alerted to the possibility of toxic effects caused by simultaneous overdoses of duloxetine and other antidepressants and that caution should be used when prescribing more than one of these drugs to patients at risk of suicide.

**Key words:** *Duloxetine, overdose, depression, HPLC-UV analysis, plasma levels*

## Introduction

Duloxetine ((γ*S*)-*N*-methyl-γ-(1-naphthalenyloxy)-2-thiophenepropanamine, DULO, Figure 1) is the most recent antidepressant drug belonging to the pharmacological class of serotonin and norepinephrine reuptake inhibitors (SNRIs), which also includes venlafaxine (VLX) and milnacipran. DULO has a potent and balanced action on both serotonin (5-HT) and norepinephrine (NE) reuptake (Bymaster et al. 2001); in vivo data support the drug dual-monoamine mechanism of action (Hunziker et al. 2005). In addition, DULO displays low affinity for human serotonergic, adrenergic, cholinergic, histaminergic and dopaminergic receptors, suggesting a low risk of adverse effects associated with action at these receptors (Hunziker et al. 2005). The drug was approved by the Food and Drug Administration (FDA) in 2002 for the treatment of major depressive disorder and in 2004 for pain associated with diabetic peripheral neuropathy (Raskin et al. 2006). It has been also approved in some countries for the treatment of stress urinary incontinence (Sweetman 2005). DULO is usually administered at daily doses ranging from 40 to 120 mg; it is well absorbed with peak plasma concentrations ($C_{max}$) occurring 6 h after administration and with an elimination half-life of about 12 h. Administration with food may delay the maximum concentration time and may decrease the extent of absorption by up to 10%. It is highly protein bound (>90%). DULO pharmacokinetics are dose-proportional over the therapeutic range. To the best of our knowledge, only one paper (Sharma et al. 2000) can be found which investigates DULO plasma levels. In this study, DULO levels were found in the 4–80 ng/ml range for daily doses from 40 to 80 mg. DULO is extensively biotransformed through hepatic oxidative metabolism mainly involving two cytochrome P450 (CYP) isozymes, CYP2D6 and CYP1A2. Excretion of unchanged DULO in the

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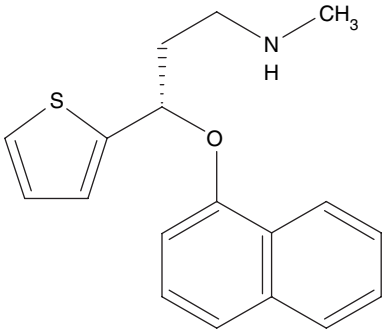


Figure 1. Chemical structure of duloxetine (DULO).

urine consists of only a trace amount (<1%) of the dose. Approximately 70% of the dose is eliminated in the urine as metabolites and 20% is excreted in faeces (Lantz et al. 2003). In clinical trials, the most common side effects associated with DULO administration were nausea, dry mouth, fatigue, insomnia, dizziness, constipation and increased sweating (Hudson et al. 2005).

The present paper reports and comments upon a case of overdose of DULO, venlafaxine, trazodone, sertraline and clonazepam with non-fatal outcome, where the toxicological analyses were carried out using a HPLC method. To the best of our knowledge, no paper describes in detail DULO overdose cases, not even in case of multi-drug intoxication. Only two papers regarding non-accidental DULO overdoses can be found in the literature (Raskin et al. 2003; Anderson et al. 2006); however, the former does not describe the cases in detail and the latter is a forensic report regarding postmortem samples.

## Case report

A 66-year-old Caucasian female with a history of depression was admitted to hospital due to a deliberate overdose of antidepressants in an attempted suicide. She reported taking nine 60-mg tablets of DULO (total 540 mg), nine 75-mg tablets of trazodone extended release (TRZ, total 675 mg) and an unknown number of 75-mg tablets of venlafaxine extended release (VLX), 50-mg tablets of sertraline (SRT) and 0.125-mg drops of clonazepam (CNZ). The patient lived alone in a protected residence and was found by a housekeeper in a confused state.

She suffered from chronic depression without history of suicide attempts. In the last few months her mood worsened due to some life events (functional impairment following physical illness, family problems) and the primary care physician prescribed her an antidepressant (SRT and then VLX). Subsequently, due to her poor clinical response, she was referred to a Community Mental Health Centre and the psychiatrist switched to DULO 60 mg once daily, TRZ 75 mg once daily and CNZ when needed.

Other than depression, the patient suffered from some physical conditions, in particular loin and sciatic pain from a slipped disk, which had been operated on 3 years previously, and chronic neuropathic pain post surgery. For these physical problems, she took gabapentin and anti-inflammatory drugs. Moreover, she suffered from relapsing herpes zoster, chronic obstructive pulmonary disease (COPD) and hypertension.

About 5 h after drug ingestion, the patient was admitted to the Emergency Department (at 10:00 h). On physical examination, she was found to be somnolent with a tendency for doziness (Glasgow Coma Scale = 14), disoriented, confused, with flat affect, slow and furred speech. She was haemodynamically stable (blood pressure 110/65 mmHg, heart rate = 60). Her blood pH was 7.4 and arterial blood gases showed $pO_2 = 65.5$ mmHg, $pCO_2 = 52.7$ mmHg and oxygen saturation = 93.3%. Scattered hisses were found on her chest examination. Shortly thereafter, the electrocardiogram (ECG) revealed a heart rate of 58 and a QTc of 423. Laboratory analyses revealed some abnormalities: serum sodium, 134 mmol/l; serum chloride, 94 mmol/l; serum potassium, 4.1 mmol/l; azotemia, 66 mg/dl. Her blood count showed leucocytosis ($16.99 \times 10^3/\mu l$) with neutrophils $10.58 \times 10^3/\mu l$, lymphocytes $4.48 \times 10^3/\mu l$ and blood platelets $455 \times 10^3/\mu l$. At the Emergency Department, a gastrolavage was carried out with 4.5 l of water, 20 g of vegetable charcoal and 20 g of magnesium sulphate that removed some tablets. The patient was also treated with intravenous fluid and oxygen therapy. Subsequently, she was transferred to the Emergency Ward and she was not co-operative, she was slowed down, soporose, with a furred speech. At 15:00 h, laboratory analyses revealed a decrease in potassium levels to 3.5 mmol/l; ECG revealed a heart rate of 69 and a QTc of 439. The day after, she was vigilant and agitated; cardiac activity parameters were: blood pressure 105/60 mmHg, heart rate 66 pulses/min and a QTc of 432. In the afternoon (at 14:00 h), she was then admitted to the Acute Inpatient Psychiatric Ward. On examination she was lucid, oriented in space and time, cooperative with low affect, she had an oxygen saturation of 96% and blood pressure 105/55 mmHg. The patient had a regular course and she was then transferred to a Psychiatric Clinic for her convalescence. Some examinations were done after 1 month: no ECG abnormalities were reported; laboratory values were normal except for her white blood cell count ($12.8 \times 10^3/\mu l$) and neutrophil

count ($9.2 \times 10^3$/μl). A sound of aortic ejection was found on heart examination.

*Drug analysis*

Thirty-five hours after the drug intake, a blood sample was taken, put into test tubes containing EDTA as the anticoagulant and immediately transported to the Pharmaco-Toxicological Analysis Laboratory. There, the sample was centrifuged at $1400 \times g$ for 15 min (4°C) and the supernatant plasma transferred into 1.5-ml polypropylene test tubes. DULO plasma levels were determined by means of an original and validated high-performance liquid chromatographic (HPLC) method; an accurate sample pre-treatment procedure was carried out by solid-phase extraction (SPE) (Mercolini et al. 2007). Assays of DULO were carried out under the following conditions: mobile phase, acetonitrile/20 mM phosphate buffer (pH 3.0); stationary phase, C8 column ($150 \times 4.6$ mm I.D., 5 μm); detection wavelength, 230 nm. Clomipramine was used as the internal standard (IS). The chromatogram of the patient's plasma sample is reported in Figure 2a. The largest peak, with a retention time ($t_R$) of 6.5 min, corresponds to DULO; the calculated plasma level was 384 ng/ml. Some of the other peaks present in the chromatogram were identified as corresponding to the other drugs involved in the intoxication (TRZ, $t_R = 4.2$ min; CNZ, $t_R = 11.3$ min; *N*-desmethylsertraline, DSR, $t_R = 13.2$ min; SRT, $t_R = 15.5$ min) by comparison with injections of standard solutions (Figure 2b). After suitable evaluation on "blank" plasma samples, it was found that the method allowed the simultaneous determination of DULO, TRZ, CNZ, SRT and its main active metabolite. The following plasma concentrations were found: TRZ, 550 ng/ml; CNZ, 83 ng/ml; SRT, 20 ng/ml; DSR, 27 ng/ml. For the analysis of VLX and its main active metabolite *O*-desmethylvenlafaxine (ODV), another original validated HPLC method with fluorescence detection was used (Mandrioli et al. 2007): the chromatogram of the plasma sample analysed with this method is reported in Figure 2c. The peak at $t_R = 6.0$ min corresponds to VLX and that at $t_R = 2.9$ min corresponds to ODV; the calculated plasma levels were 6.3 ng/ml for the former and 6.8 ng/ml for the latter.

As one can note, except for DULO, all plasma levels of central nervous system drugs are within their therapeutic ranges.

## Discussion

Buckley and McManus (2002) reported that VLX, the parent drug of SNRIs, is more dangerous in overdose than SSRIs. Thus, it is surely important to collect clinical information about DULO overdose. However, experience with DULO overdose in humans is very limited also because this drug has only been commercially available from a short time.

In fact, only two papers which report DULO overdose cases have been published. In the first paper (Raskin et al. 2003), two cases of non-fatal DULO overdose during a phase-III long-term trial are cited. However, they are reported by the authors among other "serious adverse events" and not described or commented upon in any way. In the second paper (Anderson et al. 2006), the Los Angeles County Department of Coroner released a report of a toxicological study which detected and quantified central blood levels of DULO by means of gas chromatography in 12 post-mortem cases involving suspected basic drug intake. The central blood DULO concentrations ranged from a not detectable value to 590 ng/ml. In seven of these 12 cases, the cause of death was not drug related. In the other five cases, the cause of death was multiple drug intoxication, in particular two of them were suicides and the other three accidental intoxications. DULO was not implicated as the sole cause of death in any of these cases.

The two cases of suicide were in depressed patients treated with DULO and other psychotropic drugs. The first case involved a 40-year-old female whose toxicological postmortem analyses revealed the presence of DULO, TRZ and VLX; the cause of death was established to be multiple drug intoxication. The second case involve a 42-year-old female whose toxicological postmortem analyses revealed the presence of DULO, mirtazapine, norfluoxetine, alprazolam, lorazepam, temazepam, meprobamate and morphine; the cause of death was due to morphine intoxication.

Usually, DULO is considered to be a well-tolerated drug at therapeutic doses, like most new generation antidepressants, but its pharmaco-toxicological profile is still not completely known. The most common side effects involve the gastrointestinal tract (Hudson et al. 2005). Generally, effects on the central nervous system are mild and aspecific (headache, dizziness, somnolence, insomnia). Regarding cardiovascular side effects, DULO was found to be associated with small increases in heart rate and systolic blood pressure due to potentiation of noradrenergic neurotransmission (Thase et al. 2005). Moreover, DULO at high doses (120 mg/die) caused significant decreases in PR and QRS intervals compared with a placebo; however, these differences were not clinically meaningful. On the other hand, no effects were reported on QTc even at high therapeutic doses (Thase et al. 2005). There are

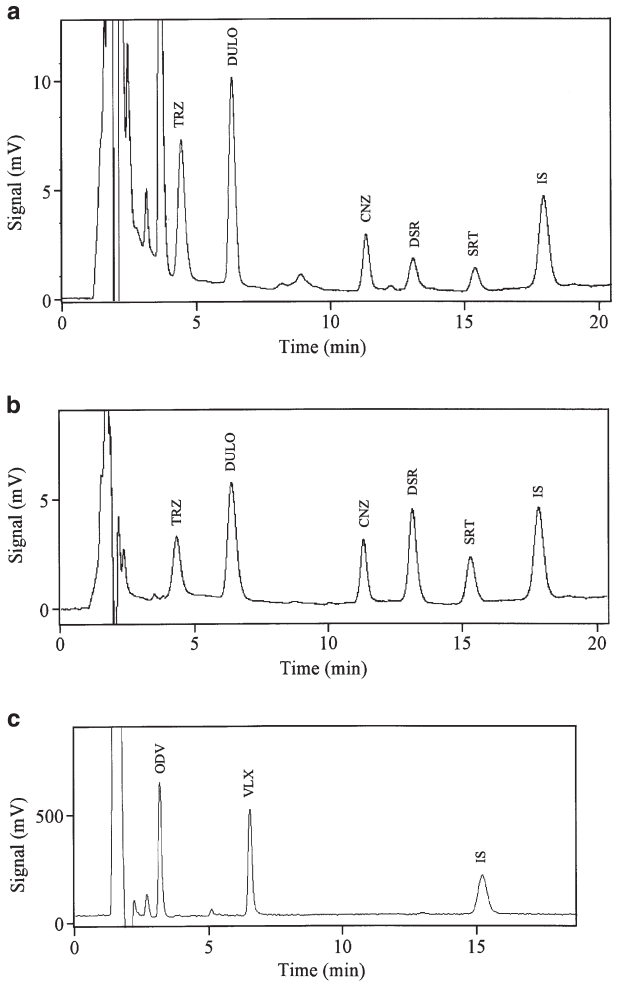


Figure 2. (a) HPLC-UV chromatogram of the patient's plasma sample; (b) HPLC-UV chromatogram of a standard solution containing 200 ng/ml of duloxetine (DULO), 200 ng/ml of trazodone (TRZ), 100 ng/ml of clonazepam (CNZ), 100 ng/ml of *N*-desmethylsertraline (DSR), 50 ng/ml of sertraline (SRT) and 100 ng/ml of clomipramine (Internal Standard, IS); (c) HPLC-FL chromatogram of the patient's plasma sample. HPLC-FL chromatographic conditions: mobile phase, acetonitrile/40 mM phosphate buffer (pH 6.8) (25/75, w/w); stationary phase, Zorbax C8 column (150 × 4.6 mm I.D., 5 μm); excitation wavelength, 238 nm; emission wavelength, 300 nm. HPLC-UV, high-performance liquid chromatography with ultraviolet detection; HPLC-FL, high-performance liquid chromatography with fluorescence detection.

no reports on the effects of DULO during multiple-drug intoxication.

The patient described in the present paper showed clinical signs and symptoms of an altered mental status and of cardiovascular alterations with hypotension, sinus bradycardia and possibly slightly prolonged QTc interval. These signs and symptoms are not reported in the literature as side effects of DULO. Moreover, the clinical experience on the other SNRI drug available on the market, VLX, showed tachycardia and hypertension (Pascale et al. 2005). Thus, a possible explanation of cardiovascular side effects might be the toxic dosage she ingested or the concomitant medications she took. Since blood sampling for the analysis of plasma levels was done several hours after gastrolavage, the acute (toxic) effects cannot be directly related to the plasma levels of the individual substances. Probably, however, the high plasma levels of DULO were responsible for the main side effects described above for the patient. In fact, several hours after gastrolavage, the plasma level of DULO was 384 ng/ml, which is about 5 times the reported (Sharma et al. 2000) plasma levels (range: 4–80 ng/ml). On the contrary, the plasma concentrations of the other co-ingested drugs (TRZ, VLX, SRT and CNZ) were subtherapeutic, as reported in the *Drug analysis* section. TRZ can cause orthostatic hypotension at therapeutic doses (50–300 mg/die) and the patient reported that she took 675 mg of this. The TRZ plasma level value found was 550 ng/ml and its therapeutic range is 800–1600 ng/ml (Sweetman 2005). If one considers that both DULO and TRZ have short elimination half-lives (6 h for DULO, 6.6 h for TRZ) and that plasma levels were measured 35 h after the overdose, both drugs could be the cause, or among the causes, of the patient's hypotension.

Even if some symptoms were alarming (altered mental status, cardiovascular alterations with hypotension, sinus bradycardia and QTc interval changes), the outcome was benign; this was also due to the quick clinical and toxicological intervention.

However, this chemical–clinical study suggests that clinicians should be alerted to the possibility of unusual cardiovascular toxic effects caused by an overdose of DULO in polypharmacy with other central nervous system drugs and that caution should be used when prescribing this drug to patients at risk of suicide.

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## Statement of interest

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**ORIGINAL INVESTIGATION**

# Lithium but not carbamazepine augments antidepressant efficacy of mirtazapine in unipolar depression: An open-label study

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**Abstract**

*Background.* The purpose of the present open-label study was to investigate the antidepressant efficacy of lithium and carbamazepine as augmentation strategies in unipolar depressed inpatients. *Method.* Forty-six patients suffering from unipolar depression (major depressive episode according to DSM-IV criteria) were pre-treated with mirtazapine for 2 weeks initially (week −2 to week 0). Thereafter, the patients received either continuation of mirtazapine monotherapy ($n=23$), combination treatment with mirtazapine and lithium ($n=13$), or combination therapy with mirtazapine and carbamazepine ($n=10$) for further 3 weeks (week 0 to week 3). Severity of depression was estimated weekly using the 21-item version of the Hamilton Depression Rating Scale (21-HAMD). Response was defined by a reduction of at least 50% in the 21-HAMD sum score after 3 weeks of pharmacotherapy (week 0–3). *Results.* Additional administration of lithium, but not adjunctive carbamazepine significantly augmented the antidepressant efficacy of mirtazapine in the unipolar depressed patients. Moreover, carbamazepine but not lithium significantly lowered the serum concentrations of mirtazapine. *Conclusion.* Whereas the clinical importance of anticonvulsants in the treatment of bipolar disorder is not in doubt, the therapeutic efficacy of antiepileptic drugs such as carbamazepine is obviously limited in the pharmacotherapy of unipolar depression.

**Key words:** *Unipolar depression, mirtazapine, lithium, carbamazepine, augmentation strategies*

## Introduction

Depression is a highly prevalent disease with significant socioeconomic and quality-of-life implications, yet frequently remains undiagnosed and undertreated in the community (Hirschfeld et al. 1997; Tylee et al. 1999). Up to half of all patients treated for depression do not respond adequately to first-line monotherapy (Nierenberg and Amsterdam 1990). For those patients, various so called second-step strategies have been proposed (e.g., increasing the dosage of the antidepressant, switching to a new class of antidepressants, combining two antidepressants, augmenting the antidepressant) of which lithium augmentation is considered to be the strategy supported by the best evidence from available research (Bauer et al. 2002).

In the 1970s several small, but controlled studies gave some evidence that the efficacy of lithium monotherapy may be superior to placebo and comparable to established tricyclic antidepressants (Mendels 1976; Souza and Goodwin 1991). Moreover, the clinical use of adding lithium to antidepressant drugs such as tricyclic antidepressants (TCAs) or monoamine oxidase inhibitors (MAOIs) was already described in 1968 and within the following years (Himmelhoch et al. 1972; O'Flanagan 1973; Zall et al. 1968), but lithium augmentation has not become common for the treatment of acute unipolar depression until the publication of de Montigny and colleagues in 1981 (De Montigny et al. 1981). They reported a dramatic response within 48 h to the addition of lithium in eight patients who had not responded to at least 3 weeks of treatment with tricyclic antidepressants. In the meantime, more than 50 clinical trials of lithium augmentation have been carried out over the past several decades, and most have shown positive effects for lithium augmentation enhancing the therapeutic effects of a broad spectrum of antidepressants (Bschor and Bauer 2006; DeBattista 2006). Bauer and colleagues (Bauer et al. 2003a) recently carried out a systematic review of lithium augmentation studies during the

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period from 1966 to 2003 in refractory, mostly unipolar depression. Of 27 prospective trials since 1981, ten were double-blind, placebo-controlled studies, four were randomized comparator studies and 13 were open-label. Overall (summarizing open and controlled data) approximately 50% of patients responded to lithium augmentation, usually within 2 to 6 weeks. In acute treatment trials, the average response rate in the lithium group was 45%, versus 18% in the placebo group ($P<0.001$) (Bauer et al. 2003a).

The clinical usefulness of antiepileptic drugs such as carbamazepine, valproate or lamotrigine as mood stabilizers is well established in bipolar disorder (Yatham et al. 2005, 2006). Their acute and prophylactic antimanic properties as well as their efficacy in the maintenance therapy of bipolar disorder in general have been repeatedly shown in controlled trials (Nemeroff 2000). However, the treatment of acute depression is the weakest element in the spectrum of psychiatric efficacy of anticonvulsants (Keck et al. 1998). In particular, studies investigating the use of antiepileptic drugs in unipolar depression are limited and have revealed conflicting results. There are no placebo-controlled studies on the use of valproate in the treatment of acute unipolar depression. However, an open study reports a substantial response rate in unipolar patients receiving valproate (Davis et al. 1996). A small number of retrospective chart reviews (Barbee and Jamhour 2002; Rocha and Hara 2003), open-label studies (Gabriel 2006; Schindler and Anghelescu 2007), or case reports (Maltese 1999), as well as two small placebo-controlled trials (Normann et al. 2002; Barbosa et al. 2003) provide evidence for some antidepressant efficacy of lamotrigine in unipolar depression. By contrast, in a number of GlaxoSmithKline-sponsored, multicentre, placebo-controlled, randomized trials in patients suffering from acute unipolar depression lamotrigine was not significantly superior to placebo in any of the efficacy measures used (Laurenza et al. 1999; Londborg et al. 1999; DeVeaugh-Geiss et al. 2000). Phenytoin was observed in a small controlled study to be as efficacious as fluoxetine in unipolar depression (Nemets et al. 2005), but was not superior to placebo in unipolar patients in another controlled trial when being added to selective serotonin reuptake inhibitors (SSRIs) (Shapira et al. 2006). Moreover, there is some evidence from a randomized, double-blind, placebo-controlled trial suggesting topiramate to be an effective agent in the reduction of depressive symptoms in female patient suffering from recurrent major depressive disorder (Nickel et al. 2005). There has also been research into the role of carbamazepine in the management of unipolar depression. In several open-label trials (Wunderlich et al. 1982a,b, 1983; Dietrich and Emrich 1998; Steinacher et al. 2002; Ciusani et al. 2004), retrospective chart reviews (Cullen et al. 1991), and case series (Prasad 1985; Schaffer et al. 1985; Schneier and Kahn 1990; De la Fuente and Mendlewicz 1992; Feiner 1997), some antidepressant effects of carbamazepine were reported. Moreover, in several controlled trials carbamazepine was found to possess comparable antidepressant effects as compared to trimipramine (Neumann et al. 1984), imipramine (Sethi and Tiwari 1984), or lithium (Rybakowski et al. 1999) and to be more efficacious in the treatment of acute depression than placebo (Post et al. 1986). However, the sample sizes of the active comparator trials were rather small having the risk of a type-II error and disallowing firm interpretations. In addition, in all controlled studies investigating putative antidepressant effects of carbamazepine a mixed population consisting of both bipolar and unipolar depressed patients was included. Since in most carbamazepine trials bipolar patients were the majority, and since carbamazepine was demonstrated to have more antidepressant efficaciousness in bipolar than in unipolar depressive disorders (Ballenger 1988), the significance of these findings in carbamazepine for unipolar depression is very limited.

In the present open-label study, the antidepressant efficacy of lithium and carbamazepine as augmentation strategies was investigated in unipolar depressed inpatients pre-treated with mirtazapine. This is the first trial comparing adjunctive lithium and carbamazepine in patients exclusively suffering from unipolar depression.

## Materials and methods

### *Patients*

Forty-six drug-free depressed inpatients (14 men, 32 women) aged between 22 and 71 years (mean age $50.78 \pm 12.27$ years) entered the study after the procedures had been fully explained and written informed consent had been obtained. The patients were the same who had been reported in former trials of our research group investigating the impact of mirtazapine, lithium and carbamazepine on the concentrations of neuroactive steroids (Schule et al. 2006, 2007). All patients entered the study after the procedures had been fully explained and written informed consent had been obtained. The patients were diagnosed by experienced and trained psychiatrists according to DSM-IV criteria using the Structured Clinical Interview for DSM-IV, German version (Wittchen et al. 1997). Inclusion criteria

for the depressed patients were: (a) a major depressive episode according to DSM-IV criteria (bipolar disorder not included); (b) a sum score of at least 18 on the 21-item version of the Hamilton Depression Rating Scale (21-HAMD) (Hamilton 1960); (c) exclusion of major medical disorders, availability of normal laboratory parameters, normal blood pressure, normal electrocardiogram, and normal encephalogram; (d) exclusion of addiction or other comorbid psychiatric diagnoses; (e) no other psychotropic drugs than the study medication for at least 5 days before and throughout the study with the exception of chloralhydrate in case of sleep difficulties; (f) exclusion of pregnancy or use of oral contraceptives. Further clinical characteristics are given in Table I.

### *Study design*

After admission to hospital and after a 5-day washout period, all depressed patients were treated with mirtazapine at a standard dosage of 45 mg/day for 2 weeks (week −2 up to week 0) to achieve steady state conditions of this antidepressant before optional additional administration of lithium or carbamazepine. After being treated with a stable dosage of mirtazapine for 2 weeks, the patients were subdivided into three different treatment groups for the following 3 weeks (week 0–3): half of the patients were treated with mirtazapine monotherapy at a dosage of 45 mg/day (group 1, $n=23$), another half received combination treatment with mirtazapine (45 mg/day) on the one hand and either lithium (group 2, $n=13$) or carbamazepine (group 3, $n=10$) on the other hand. In case of sleep difficulties, chloralhydrate (up to 1000 mg/day) was allowed as concomitant medication. The allocation to the treatment groups (monotherapy versus combination treatment) was carried out at the discretion of the doctor in attendance considering individual contraindications in the patients with regard to additional lithium or carbamazepine. The dosages of lithium and carbamazepine were not predefined but were adapted according to clinical requirements to reach

Table I. Pre-treatment, clinical and demographic data in depressed patients treated with mirtazapine monotherapy ($n=23$), combination therapy with mirtazapine and lithium ($n=13$), or combination therapy with mirtazapine and carbamazepine ($n=10$). Each patient of the combination therapy groups was treated with mirtazapine monotherapy for 2 weeks (week −2 up to week 0) before adding lithium or carbamazepine (week 0–3). Data represent mean±SD.

| | Mirtazapine monotherapy ($n=23$) | Mirtazapine+lithium ($n=13$) | Mirtazapine+carbamazepine ($n=10$) |
|---|---|---|---|
| First depressive episode | 3 out of 23 | 2 out of 13 | 2 out of 10 |
| No pretreatment | 5 | 4 | 3 |
| Tricyclics | 8 | 4 | 3 |
| SSRIs | 6 | 3 | 1 |
| Reboxetine | 1 | 1 | 2 |
| Venlafaxine | 1 | 0 | 1 |
| St. John's wort | 2 | 1 | 0 |
| TRD before trial | 5 out of 23 | 3 out of 13 | 3 out of 10 |
| Gender | 4M, 19F | 6M, 7F | 4M, 6F |
| Age (years) | 52.26±11.92 | 45.92±13.47 | 53.70±10.67 |
| Height (cm) | 166.70±8.90 | 170.08±8.21 | 170.50±8.85 |
| weight (kg) | 72.48±14.86 | 73.88±16.12 | 74.03±15.42 |
| MAP at baseline (mmHg) | 99.74±9.62 | 100.64±11.83 | 99.90±6.81 |
| Age of onset (years) | 41.26±11.77 | 35.54±13.85 | 35.10±18.79 |
| Number of episodes | 5.09±4.55 | 4.08±3.52 | 6.50±6.29 |
| $C_{[mir]}$ week 0 (nmol/l) | 279.85±120.93 | 255.94±80.41 | 281.84±103.67 |
| $C_{[mir]}$ week 3 (nmol/l) | 297.33±127.70 | 256.11±97.11 | 149.65±70.88 |
| Dosage of lithium, week 3 (mg/day) | – | 917.31±144.12 | – |
| $C_{[lithium]}$ week 3 (mmol/l) | – | 0.71±0.13 | – |
| Dose of carbamazepine, week 3 (mg/day) | – | – | 370.00±67.49 |
| $C_{[carbamazepine]}$ week 3 (μmol/l) | – | – | 32.39±8.16 |
| 21-HAMD week -2 | 26.26±4.05 | 29.08±5.91 | 28.00±7.15 |
| 21-HAMD week 0 | 19.48±5.16 | 21.31±8.74 | 17.90±9.30 |
| 21-HAMD week 1 | 15.61±6.04 | 17.38±10.07 | 17.80±8.74 |
| 21-HAMD week 2 | 14.48±6.29 | 15.31±10.49 | 16.80±9.54 |
| 21-HAMD week 3 | 13.83±7.20 | 12.62±11.01 | 15.10±10.40 |
| Duration of inpatient status (days) | 67.96±30.62 | 66.62±21.68 | 89.50±45.93 |

TRD, treatment-resistant depression (>2 treatment failures before entering the trial). M, males; F, females; MAP, mean arterial blood pressure. $C_{[mir]}$, $C_{[lithium]}$, $C_{[carbamazepine]}$, serum concentrations of mirtazapine (nmol/l), lithium (mmol/l), or carbamazepine (μmol/l).

the intended blood levels in the patients of the combination therapy groups (target levels: lithium 0.5–1.0 mmol/l; carbamazepine 20–40 μmol/l). Before lithium augmentation, normal TSH and thyroid hormone levels, regular renal function (including normal creatinine clearance) and normal cervical circumference (exclusion of pre-existing struma) were established. After completion of the study period (week 3), the depressed patients were treated ad libitum according to routine clinical requirements. Further details of the treatment design are outlined in Figure 1.

Severity of depression was estimated at baseline (week -2) and weekly within the actual study period (week 0–3) using the 21-HAMD in the morning between 09:00 and 11:00 h. In each patient, the same rater was used throughout the whole study period. All raters were experienced psychiatrists. Treatment resistant depression (TRD) before entering the study was defined as failure to respond to at least two trials of antidepressants at an adequate dose and duration from at least two different classes (Ananth 1998).

The study was carried out according to the Declaration of Helsinki (http://www.wma.net) and had been approved by a local ethics committee (intramural review panel of the Ludwig-Maximilian-University of Munich, Faculty of Medicine).

*Measurement of mirtazapine, lithium, and carbamazepine serum concentrations*

At weeks 0 and 3, the mirtazapine concentrations were determined using blood samples drawn at 08:00 h. The detection of mirtazapine was accomplished via an isocratic reversed-phase high-performance liquid chromatography (HPLC) separation and ultraviolet (UV) detection at 214 nm as described earlier (Schule et al. 2006). The limits of quantification were 2.5 ng/ml for mirtazapine. SI units (nmol/l) were calculated considering the molecular weight of mirtazapine (molecular weight of mirtazapine: 265.36).

Lithium serum levels were measured by flame photometry using an EFOX 5053 (Eppendorf AG, Hamburg, Germany). Carbamazepine levels were determined by use of CEDIA Carbamazepine assay on a Roche/Hitachi 912 analyser (Roche Diagnostics, Mannheim, Germany).

*Statistical analysis*

Homogeneity in demographic variables at baseline between the three treatment groups was analyzed by the Chi-square test for contingency tables with respect to qualitative variables (gender; type of previous medication subdivided into no medication, tricyclics, SSRIs, or other antidepressants) or by

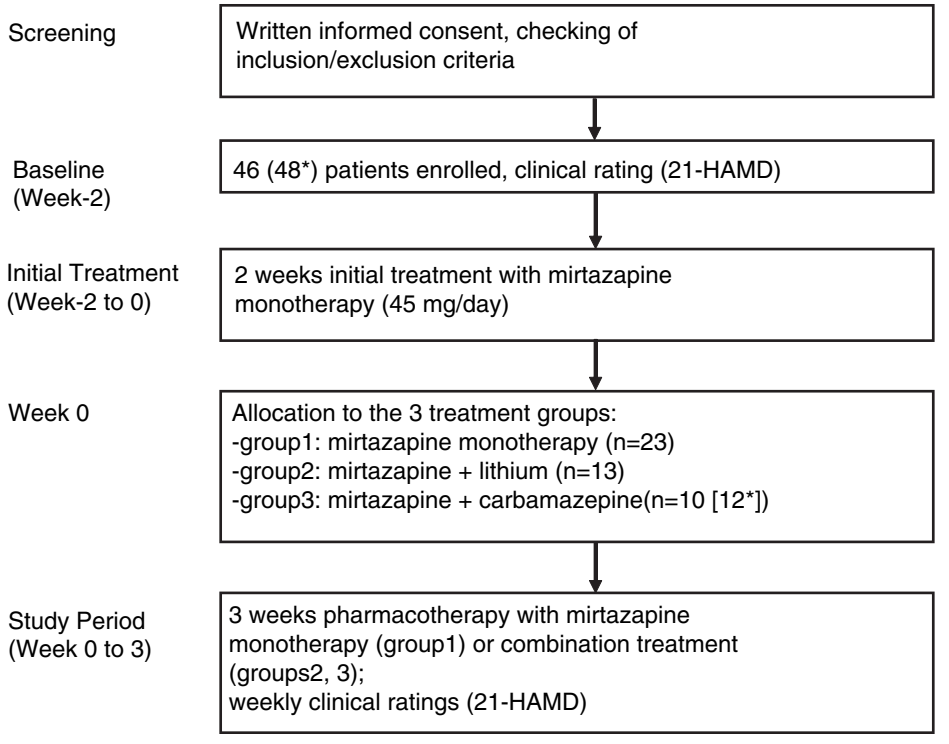


Figure 1. Flow chart of the study design. *Two dropouts in the carbamazepine group.

one-way ANOVA procedure with regard to quantitative variables (age, height, weight, mean arterial blood pressure, age of onset of the depressive disease, number of hitherto existing episodes, 21-HAMD sum score on week −2 and week 0). All patients who were subjected to the statistical analysis participated in the study up to the end of the observation period (week 3). Moreover, one-way ANOVA was employed to compare mirtazapine concentrations at baseline (week 0) and at week 3 between the three treatment groups.

The primary efficacy variable was the change in the 21-HAMD sum score between week 0 (baseline) and week 3 (end of study period). Clinical response was defined by a reduction of at least 50% in the 21-HAMD sum score after 3 weeks of pharmacotherapy (week 0–3). Response rates were compared between mirtazapine monotherapy and lithium augmentation and also between mirtazapine monotherapy and augmentative treatment with carbamazepine using the Chi-square test for contingency tables, respectively.

As a nominal level of significance, $\alpha = 0.05$ was accepted. The software program SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

## Results

At baseline, patients receiving mirtazapine monotherapy (group 1), additional lithium (group 2), or additional carbamazepine (group 3) did not differ significantly with regard to proportion of treatment resistant depressives ($\chi^2 = 0.268$; df = 2; $P = 0.874$), gender distribution ($\chi^2 = 3.798$; df = 2; $P = 0.150$) or type of pre-treatment ($\chi^2 = 1.975$; df = 6; $P = 0.922$) (Table I). Moreover, one-way ANOVA did not reveal any baseline differences between the treatment groups with respect to age ($F = 1.503$; df = 2, 43; $P = 0.234$), height ($F = 0.972$; df = 2, 43; $P = 0.387$), weight ($F = 0.053$; df = 2, 43; $P = 0.948$), mean arterial blood pressure ($F = 0.036$; df = 2, 43; $P = 0.964$), age of onset of the depressive disease ($F = 1.016$; df = 2, 43; $P = 0.371$), number of episodes ($F = 0.743$; df = 2, 43; $P = 0.482$), 21-HAMD sum score on week -2 ($F = 1.213$; df = 2, 43; $P = 0.307$), and 21-HAMD sum score on week 0 ($F = 0.632$; df = 2, 43; $P = 0.537$) (Table I). At baseline, the mirtazapine levels were comparable between the three treatment groups (Table I; $F = 0.244$; df = 2, 43; $P = 0.784$). However, there was a highly significant difference in mirtazapine concentrations between the three groups at week 3 ($F = 6.325$; df = 2, 43; $P = 0.004$). Post-hoc tests demonstrated significantly lower mirtazapine levels after additional administration of carbamazepine as compared to mirtazapine monotherapy ($P = 0.003$), whereas the mirtazapine levels in the monotherapy group and the lithium group were comparable at week 3 ($P = 0.854$), suggesting a lowering effect of carbamazepine on the mirtazapine concentrations (Table I).

During the 2-week initial treatment with mirtazapine (week −2 to week 0), the majority of patients experienced sedation, in particular within the first days of therapy. Otherwise, mirtazapine was well tolerated throughout the whole study period. With regard to patients receiving combination of mirtazapine and lithium, one patient complained about fine hand tremor to moderate degree which persisted up to week 3, but did not necessitate premature termination of the study. In one further patient, a mild lid oedema was observed after addition of lithium which disappeared after 1 week of adjunctive treatment. Moreover, one female patient reported a moderate weight gain during combination treatment with mirtazapine and lithium, but agreed to further participate in the study up to week 3. In all other cases, lithium augmentation was well tolerated without any clinically relevant side effects. In the carbamazepine group, two patients were dropouts after a few days of adjunctive carbamazepine therapy because of marked sedation, dizziness and nausea and are not reported in the evaluation of the clinical parameters. Out of the 10 patients treated with additional carbamazepine for 3 weeks, three patients reported moderate, but tolerable sedation. Moreover, one patient out of the carbamazepine group indicated mild and transient disturbances of accommodation during the first week of carbamazepine treatment which were no longer obvious for the remaining treatment period. In one patient a moderate increase of liver enzymes was seen which was not clinically significant and allowed continuation of pharmacotherapy. In other respects, adjunctive carbamazepine was tolerated without adverse effects of clinical significance.

During the study period (week 0–3), 21.7% (5 out of 23) of the patients receiving mirtazapine monotherapy (group 1) showed a clinical response (more than 50% reduction in the 21-HAMD sum score), whereas 53.8% (7 out of 13) of the patients treated with mirtazapine and additional lithium (group 2) and 20.0% (2 out of 10) of the patients receiving mirtazapine and additional carbamazepine (group 3) were responders. Moreover, these different response rates were also reflected by diverse amounts of 21-HAMD sum score reductions between week 0 and 3 (group 1: −5.65; group 2: −8.69; group 3: −2.80), being the highest in patients treated with additional lithium and the lowest in patients receiving additional carbamazepine (Figure 2). Using the Chi Square test for contingency tables, additional

administration of lithium ($\chi^2 = 3.853$; df = 1; $P = 0.050$), but not of carbamazepine ($\chi^2 = 0.013$; df = 1; $P = 0.911$) significantly enhanced the response rates as compared to mirtazapine monotherapy (Figure 3). It is also remarkable that patients receiving additional carbamazepine treatment were prone to have a longer stay in the psychiatric hospital (89.50 ± 45.93 days) than patients of the monotherapy group (67.96 ± 30.62 days) or of the lithium group (66.62 ± 21.68 days), although this observation did not reach statistical significance (one-way ANOVA: $F = 1.812$; df = 2, 43; $P = 0.176$).

## Discussion

The main result of our study is the finding that additional administration of lithium, but not adjunctive carbamazepine augments the antidepressant efficacy of mirtazapine in unipolar depressed patients. Most patients included in the present trial were not treatment-refractory and already showed some improvement within the first 2 weeks of initial mirtazapine monotherapy (week −2 to week 0). Apparently, lithium augmentation is not only useful in treatment-resistant depression as it has been demonstrated in a substantial number of randomized controlled trials (Bauer et al. 2003a), but also enhances the amelioration of depressive symptoms in non-refractory, partially improved depressed patients. According to our results, the additive antidepressant effect of lithium already begins 2 weeks after the initiation of adjunctive lithium administration. Moreover, our patient sample was restricted to unipolar depression. Whereas the efficacy of lithium augmentation in the treatment of acute unipolar depression is well documented (Katona et al. 1995; Bauer et al. 2003a; Bschor and Bauer 2006), the effectiveness of adjunctive lithium is still a matter of debate in the therapy of acute bipolar depression. Since only 6% of patients in the trials included in the meta-analysis of Bauer and colleagues (Bauer et al. 2003a) were reported to have bipolar depression, no firm conclusions can be drawn from double-blind, controlled studies as to whether depressed patients suffering from bipolar disorder respond differently to lithium augmentation than patients with unipolar depression do (Bauer et al. 2003b). Although two uncontrolled trials demonstrated even better antidepressant effects of lithium augmentation in bipolar depression than in unipolar depression (Nelson and Mazure 1986; Rybakowski and Matkowski 1992), randomized controlled studies in bipolar depressed patients are warranted.

Lithium has been found to augment the therapeutic effects of a broad spectrum of antidepressants (De Montigny 1994; Bauer 1995; Baumann et al. 1996; Zullino and Baumann 2001). With regard to mirtazapine, successful pharmacotherapy of treatment-refractory depression using the combination of

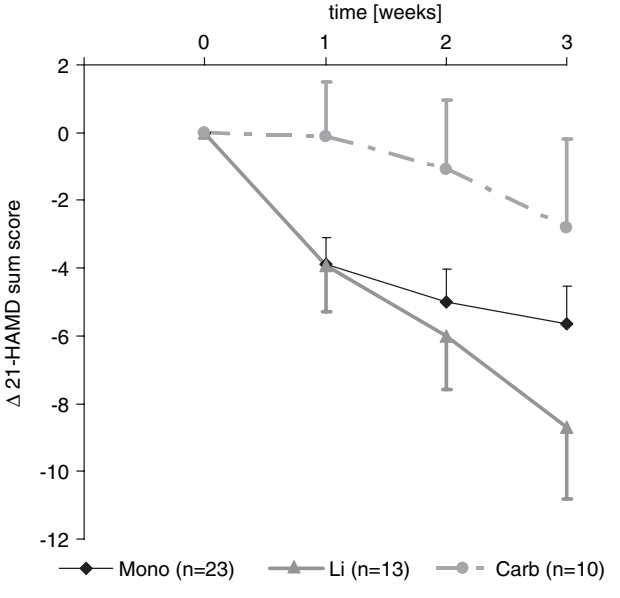


Figure 2. 21-HAMD sum score reduction in depressed patients during treatment with mirtazapine monotherapy ($n = 23$), combination treatment with mirtazapine and lithium ($n = 13$), and combination treatment with mirtazapine and carbamazepine ($n = 10$), expressed as differences versus baseline (week 0). Means ± SEM are indicated. Mono, mirtazapine monotherapy; Li, mirtazapine + lithium; Carb, mirtazapine + carbamazepine.

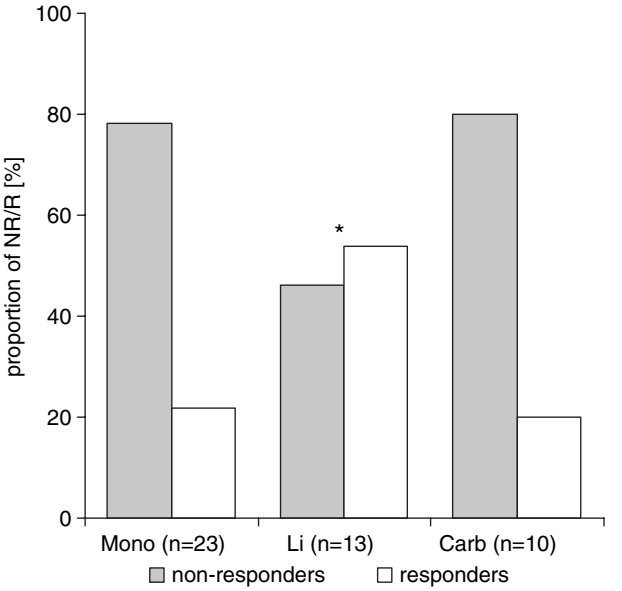


Figure 3. Proportion of non-responders and responders (%) in depressed patients treated with mirtazapine monotherapy ($n = 23$), combination treatment with mirtazapine and lithium ($n = 13$), and combination treatment with mirtazapine and carbamazepine ($n = 10$). *Significant result in the $\chi^2$-test compared to mirtazapine monotherapy ($P < 0.05$). NR, non-responders; R, responders; Mono, mirtazapine monotherapy; Li, mirtazapine + lithium; Carb, mirtazapine + carbamazepine.

mirtazapine and lithium was described in a case report (Moustgaard 2000). However, in a randomized, but open-label study comparing the efficacy of lithium addition in imipramine- and mirtazapine-treated depressed patients, the combination of imipramine and lithium was superior to the same treatment strategy with mirtazapine, but more serious side effects were observed in imipramine (Bruijn et al. 1998). In our study, combination treatment with mirtazapine and lithium was clinically effective and was in general well tolerated, although some patients sporadically complaint about lithium's well-known side effects (Rouillon and Gorwood 1998; Freeman and Freeman 2006), such as fine hand tremor or weight gain. Our finding that the mirtazapine levels remained stable after addition of lithium is in line with a former study suggesting lithium not to alter the pharmacokinetics of mirtazapine (Sitsen et al. 2000).

The neurobiological basis of the mechanism of action of lithium augmentation involves in particular a lithium-induced enhancing effect on serotonin function (Bschor and Bauer 2006). In humans, this assumption is supported by neuroendocrinological challenge test showing that additional administration of lithium increases both the prolactin response to l-tryptophan in patients receiving tricyclic antidepressants (Cowen et al. 1991) and the cortisol and ACTH secretion during the dexamethasone/CRH test in depressed patients treated with different antidepressants (Bschor et al. 2002), probably via serotonergic mechanisms. It is not clear whether the response to adjunctive lithium is a pure augmentative interaction effect, resulting from a specific pharmacological interaction between lithium and the antidepressant (e.g., in our trial enhancing the impact of mirtazapine on serotonergic transmission), or whether it is simply the antidepressant effect of lithium alone which has been shown in a series of controlled studies in the 1970s (Mendels 1976; Souza and Goodwin 1991) and which may additively enhance the efficacy of the respective antidepressant.

In contrast to lithium, adjunctive carbamazepine did not improve the antidepressant efficacy of mirtazapine in our study but even tended to rather deteriorate the depressive symptomatology as compared to mirtazapine monotherapy. Moreover, two out of 12 patients treated with carbamazepine were dropouts due to side effects (especially sedation, dizziness and nausea). Our results support the view that anticonvulsants are of limited clinical usefulness in unipolar depression and point to the fact that clinical and pathophysiological distinctions between bipolar and unipolar depression are also reflected by a different responsiveness to antiepileptic drugs (Bowden 2005). There is some evidence that anticonvulsants such as valproate (Calabrese et al. 1992; Winsberg et al. 2001; Davis et al. 2005), lamotrigine (Walden et al. 1996; Fogelson and Sternbach 1997; Kusumakar and Yatham 1997; Sporn and Sachs 1997; Calabrese et al. 1999a,b; Suppes et al. 1999; Frye et al. 2000; Van der Loos and Nolen 2006), topiramate (McIntyre et al. 2002), or carbamazepine (Ballenger and Post 1980; Post et al. 1986; Yatham et al. 1997) may be useful in the treatment of bipolar depressed patients. In particular lamotrigine has been demonstrated to be effective in acute bipolar depression with a low risk for switching into mania and is therefore regarded as one of the first-line treatment options in this disease (Yatham et al. 2006, 2005). However, in unipolar depressed patients placebo-controlled trials with sufficient methodological quality testing augmentation strategies with antiepileptic drugs such as carbamazepine or others are lacking (Coryell 2000). Therefore, augmentation with carbamazepine can not be recommended as well proven or high-level evidence-based treatment option in clinical guidelines for unipolar depression (Bauer et al. 2002). Moreover, it has been concluded that carbamazepine is less effective in unipolar depression than established antidepressants and that its use is only justifiable as an experimental pharmacotherapy option when other treatments have failed (Elphick 1989).

One reason for the lacking antidepressant efficacy of carbamazepine in our study may involve pharmacokinetic interactions between mirtazapine and carbamazepine. Carbamazepine has been shown to induce cytochrome P450 isoforms such as CYP1A2, CYP3A4, and others (Ketter et al. 1999; Patsalos and Perucca 2003). The biotransformation of mirtazapine is mainly mediated by the CYP2D6, CYP1A2, and CYP3A4 isoenzymes (Stormer et al. 2000; Timmer et al. 2000). Moreover, in young males, a 60% decrease in mirtazapine levels was found when carbamazepine was added to mirtazapine at steady-state conditions (Ebes et al. 1998; Timmer et al. 2000). In line with this finding, in the present investigation the additional administration of carbamazepine significantly lowered the mirtazapine levels up to the end of the study period, which may at least in part account for the absence of clinical improvement after addition of carbamazepine. This is obviously also true for other antidepressant drugs, since many clinical trials or case series report on a carbamazepine provoked decrease of plasma or serum concentrations of tricyclic antidepressants such as nortriptyline (Brosen and Kragh-Sorensen 1993) or desipramine (Spina et al. 1995), and of SSRIs such as sertraline (Khan et al. 2000), fluvoxamine (Martinelli et al. 1993; Spina et al. 1993), fluoxetine (Spina et al. 1993), nefazodone (Laroudie

et al. 2000), citalopram (Leinonen et al. 1996; Steinacher et al. 2002) and mianserin (Eap et al. 1999).

Although the conclusions which can be drawn from the present study are restricted due to the open-label, non-randomized design, our results suggest that lithium but not carbamazepine augments the antidepressant efficacy of mirtazapine in unipolar depressed patients. Whereas the clinical importance of anticonvulsants in the treatment of bipolar disorder is undoubtful, the therapeutic efficacy of antiepileptic drugs such as carbamazepine is obviously limited in the pharmacotherapy of unipolar depression.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# Are there differences between serotonergic, noradrenergic and dual acting antidepressants in the treatment of depressed women?

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**Abstract**

*Background.* This study aims to investigate if there is a differential outcome of serotonergic and noradrenergic antidepressant treatment and if menopausal status has an impact on antidepressant response in depressed women. *Methods.* Data of the 111 depressed women who were included and completed the previous four open-label studies where patients were evaluated six times during a 10-week period, were pooled in the current study. Each of the reboxetine, sertraline and venlafaxine groups consisted of 37 depressed women. Patients were also divided into two subgroups of age, determining the 44 years as the cut-off point representing the menopausal status. *Results.* No significant difference was observed in the percent change of Hamilton Depression Rating Scale-17 (HDRS) and remission rates among treatment groups. Percent changes in Clinical Global Impression-Severity of Illness scale (CGI-S) and response rates were in favour of venlafaxine group at week 10. Individual HDRS items 2, 3, 4, 5 and 6 demonstrated significant improvement in the sertraline group, whereas HDRS item 7 demonstrated significant improvement in the venlafaxine group. An early reduction in anxiety subscale was observed in the venlafaxine group. Menopausal status had no impact on the outcome measures. *Conclusions.* These results suggest that noradrenergic and serotonergic activity do not differ from each other in treating depressed women. However, serotonergic activity appears to be more prominent in some particular symptoms such as feelings of guilt, suicidal ideation and sleep. Also, menopause does not appear to affect antidepressants' benefit in depressed women.

**Key words:** *Gender, depression, antidepressants*

## Introduction

Epidemiological studies report a greater prevalence of major depressive disorder (MDD) in women compared to men (Kessler et al. 1994). Depressed women are reported to demonstrate higher levels of somatic symptoms, coronary artery disease, anxiety, psychomotor retardation, increased appetite, feelings of worthlessness and guilt, poor social adjustment and impaired quality of life compared to depressed men (Hirschfeld et al. 1984; Kornstein 1997; Silverstein 1999; Kornstein et al. 2000a; Möller-Leimkühler 2008). Despite several significant gender-related differences, some aetiological factors are proposed to explain the reported differences. Psychosocial (Bebbington et al. 1998; Wang 2004), genetic (Kendler et al. 1993) and personality factors (Goodwin and Gotlib 2004) are suggested as risk factors for developing MDD in women.

Gonadal hormones which are thought to involve in the development of depression, have an interaction with neurotransmitters such as noradrenaline, glutamate, dopamine and particularly serotonin. In animal studies, higher rate of serotonin synthesis (Halem et al. 1990) and higher concentrations of 5-hydroxyindolacetic acid in several regions of the female rat brain (Dickinson and Curzon 1986; Carlsson and Carlsson 1988) have been reported. Moreover, oestrogen treatment has been found to increase 5-HT2A receptor and its binding in several regions of the female rat brain (Sumner and Fink 1995; Cyr et al. 1998). Data from animal studies support that decreased levels of oestrogen are associated with decreased activity of serotonin (Amin et al. 2005).

Animal data provide robust information about the interactions between oestrogen and the serotonergic system. However, from human data it is known that

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gonadal hormones have an impact on the synthesis and release of neurotransmitters and their receptor functioning (Fink et al. 1996; McEwen et al. 1997; Rubinow et al. 1998). Findings from Nishizawa et al. (1997) and Ellenbogen et al. (1996) reveal that depressed women have a vulnerable serotonergic system and high local cerebral serotonin synthesis (Chugani et al. 1998). Studies demonstrate that oestrogen enhances monoaminergic activity and augments serotonergic postsynaptic responsiveness, and increases both the number and the uptake of serotonergic receptors (Halbreich et al. 1995; McEwen et al. 1997).

Though, the early evidence indicates that some noradrenergic neurons in the central nervous system are oestrogen-receptive and it is likely that oestrogen may enhance noradrenergic transmission within the gonadotropin releasing hormone network, the role of the noradrenergic system in depressed women is hardly mentioned (Herbison 1997; Heritage et al. 1977).

Accumulated data to date suggest that depressed women may differ in response to treatment (Raskin 1974; Kornstein et al. 2000b). There are several reports suggesting that women are more likely to respond and tolerate selective serotonin reuptake inhibitors (SSRI) (Kornstein et al. 2000b; Khan et al. 2005). On the contrary, however, some studies have failed in finding such differences with various types of antidepressants (Quitkin et al. 2002; Hildebrandt et al. 2003). The evidence is sparse whether depressed women improve better on serotonergic or noradrenergic antidepressants, or both.

If women respond differently to antidepressants, it is necessary to compare drugs with different types of effects on neurotransmitter systems. Considering this, we aimed to investigate two areas; first, to compare the efficacy of reboxetine, sertraline and venlafaxine which exert their mechanisms of action through different pharmacological properties in depressed women. Second, since female hormones may be a factor in causing different responses to antidepressants in depressed women, in addition to the whole sample, patients over 44 years of age or younger were analysed separately.

## Methods

### *Patient population*

Subjects were recruited from the databases of previous four studies with same study design. These studies compared reboxetine and venlafaxine (Akkaya et al. 2003), reboxetine and sertraline (Eker et al. 2005), venlafaxine and combination of reboxetine and sertraline (Yazicioglu et al. 2006) and sertraline and combination of reboxetine and sertraline (data on file). Patients who were in the venlafaxine, reboxetine, and sertraline groups and completed the above-mentioned studies were included in the current study. Patients under combination therapy were excluded from the study.

According to the patient eligibility criteria of the above-mentioned studies, patients aged 18–65 years and were diagnosed with MDD, as determined on the basis of the Diagnostic and Statistical Manual of Mental Disorders, 4th ed, Text Revision (DSM-IV-TR) (APA 2000), were eligible for participation in the present study. The patients in these four studies had been required to have a score of at least 16 at baseline on the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton 1960). Patients fulfilling the criteria for a DSM-IV Axis I disorder other than MDD or a DSM-IV Axis II disorder, patients having MDD with psychotic features or patients who had a history of psychoses and patients with significant suicide risk were excluded from the study after the psychiatric interview. Patients who had not responded to venlafaxine, reboxetine or sertraline in previous episodes of depression, patients who have or had history of treatment resistance, patients who have had electroconvulsive therapy within the last 6 months, patients having a history of drug sensitivity (especially to psychotropic drugs), patients with any clinically significant medical disorder or laboratory abnormality, patients with a history of substance abuse within a year were not eligible for participation in the study. Women were excluded if pregnant or if not practicing an adequate birth control method. None of the subjects received hormonal therapy during the trials.

The age subgroups were chosen to be representative of female's reproductive (18–43 years) and peri- or postmenopausal periods ($44 \geq$ years). Several endocrine changes precede the clinical menopause, such as the pattern of pulsatile GnRH secretion (Crowley and Flicori 1985); therefore we used 44 years as the cut-off for the split of fertile period from peri/postmenopause. The same method was also previously used by Martenyi et al. (2001) and Naito et al. (2007).

### *Study design*

The data of the previous four studies were pooled and depressed women were separately analysed. These four studies had been designed as open-label studies; thus researchers and patients were not blind to the study drugs. Throughout the studies, the patients were assessed six times; on the day of the screening visit (–7th day), at baseline or first visit (day 0), and on the 14th (visit 2), 28th (visit 3), 49th (visit 4) and 70th (visit 5) days after the baseline. All patients underwent a detailed psychiatric evaluation

on the screening visit where inclusion and exclusion criteria as well as MDD diagnosis were assessed according to DSM-IV criteria. Physical examination and laboratory tests including biochemical blood and urine analysis, complete blood count, electrocardiography were carried out and vital signs were measured at the screening visit and at the end of study. The study protocols were approved by the relevant ethics committee, and were conducted in accordance with Declaration of Helsinki. All subjects gave written informed consent to participate.

### *Drug administration*

Patients who met the study inclusion criteria were assigned to venlafaxine XR capsules, reboxetine tablets, sertraline tablets at baseline visit. The initial dose of venlafaxine XR was 75 mg/day which was increased to 150 mg/day at the second week. There are several papers claiming that venlafaxine 150 mg/day is effective in the treatment of depression (Ballus et al. 2000; De Nayer et al. 2002). Reboxetine was started with 4 mg/day and the dose was increased to 8 mg/day at the second week of the study. The optimum dose of reboxetine suggested by Pfizer is 8–10 mg/day. There are also papers claiming that 8 mg/day reboxetine is effective (Scates and Doraiswamy 2000; Hajós et al. 2004). Sertraline was started at 50 mg/day and was not changed throughout the study. The minimum optimum dose of sertraline suggested by Pfizer is 50 mg/day. There are also papers claiming that 50 mg/day sertraline is effective (Schweizer et al. 2001; Lepine et al. 2004). The doses remained at those levels throughout the study. At the end of 11th week the study was completed. Patients were not allowed to take concomitant medications. However, in cases of sleep disturbance or increase of anxiety, patients were allowed to take low doses of hydroxyzine (12.5 mg/day) when required. The study was designed as an open-label study; thus researchers and patients were not blind to the study drugs.

### *Assessment instruments*

The Turkish version of HDRS-17 item (Akdemir et al. 1996) was applied at all assessments. Response to an antidepressant was defined as 50% or more decrease in the HDRS score with comparison to the baseline value, and the remission was defined as being of HDRS scale score $\leq 7$.

To define the effects of antidepressants on different components of depression, HDRS item groups were defined as follows: Core symptoms of depression (HAM-D6) subscale (Bech et al. 1975): HDRS items 1, 2, 7, 8, 10 and 13; Psychomotor retardation subscale: HDRS items 1, 7, 8 and 14; Anxiety subscale: HDRS items 10, 11, 12, 13, 15 and 17 (Guy 1976).

Clinical Global Impression-Severity of Illness scale (CGI-S) (Guy 1976) was also applied at all assessment points.

One investigator was appointed for each study. There was a substantial agreement among these four investigators ($P = 0.8833$, $\kappa = 0.766081$).

### *Data analysis*

Statistical analyses were performed using SPSS for Windows 13 (SPSS Inc., IL, USA). For categorical variables we calculated frequency statistics and descriptive statistics were calculated for continuous variables. We examined the related variables using Kolmogorov–Smirnov and Shapiro–Wilk tests whether the assumptions of normal distribution are convenient homogeneity of groups. For group homogeneity HDRS and CGI-S variables which were used as tools for clinical evaluation, were computed before treatment. For time-dependent variables (HDRS, CGI-S, each item of HDRS, and subscales) percent changes were computed. One way variance analyses, Kruskal–Wallis test and/or Mann Whitney *U*-tests were performed for comparisons between groups concerning the number of groups (age-specified groups and treatment groups) and the convenience of variables to the normal distribution. Comparisons within groups were evaluated by the Wilcoxon *t*-test. The Pearson Chi-Square test was used to determine whether there are differences between groups. Effect sizes (ES) were calculated by using Morse' algorithm (Morse 1999). Siegel and Castellan's (1988) fixed-marginal multirater kappa statistics was used to calculate the interrater reliability.

## Results

A total of 156 patients were included in the study, 26 dropped out. Of the remaining 130 patients, 111 were women and 19 were men. A total of 111 women were enrolled to the current study. The mean age of the study sample was $40.5 \pm 10.8$ years (range 19–65 years). There were 37 patients in each treatment group. There were not any significant differences between treatment groups in terms of demographic and clinical characteristics of the patients as presented in Table I.

Each treatment arm demonstrated significant improvement at the end of the trial. No significant differences were found among treatment groups in terms of reduction in percent changes of HDRS scores at the end of the trial. However, an early reduction which did not continue throughout the trial

Table I. Patient demographics and baseline scores.

| | Reboxetine ($n=37$) | Venlafaxine ($n=37$) | Sertraline ($n=37$) |
|---|---|---|---|
| Mean years of age (±SD) | 39.8 (±11) | 41.9 (±10.1) | 39.9 (±11.4) |
| Mean number of previous episodes (±SD) | 1.9 (±1.3) | 2.3 (±2.1) | 1.6 (±0.9) |
| Mean basal HDRS score (±SD) | 21.2 (±3.9) | 20.5 (±3.6) | 20.2 (±2.8) |
| Mean basal CGI-Severity (±SD) | 4.6 (±0.6) | 4.7 (±0.5) | 4.5 (±0.6) |
| Mean duration of episode- in months (±SD) | 5.3 (±4.1) | 6.3 (±4.1) | 4.5 (±2.7) |
| Family history of depression | 27% | 32.4% | 16% |
| Mean years of age (±SD) | 39.8 (±11) | 41.9 (±10.1) | 39.9 (±11.4) |

HDRS, Hamilton Depression Rating Scale; CGI-S, Clinical Global Impression Severity Scale. Standard deviation values are given in parentheses.

in HDRS scores was observed between the venlafaxine and sertraline groups at visit 2. The venlafaxine and sertraline groups significantly differed from the reboxetine group in reduction of percent changes of CGI-S scores at visit 5. Table II presents HDRS and CGI-S scores and statistical values.

There were no significant differences between treatment groups in terms of remission rates; whereas significant difference was detected between the venlafaxine and reboxetine groups in terms of response rates in favour of the venlafaxine group at visit 5. Table III presents response and remission rates and statistical values.

There were 21 patients in reboxetine, 19 patients in venlafaxine and 21 patients in sertraline subgroups of <44 years. No statistical differences were found in terms of educational status, basal HDRS and CGI-S scores, number of previous depressive episodes, family history of depression, duration of current episode between two age-specified subgroups. No significant differences were found between age specific subgroups in terms of percent changes of HDRS and CGI-S scores and rates of remission and response regardless of treatment groups at the end of the trial. There were no statistical differences between age-specified subgroups of each treatment group with regard to the percent changes of HDRS and CGI-S scores at the end of the trial. In <44 age-specified subgroup a significant difference was found between the venlafaxine and reboxetine subgroups regarding the percent changes of CGI-S scores (Mann–Whitney *U*-test: $z=-2.467$, $P=0.015$, ES: 0.1561 (large)) where venlafaxine demonstrated significant decline at visit 3. However, this significance did not persist throughout the trial. Within each treatment groups, there were not any significant differences between age-specified subgroups regarding the percent changes of HDRS and CGI-S scores. There were not any significant differences among treatment groups in terms of remission and response rates by age.

Sertraline produced significantly greater change in HDRS items of "feelings of guilt", "thoughts of suicide", "insomnia early", "insomnia middle" and "late insomnia" at the end of the trial. However, significant difference was observed in HDRS item of "work and activities" in favour of venlafaxine group at the end of the trial. Significant differences were found in percent changes of HDRS items of "psychic anxiety" and "somatic symptoms" (Kruskal–Wallis $\chi^2=8.295$, df = 2, $P=0.016$, ES: 0.0754 (medium) and Kruskal–Wallis $\chi^2=6.825$, df = 2, $P=0.003$, ES: 0.0632 (medium), respectively) in favour of venlafaxine at visit 3. For HDRS item of "genital symptoms", there was a significant difference in percent changes between treatment groups in favour of reboxetine group (Kruskal–Wallis $\chi^2=7.301$, df = 2, $P=0.026$,

Table II. HDRS and CGI-S values at each visit.

| | Reboxetine | | Venlafaxine | | Sertraline | |
|---|---|---|---|---|---|---|
| | HDRS | CGI-S | HDRS | CGI-S | HDRS | CGI-S |
| Visit 1 | 21.2 (±3.9) | 4.6 (±0.6) | 20.5 (±3.6) | 4.7 (±0.5) | 20.2 (±2.8) | 4.5 (±0.6) |
| Visit 2 | 17.4 (±4.09) | 4.3 (±0.5) | 15.9 (±3.9)[a] | 4.1 (±0.7) | 17.1 (±3.7) | 4 (±0.6) |
| Visit 3 | 13.3 (±4.8) | 3.6 (±0.8) | 11.7 (±4.6) | 3.3 (±0.9) | 13.1 (±4.2) | 3.4 (±0.8) |
| Visit 4 | 10.2 (±4.3) | 3 (±0.9) | 9.4 (±4.7) | 2.7 (±0.9) | 10.1 (±4.6) | 2.5 (±1.1) |
| Visit 5 | 8.7 (±5.1) | 2.4 (±1.1) | 7 (±4.3) | 1.8 (±0.9) [b1] | 7.8 (±4.2) | 1.8 (±1) [b2] |

HDRS, Hamilton Depression Rating Scale; CGI-S, Clinical Global Impression Severity Scale. Standard deviation values are given in parentheses.

[a] Between venlafaxine and sertraline groups →Mann–Whitney *U*-test $z=-2.774$, $P=$ 0,006, ES: 0.1054 (medium).

[b1] Between venlafaxine and reboxetine groups →Mann–Whitney *U*-test $z=-2.815$, $P=$ 0,005, ES: 0.1086 (medium).

[b2] Between sertraline and reboxetine groups →Mann–Whitney *U*-test $z=-2.269$, $P=$ 0,023, ES: 0.0705 (medium).

Table III. Response and remission scores at visit 5.

| | Response *n* (%) | Remission *n* (%) |
|---|---|---|
| Reboxetine | 22 (59.5) | 17 (45.9) |
| Venlafaxine | 32 (86.5)* | 23 (62.2) |
| Sertraline | 29 (78.4) | 18 (48.6) |

*$\chi^2 = 7.546$, df = 2, $P = 0{,}023$, ES: 0.2607 (small).

ES: 0.0716 (medium)) at visit 2. Also for anxiety subscale there was a significant difference in percent changes between treatment groups in favour of venlafaxine group at visit 2 ((Kruskal–Wallis $\chi^2 = 6.717$, df = 2, $P = 0.035$, ES: 0.0611 (medium)) and visit 3 (Kruskal–Wallis $\chi^2 = 11.592$, df = 2, $P = 0.003$, ES: 0.1054 (medium)). There were no significant differences between treatment groups in terms of percent changes in rest of the HDRS items. We also did not find any significant differences between treatment groups in terms of percent changes in subscales of core symptoms of depression, psychomotor retardation and anxiety at the end of the trial. Figure 1 illustrates the percent changes of HDRS items and subscales at visit 5. There were not any significant differences between age-specified subgroups in terms of percent changes in individual HDRS items and subscales at the end of the trial.

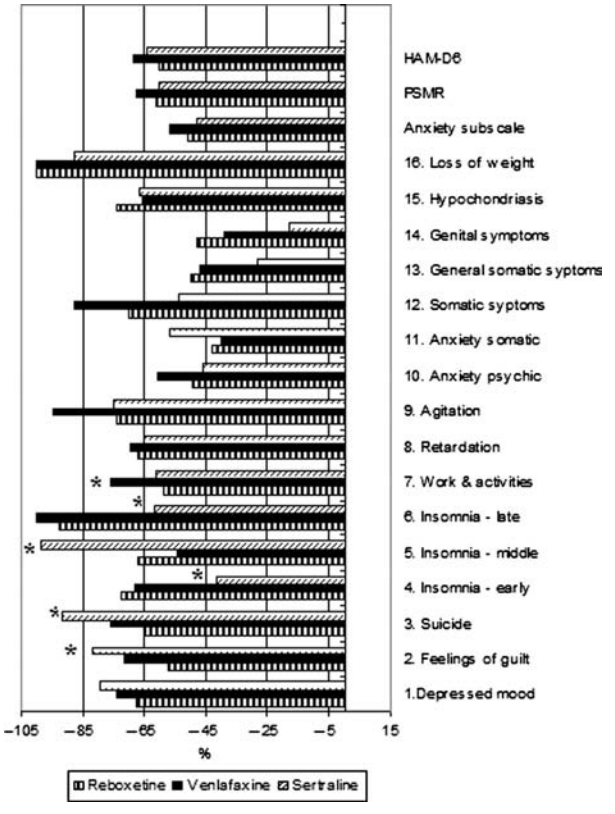


Figure 1. Percent changes of HDRS items and subscales at visit 5.

## Discussion

The aim of this study was to investigate the outcome of serotonergic and noradrenergic antidepressants in depressed women. The results of this trial demonstrate that there are no significant differences between serotonergic and noradrenergic antidepressants in terms of efficacy and remission regardless of menopausal status. However, drugs that have total or in part serotonergic activity revealed better results in response rates.

Though it is suggested that all kinds of antidepressant treatments have similar efficacy (Ayuso-Gutiérrez 2005), recently the differential effects of antidepressants in depressed women become the focus of attention. Accumulated data regarding the effects of antidepressants acting through different mechanisms of action on depressed women demonstrate contradictory results (Kornstein et al. 2000b; Quitkin et al. 2002). In the present study, there were no significant differences between treatment groups in terms of improvement in HDRS scores at the end of the trial. Though the evidence is scarce with regard to the differential effects of antidepressants in depressed women, our results are in line with the Hildebrandt et al. (2003) findings which did not report significant difference between antidepressants in terms of changes in HDRS. The significant difference between the venlafaxine and sertraline groups in this study at visit 2 in terms of reduction in percent changes of HDRS scores, though did not continue throughout the trial, may be attributed to the rapid action of venlafaxine (Stahl et al. 2002). On the contrary, there are some reports which demonstrate differential effects of antidepressants particularly in favour of serotonergic activity in depressed women. Martenyi et al. (2001) reported that female depressed patients had higher reduction in HDRS scores on fluoxetine compared to maprotiline, which has predominantly noradrenergic activity; and citalopram was found superior over reboxetine in terms of remission and response rates (Berlanga and Flore-Ramos 2006). A more recent study by Pjrek et al. (2009) also reported higher response rates with citalopram than with reboxetine in women with seasonal affective disorder. Joyce et al. (2002) also found that depressed women had a superior response to fluoxetine than nortriptyline. In another study, statistically significant differences were found between women taking sertraline and women taking imipramine, which has both noradrenaline and serotonin reuptake properties with respect to reductions in HDRS and in CGI-S scores in favour of sertraline (Baca et al. 2002). However, for women, rates of response with venlafaxine were found greater than those for women receiving SSRIs

in the meta-analysis of Entsuah et al. (2001). Though, there were no significant differences between treatment groups in terms of improvement in HDRS scores at the end of the present study, higher response rates and reductions in CGI-S scores were found in favour of the sertraline and venlafaxine groups compared to the reboxetine group. These data may be interpreted as compatible with the studies that demonstrate higher benefit with serotonergic agents (Martenyi et al. 2001; Berlanga and Flore-Ramos 2006) and may indicate a possible superiority of the serotonergic system over the noradrenergic system in depressed women.

An early study by Raskin (1974) found a differential response to antidepressant therapy between premenopausal and postmenopausal depressed women. Similar findings were replicated by Kornstein et al. (2000b) several decades later. In the present study we did not find a significant difference between age-specific subgroups in terms of percent changes of HDRS and CGI-S scores regardless of treatment groups. We also did not observe a significant difference between age-specified subgroups of each treatment group with regard to the percent changes of HDRS and CGI-S scores at the end of the trial. Significant difference, which did not persist throughout the trial, between venlafaxine and reboxetine in $<44$ age-specified subgroup regarding the percent changes of CGI-S scores at visit 3 may be attributed to the rapid action of venlafaxine (Stahl et al. 2002). In each treatment group again we did not find any significant difference between age-specific subgroups in terms of percent changes of HDRS and CGI-S scores. Moreover, remission and response rates did not differ between age specific subgroups in each treatment group. In line with the present study, Quitkin et al. (2002) and Thiels et al. (2005) found that women age 50 or older and those younger than age 50 had similar response rates with fluoxetine sertraline, respectively. On the other hand, women younger than 44 years were reported to respond better on fluoxetine compared to maprotiline (Martenyi et al. 2001). In a Japanese study, fluvoxamine was found to produce a significant difference in the time course of Montgomery and Åsberg Depression Rating Scale score change in younger female depressive patients ($<44$ years of age) compared with older female ($\geq 44$ years of age), whereas milnacipran, which has both serotonergic and noradrenergic activity, produced no difference between age specific groups (Naito et al. 2007). Thase et al. (2005) reported that women age $>50$ had significantly lower remission rates with SSRI therapy than did younger women; however, this pattern of response was not observed with venlafaxine. Moreover, women older than 50 were reported to remit better on venlafaxine compared to SSRIs. In another study involving SSRIs, nefazodone and venlafaxine, premenopausal ($\leq 44$ years) depressed women compared with postmenopausal ($>50$ years) depressed women had significantly lower HDRS scores at the end of the trial (Grigoriadis et al. 2003). When they compared the response to SSRIs only, they found that premenopausal depressed women still had significantly lower HRDS scores and had higher remission rates compared to postmenopausal depressed women. The data from several studies mentioned above indicate that it is still unclear whether a difference exists in treatment response to different antidepressants between premenopausal and postmenopausal depressed women.

The antidepressants administered in the present study have different mechanisms of actions through serotonin, noradrenalin or both. This could be critical to understand the different antidepressant response in women considering the modulatory effects of oestrogen by its interaction with neurotransmitters in the mechanisms of depression and its treatment in depressed women. There is evidence, that oestrogen can affect neurotransmitter synthesis, release and reuptake which cause alteration in mood (Fink et al. 1997; McEwen et al. 1997). Furthermore, oestrogen alone is suggested to be effective in the treatment of perimenopausal depression (Schmidt et al. 2000; Soares et al. 2001) but not for postmenopausal women (Cohen et al. 2003; Morrison et al. 2004). However, the data with regard to the effect of oestrogen as an augmentation agent to antidepressants is also not consistent (Amsterdam et al. 1999; Schneider et al. 2001; Soares et al. 2003). Our data from age-specific subgroup analysis suggest that menopause does not appear to affect antidepressants' benefit in depressed women.

The issue of differences in the symptoms that respond to serotonergic or noradrenergic antidepressants is one of the challenges of clinicians. It is suggested that symptoms of loss of interest, anhedonia, and lack of energy or motor retardation improve better on noradrenergic drugs, whereas serotonergic drugs are more likely to improve anxiety and mood (Nelson et al. 2005a). There are some papers that do not find significant differences between the effects of serotonergic and noradrenergic antidepressants on individual symptoms of HDRS (DeJonghe et al. 1991; Bowden et al. 1993). Similarly Nelson et al. (2005b) concluded that the pattern of symptom response was very similar for fluoxetine and reboxetine. However, it should be noted that the data from these studies are irrespective of gender influence. To the best of our knowledge there is no published work about differential effects of serotonergic and noradrenergic

antidepressants on individual HDRS items in depressed women. In the present study, symptom analysis of each individual HDRS item revealed that symptoms with "feelings of guilt" and "thoughts of suicide" improved better with sertraline treatment. Individual symptoms regarding "insomnia early", "insomnia middle" and "late insomnia" improved better on sertraline treatment. This may indicate that insomnia complaints may improve better on SSRI treatment. Individual item "work and activities" improved better on venlafaxine treatment. This may be associated with the additional noradrenergic property of venlafaxine (Harvey et al. 2000) since noradrenergic antidepressants have been reported to have prominent effects on motivation and driving (Healy and McMonagle 1997), which might be reflected in improvement of loss of interest, anhedonia, lack of energy or motor retardation (Montgomery 1997).

Patients demonstrated an early improvement on venlafaxine treatment in individual symptoms "psychic anxiety" and "somatic symptoms general" and cluster of somatization, though no significant difference detected at the end of the trial. Similarly reboxetine-treated patients demonstrated an early improvement in "genital symptoms". These data are in line with literature finding that the sexual dysfunction may appear as a consequence of SSRI treatment (Nurnberg 2008).

The definitive association between gonadal hormones and depression in women remains unclear in many aspects; however, there are biological findings that could explain why women respond better to serotonergic agents. Acute tryptophan depletion using PET and MRI scans in depressed men and women revealed that compared to female counterparts men had 52% higher mean serotonin synthesis (Nishizawa et al. 1997). Similarly, Neumeister (2003) demonstrated that women are more likely to develop depressive symptoms when serotonin synthesis is reduced compared to men. These findings indicate that women may have a more vulnerable serotonergic system. Another study also suggested that tryptophan pyrrolase which reduces blood tryptophan levels, is found to be overactive in women (Bano et al. 2004). These findings may indicate a vulnerable serotonin system in women. Significantly higher response rates and reductions in CGI-S scores and improvement in individual HDRS items mentioned above with sertraline and venlafaxine, but not reboxetine may indicate the mentioned vulnerability.

This study has several limitations. Most importantly this study bears the weaknesses of all open label studies. It is open to bias and the findings should be replicated in a double-blind study. The small patient number and the missing placebo arm is one of the major limitations of the present study. The high rates of remission and response should also be considered as another limitation of this open label study. Though, there are papers claiming that daily dose of 50 mg of sertraline is effective in the treatment of MDD, the dose of sertraline administered in the current study might be considered as low compared to the dosage of venlafaxine and reboxetine. Lack of data on tolerability should also be considered as another limitation of this study. On the other hand this is the first study comparing the efficacy of three different pharmacological types of antidepressants and their effects on individual depressive symptoms in depressed women.

## Conclusion

The effects of antidepressants with different pharmacological properties in specific populations, such as women, have become the interest of researches recently. There is still debate on which monoaminergic system in particular should be manipulated while treating depressed women. Also, observation of which symptoms improve better on specific antidepressant is also important in achieving full remission. We conclude that both noradrenergic and serotonergic antidepressants are effective in depressed women. However, depressed women are more likely to benefit from antidepressants with total or partial serotonergic activity. Since women are more likely to suffer from depression than men and full remission is the main goal of depression treatment, double blind, randomized trials are required to differentiate the proper antidepressant treatment in depressed women. Also, the interaction between noradrenergic system and oestrogen and their impact on depressed women should be more extensively studied in future studies since the evidence is focused on oestrogen and serotonergic system so far.

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## Statement of interest

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**ORIGINAL INVESTIGATION**

# Treatment of unipolar psychotic depression: The use of evidence in practice guidelines

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**Abstract**

*Introduction.* In a recent meta-analysis we found no evidence that an antidepressant plus an antipsychotic is more effective than an antidepressant alone in unipolar psychotic depression. However, most current guidelines recommend the combination over an antidepressant alone. *Method.* We assessed available guidelines by the AGREE instrument and discuss their recommendations in relation to the evidence as referred to in the guidelines. *Results.* The UK-NICE guideline had the highest AGREE quality score, followed by the Dutch, Australian, and US-APA guidelines. Guidelines are not always consistent with at date of publication available evidence and (with exception of the UK-NICE and Dutch guidelines) also not with the in that guideline referred evidence. *Conclusion.* Physicians (and patients) should be aware that in guidelines treatment recommendations may be less evidence-based than asserted, even when treatment recommendations are stated as being based on the highest level of evidence.

**Key words:** *Affective disorders, psychotic, practice guidelines*

## Introduction

It is unclear whether unipolar psychotic depression should be treated initially with an antidepressant (AD) and an antipsychotic (AP) in combination or first with an AD alone and with an AP being added if there is no or an insufficient response. In a recent Cochrane systematic review and meta-analysis of 10 randomized controlled trials, we found no evidence that the AD plus AP combination was more effective than an AD alone, whereas the combination was statistically more effective than an AP alone (Wijkstra et al. 2005, 2006). We concluded that both AD monotherapy with an AP added if required and combination treatment with an AD and an AP appear to be appropriate options for patients with unipolar psychotic depression. In addition, the balance between risks and benefits would make the first option preferable for many patients. However, most current guidelines quite explicitly recommend the combination as initial treatment for patients with psychotic depression. To investigate this apparent discrepancy, we evaluated the quality of these guidelines and the evidence on which they are based.

## Method

To identify relevant guidelines, we searched (in January 2006) Medline® using the search terms 'depressive disorder and guideline', the Internet (via Google®) with the search terms 'depression, depressive disorder, guideline and practice guideline', guideline databases (National Guideline Clearinghouse, Guideline international Network), and also the website of the World Psychiatric Association to contact national psychiatric organisations in different countries (Argentina, Austria, Azerbaijan, Bolivia, Brazil, Bulgaria, China, Greece, Hungarian, India, Mexico, Nigeria, South Africa, Switzerland, Thailand, Venezuela, and Uruguay). We only excluded all those depression guidelines in which there were no specific recommendations regarding the treatment of unipolar psychotic depression. We identified eight relevant guidelines: the Australian and New Zealand guideline (AU/NZ) (Royal

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Australian and New Zealand College of Psychiatrists Clinical Practice Guideline Team for Depression 2004), the Canadian guideline (CA) (CANMAT Depression Workgroup 2001), the World Federation of Societies of Biological Psychiatry guideline (WFSBP) (Bauer 2002), the Dutch multidisciplinary guideline (NL) (Landelijke stuurgroep Multidisciplinaire Richtlijnontwikkeling in de GGZ 2005), the South African guideline (SA) (South African Society of Psychiatrists, unknown year), the British NICE guideline (UK-NICE) (National Institute for Clinical Excellence 2004), the American Psychiatric Association guideline (US-APA) (American Psychiatric Association 2000), and the Texas Implementation of Medication Algorithms guideline (US-TIMA) (Trivedi et al. 2000).

Two authors (JW, CS) independently scored the quality of the reporting of the guidelines and the quality of the guideline development process, using the AGREE (Appraisal of Guidelines for Research & Evaluation) instrument (Agree Collaboration 2001). This is an instrument for assessing the quality of clinical practice guidelines. Besides the general items (general because there is no relation with diagnostic category) of the AGREE instrument (1. Overall objectives; 2. Clinical question; 3. Patient group; 4. Professional groups included; 5. Patients' views; 6. Target users; 7. Pre-testing among target users; 8. Search methods; 9. Evidence criteria; 10. Methods for decisions; 13. External review; 14. Update procedure; 18. Application tools; 19. Organisational barriers; 20. Cost implications; 21. Key review criteria; 22. Editorial independence; 23. Conflicts of interest), five (out of 23) items were specifically scored with regard to recommendations for unipolar psychotic depression: 11. Health benefits, side effects, and risks have been considered in formulating the recommendations; 12. There is an explicit link between the recommendations and the supporting evidence; 15. The recommendations are specific and unambiguous; 16. The different options for management of the condition are clearly presented; and 17. Key recommendations are easily identifiable. These five specific items are linked to diagnostic category, in our case psychotic depression. We also retrieved from the guidelines the studies on which the recommendations were based and evaluated their methodological quality, in terms of controlled or uncontrolled studies, double-blind or open studies, inclusion of patients with unipolar psychotic depression or also other diagnoses (e.g. bipolar psychotic depression), and data analysis. Both authors had to reach agreement about their conclusions. In case of disagreement the case could be discussed with the third author (WN) to reach final agreement (which in no case was necessary).

## Results

### *Quality*

According to AGREE, the UK-NICE guideline (2004) has the highest quality score followed by the Dutch (2005), Australian (2004), and US-APA (2000) guidelines (see Table I), with 'rigor of development scores' (which covered the following items: search strategy, evidence inclusion criteria, methods for formulating the recommendations, considering benefits as well as risks, link between references and recommendations, external review, update procedure) being 100, 79, 69 and 64%, respectively (expressed as a percentage of maximum possible score). Editorial independence scores (which covered the following items: explicit statement about external funding and statement about conflicts of interest) are 67, 50, 58, and 83%, respectively. The other four guidelines (Trivedi 2000; CANMAT 2001; Bauer 2002; SASOP year unknown) have significantly lower scores on these two important domains.

Table I. Quality of guidelines (AGREE 2001): standardized domain scores.

| Guideline | | | Domain and percentage of maximum possible domain score | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Country | Year | Developer | Scope and purpose | Stakeholder involvement | Rigor of development | Clarity and presentation | Applicability | Editorial independence | Sum score |
| UK | 2004 | NICE | **100%** | **88%** | **100%** | **71%** | **61%** | **67%** | 487% |
| NL | 2005 | Trimbos Inst. | **100%** | **75%** | **79%** | **92%** | 17% | 50% | 413% |
| USA-APA | 2000 | APA | **78%** | 42% | **64%** | **83%** | 0% | **83%** | 350% |
| AU/NZ | 2004 | RANZCP | **78%** | 58% | **69%** | 58% | 0% | 58% | 321% |
| CAN | 2001 | CPA/CANMAT | **100%** | 50% | 38% | **83%** | 0% | 17% | 288% |
| Internat. | 2002 | WFSBP | **100%** | 33% | 36% | 29% | 0% | 33% | 231% |
| USA-TIMA | 2000 | TIMA | **78%** | 38% | 7% | **67%** | 22% | 0% | 212% |
| SA | ? | SASOP | 39% | 0% | 0% | 42% | 0% | 0% | 81% |

Bold characters: >60% of maximum possible score.
AGREE, Appraisal of Guidelines for Research & Evaluation.

*Recommendations*

Two guidelines are cautious in their formulation of treatment recommendations. The UK-NICE guideline (2004) states that 'augmenting the current treatment plan (i.e. the NICE recommendations regarding the use of ADs for the treatment of unipolar depression without psychotic features) with an AP should be considered'. The Dutch guideline (2005) considers 'starting with an AD and adding an AP if the patients does not respond' a reasonable option. The other six guidelines strongly recommend treatment with the combination of an AD and an AP instead of an AD alone.

*Supporting evidence*

Two guidelines do not mention the evidence (studies) on which the guidelines are based (USA-TIMA and South Africa). The other six guidelines all refer to Spiker et al. (1985). Parker et al. (1992) is mentioned by the Canadian and the Dutch guideline and Zanardi et al. (1996) by the Canadian and the UK-Nice guideline. The following studies are mentioned by one guideline: Anton and Burch (1990), Bellini et al. (1994), Zanardi et al. (2000) and Mulsant et al. (2001) by theUK-NICE guideline, Minter and Manderl (1979), Kaskey et al. (1980), Charney and Nelson (1981), Brown et al. (1982), Spiker et al. (1982) by the Australian guideline, Bruijn et al. (2001) by the Dutch guideline, Rothschild et al. (1993) by the WFSBP guideline, Zanardi et al. (1998) and Rothschild et al. (1999) by the Canadian guideline. A critical short overview of all the references is presented in Table II.

The UK-NICE guideline (2004) refers to six randomised clinical trials (RCTs) (Spiker 1985; Anton and Burch 1990; Bellini 1994; Zanardi 1996, 2000; Mulsant 2001), and data were re-analysed according to intention to treat (ITT) principles (i.e. drop-outs after randomisation were included in the analysis). The guideline states that only trials with fewer than 15% patients with bipolar disorders were included. However, in the study of Bellini et al. (1994) 25% (12/48) of the included patients had bipolar disorders and in the study of Spiker et al. (1985) 15.5% (9/58). Therefore, according to the UK-NICE criteria, only the studies by Mulsant et al. (2001) (no patients with bipolar disorders included) and Zanardi et al. (1996) (excluding patients with bipolar disorders in the analysis was possible) should have been included. Of these two RCTs, Mulsant et al. (2001) found no significant difference between treatment with an AD alone or combination treatment with an AD plus an AP, whereas Zanardi et al. (1996) compared two ADs without an AP. Thus, to our opinion and in fact also according to their own criteria, the UK-NICE guideline does not provide evidence on which to base their cautious recommendation that '... augmenting AD with an AP should be considered'.

The Dutch guideline (2005) refers to three studies (Spiker 1985; Parker 1992; Bruijn 2001) The data of these studies were not re-analyzed. The study of Spiker et al. (1985) included 15.5% patients with bipolar disorders and in the ITT analysis the difference between an AD and an AD plus an AP was no longer statistically significant. The conclusions of the review by Parker et al. (1992) were based on data from mostly uncontrolled studies. The cautious recommendations of this guideline 'Starting treatment with a TCA and if after 4 weeks there is still no response adding an AP is a reasonable option. Starting with the combination of a TCA and an AP is also a reasonable option' are in accordance with the evidence provided by these three studies.

In the US-APA guideline (2000), the recommendation 'a combination of AP and AD medications (or ECT) should be used for psychotic depression' is based on one referred study: the study of Spiker et al. (1985) which was rated as providing a level I evidence (the highest level in this guideline), meaning 'with substantial clinical confidence'. The guideline does not mention that in this trial the difference between the combination of amitriptyline plus perphenazine versus amitriptyline alone was statistically significant only when the data of study completers were analysed and not in the ITT analysis which was not presented in that paper. Thus, we conclude that the recommendation of the US-APA guideline is in fact not based on level I evidence and to our opinion should not be considered evidence based on an ITT analysis of the study by Spiker et al. (1985)

The Canadian guideline (2001) refers to five studies (Spiker 1985; Parker 1992; Zanardi 1996, 1998; Rothschild 1999); the data from these studies were not re-analysed. The guideline seems inconsistent in its evaluation of the available evidence. The study of Spiker et al. (1985) was scored as a level I evidence and those of Zanardi et al. (1996, 1998) as a level II evidence, without further clarification, while the Spiker (1985) study looses significance with ITT recalculation and both Zanardi (1996, 1998) studies do not. Of the study of Parker et al. 1992), the guideline states that '... the results of this meta-analysis are limited by the inclusion of open-label trials'. Nevertheless, the guideline reaches a clear recommendation: 'First-line treatment is (ECT or) an AP plus AD (level I evidence) and as second-line treatment olanzapine plus AD (level III evidence)'. The guideline does not recommend

Table II. Review of references.

| Reference | Description of study |
|---|---|
| Minter and Manderl 1979 | A prospective open case report of 11 patients with a psychotic major depression. Nonresponders to single treatment were included again. Treatment was with TCA till maximum tolerated dose (mean dose 150 mg) for at least 3 weeks, or with an AP. Responders with first treatments only: TCA 0/3 (0%), AP 2/4 (50%). |
| Kaskey et al 1980 | A retrospective study of 23 patients with depression and delusions, including 30% with bipolar disorder or schizoaffective disorder. Treatment was with imipramine 100 mg for more than 3 weeks (without blood level control), with an AP, or with the combination. Responders: AD plus AP 7/11 (63%), AP 2/6 (33%) and AD 3/10 (30%) |
| Charney and Nelson 1981 | A retrospective study of patients with unipolar depression with ($n=54$) or without delusions ($n=66$). Nonresponders to single treatment were included again. Treatment was with TCA mostly 250 mg for a minimum of 3 weeks (without blood level control), or with an AP (or with the combination if nonresponder to AD or AP). Responders to first treatment only: TCA psychotic 2/9 (22%) and nonpsychotic 32/40 (80%), AP psychotic 8/26 (31%) and nonpsychotic 0/1 (0%) |
| Brown et al 1982 | Retrospective study with 34 patients with unipolar depression without psychotic features (NP) and 30 with psychotic features (P). Twenty-three NP and 18 P were treated for at least 3 weeks with at least 150 mg TCA (without blood levels), 1 NP and 0 P with AP, and 0 NP and 3 P with the combination (rest of patients with psychotherapy or ECT). Responders: TCA: NP 12/23 (52%) and P 2/18 (11%), AP: NP 0/1 (0%) and P 0/0, TCA plus AP: NP 0/0 and P 0/3 (0%) |
| Spiker et al 1982 | This study describes the results of the first 2 years of the Spiker et al, 1985 study. [12] |
| Spiker et al 1985 | RCT of patients with psychotic depression (only patients with delusions); 9/58 (15.5%) of the patients had bipolar disorders. Three treatments: perphenazine versus amitriptyline versus amitriptyline plus perphenazine. Treatment period 4 weeks. In the original publication 7 dropouts were excluded from the analysis. Responders (ITT): perphenazine 3/17, amitriptyline 7/19, combination 14/22. Difference between AD plus AP and AD was not statistically significant. |
| Anton and Burch 1990 | RCT comparing amoxapine versus amitriptyline plus perphenazine. DSM-III psychotic major depressive episode. No blood levels. Treatment period 4 weeks. Dropouts: 8/46 (17%). Bipolar disorders: 6/38 (15.8%). Responders (ITT): amoxapine 12/21 (57%) and amitriptyline plus perphenazine 17/25 (68%). This difference was not statistically significant. |
| Parker et al 1992 | In this review included studies are from before 1988. Most of the included studies were retrospective or open prospective case reports. Some did not use clear classification criteria and many included unknown numbers of patients with bipolar disorders. As a consequence, the conclusion of this review is cautious: "TCA plus AP is not significantly better than TCA" and "Prescriptive statements about the comparative efficacy of drug treatments should not be accepted with confidence"' |
| Rothschild et al 1993 | An open prospective study of 23 patients with unipolar psychotic depression (DSM-III-R). Treatment was for 5 weeks with fluoxetine 20 mg (or, if after 3 weeks no response, 40 mg) plus perphenazine 32 mg. Responders: 18/23 (78%). |
| Bellini et al 1994 | Underpowered RCT with 4 study arms. DSM-III-R psychotic major depressive episode. Number of patients 10, 14, 13, and 11. Treatment period 6 weeks. No blood levels. Dropouts: 0. Bipolar disorders: 12/48 (25%). Responders: desipramine+placebo 4/10 (40%), desipramine+haloperidol 9/14 (64%), fluvoxamine+placebo 9/13 (10%), fluvoxamine+haloperidol 5/11 (45%) |
| Zanardi et al 1996 | RCT comparing 150 mg sertraline versus 30 mg paroxetine. Included 46 patients with psychotic depressive episode (DSM-III-R); patients with bipolar disorders could be excluded from analysis. Treatment period 5 weeks. Dropouts: paroxetine 5/14 (36%), sertraline 0/18. Responders (ITT): sertraline 13/18 (72%), paroxetine 3/14 (21%).<br>Comment: Rothschild and Philips (1999a) have raised question to this study because of the high response rate without a placebo arm. They suggested the possibility of misdiagnosis. Zanardi replied that a placebo arm was ethically impossible, that the lack of placebo response in psychotic depression is well documented, and that diagnosis was strictly according to DSM-III-R. |
| Zanardi et al 1998 | RCT comparing 300 mg fluvoxamine plus pindolol versus 300 mg fluvoxamine plus placebo and including 50 patients with psychotic unipolar depression and 22 patients with bipolar disorders (30.5%). Treatment period 6 weeks. Dropouts: 1/36 (3%) in fluvoxamine plus pindolol group. Responders (ITT): fluvoxamine plus pindolol 29/36 (81%), fluvoxamine plus placebo 28/35 (80%), with a significant more rapid improvement with pindolol addition. |
| Rothschild et al 1999b | A retrospective study of 22 hospitalised patients with unipolar psychotic depression. They were treated with olanzapine or other neuroleptics and 80% of the patients also with various types of ADs in unknown dose. Length of treatment is unknown (until discharge, partly less than 2weeks). Responders: olanzapine plus an AD 8/11 (73%), other neuroleptics plus an AD 3/11 (27%). |
| Zanardi et al 2000 | RCT comparing 300 mg venlafaxine versus 300 mg fluvoxamine in 22 patients with major depressive disorder with psychotic features (DSM-IV) including 6 (21.4%) patients with bipolar disorders. We received additional data from the author to exclude the patients with bipolar disorders from the analysis. Treatment period 5 weeks. Dropouts: venlafaxine 2/11 (18%), fluvoxamine 0/11. Responders (ITT): venlafaxine 6/11 (55%); fluvoxamine 9/11 (82%). |

Table II (*Continued*)

| Reference | Description of study |
|---|---|
| Bruijn et al 2001 | A subgroup analysis of the patients with unipolar psychotic depression of the Bruijn et al, 1996 [28] study comparing imipramine and mirtazapine in the treatment of major depression. A well-designed RCT including blood level guided dosing of imipramine. Responders in the unipolar psychotic depression group: imipramine 9/15 (60%), mirtazapine 3/15 (20%) |
| Mulsant et al 2001 | RCT with inpatients older than 50 years. DSM-III-R psychotic major depressive episode. (Manic episode in history excluded). Open treatment with nortriptyline until therapeutic plasma level followed by randomisation to nortriptyline or nortriptyline plus perphenazine. With blood levels. Treatment period after randomisation was 2–16 weeks, total treatment was at least 4 weeks. The randomised group was a selected group because responders on nortriptyline and patients with adverse effects and for other reasons were excluded (18/54, 33%). Responder in ITT analysis: nortriptyline 7/19 (37%), nortriptyline+perphenazine 7/17 (41%). This difference is statistically not significant. |

monotherapy with a SSRI despite, according to the authors, there being level II evidence for this recommendation, while it advises using olanzapine, which, again according to their own classification, is supported by only level III evidence (Rothschild et al. 1999).

The Australian New Zealand guideline (2004) refers to six studies (Minter and Manderl 1979; Kaskey 1980; Charney and Nelson 1981; Brown 1982; Spiker 1982, 1985) in their conclusion that the treatment with a TCA plus an AP is superior to a TCA alone, indicated by a number needed to treat of 2 (!). However, the guideline does not take into account the many methodological problems of all six studies. The only prospective RCT was the study of Spiker et al. (1985) (the 1982 study of Spiker et al. is part of that study); three studies were retrospective (Minter and Manderl 1979; Charney and Nelson 1981; Brown 1982); two studies used too low doses of a TCA and for only a very short treatment period (Kaskey 1980; Brown 1982); and one study included 30% patients with bipolar disorders Kaskey (1980). As already mentioned, based on ITT analysis, the Spiker et al. (1985) study did not find combination treatment (AP+AD) to be superior to AD monotherapy. Thus, considering the referred studies, the conclusion of this guideline can in fact not be considered evidence based.

The US-TIMA guideline (Trivedi 2000) does not refer to studies. Therefore, we cannot evaluate on which evidence its recommendation 'treatment with AD+AP has been shown to be significantly more effective than either alone' has been based.

The WFSBP guideline (Bauer 2002) refers to two studies (Spiker et al. 1985), and an open uncontrolled study by Rothschild et al. (1993), the data of which were not re-analysed. On the basis of these two studies, the guideline concludes that 'the combination of an AD plus an AP has a considerably better response rate than either component alone', a conclusion that is 'supported' by the highest level of evidence (in this guideline level A means more than three RCTs, at least one with placebo). Given the quality of the studies (and the ITT outcome of the Spiker et al. (1985) study), this conclusion seems not warranted.

The South-African guideline (year unknown) is in fact an enumeration of algorithms for the most prevalent psychiatric conditions. It does not mention any evidence and has no date of publication. It advises combination treatment with an AD and an AP.

## Discussion

### *Quality*

With the AGREE method, the UK-NICE guideline (2004) was the best of these eight guidelines. It is cautious in its recommendations with regard to the treatment of unipolar psychotic depression. The Dutch guideline (2005) was second and is also cautious in its recommendations. Given their referred evidence, these two guidelines can be considered as the most evidence based. The other six guidelines scored lower with the AGREE method and all of them recommend using the combination of an AD and an AP.

There is a small correlation between AGREE scores and year of publication. From higher to lower scores: 2004, 2005, 2000, 2004, 2001, 2002, 2000, and one unknown.

### *Evidence and recommendations*

Surprisingly, not all guidelines were based on the same or, considering the year of publication, on the evidence available at date of publication. A possible explanation is that a systematic review, including an extensive literature search and analysis, is too time-consuming for just a small paragraph in the often large guidelines on the treatment of depression. In this respect, it is noteworthy to indicate that there is no MESH term for psychotic depression, making it very complicated to find all the relevant references.

In our review we had to check 3333 abstracts to find 10 RCTs into the pharmacological treatment of bipolar depression (Wijkstra 2005, 2006)

A serious problem is that most of the referred studies methodologically can be criticised (see also Table II). Many of them are retrospective and open studies. Even the few randomised clinical trials have been criticised. Rothschild and Phillips (1999) have raised question to the 1996 study by Zanardi et al. because of the high response rate without a placebo arm. They suggested the possibility of misdiagnosis. The landmark study of Spiker (1985), referred to in all guidelines, excluded drop outs in the results. With intention to treat recalculation in this study the difference between an AD plus an AP and an AD alone looses statistical significance.

The treatment of psychotic depression is a challenge and is under studied. Reasons for the overall scarcity of studies are probably manifold. In most or even almost all clinical trials, also registration trials, patients with psychotic symptoms are excluded. Diagnostics are difficult. Differential diagnosis between psychotic bipolar and unipolar depression for instance depends greatly on information from others about prior illness history. In some, especially the older studies, psychotically depressed bipolar patients were included. And how to differentiate between a first psychotic episode in schizophrenia and psychotic depression? Most schizophrenic patients also have affective symptoms. Besides problems with diagnostics, obtaining informed consent is a problem with psychotic depressed patients. Many of them have extreme difficulties in decision making. Psychotic patients often are incompetent to give informed consent. Informed consent from legal representatives can be difficult to get. In addition, to our own experience, a necessary medication free period prior to randomisation and start of study treatment is emotionally difficult for many doctors and nurses because of the severe suffering of their patients. If family and nurses do not see progress drop out chances quickly rise. And last but not least getting funds for research can be difficult because most pharmaceutical companies are not interested to obtain licensing for only a subcategory of depression.

## Implications

Although guidelines not always influence clinical practice as they should (Weinmans 2005), clinical guidelines are in general considered of great importance to clinical practice (Miller 2004) Our previous systematic review of available studies in psychotic depression and subsequently our review of conclusions and treatment recommendations in guidelines based on (part of) these studies, shows that the conclusions and recommendations given in these guidelines appear not as evidence based as being claimed. Physicians (and patients) should be aware of this potential limitation.

In general in could be useful to study guidelines on other treatment issues using the methodology described here.

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## Statement of interest

J Wijkstra and W Nolen are currently conducting a randomized controlled trial involving patients with unipolar psychotic depression that is financially supported by Wyeth and AstraZeneca.

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**ORIGINAL INVESTIGATION**

# Use of aripiprazole in tardive dyskinesia: An open label study of six cases

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**Abstract**

Aripiprazole, a partial dopamine agonist has been reported to help reduce symptoms of tardive dyskinesia (TD). In a prospective, open label study of a series of cases, we examined the effectiveness of aripiprazole in reducing TD symptoms. Six clinically stable patients with schizophrenia or Schizoaffective disorder and a moderate to severe TD participated in this study. They were systematically cross-titrated from their current medication to aripiprazole and maintained for 16 weeks. The mean extra pyramidal symptom score measured by Abnormal Involuntary Movement Scale (AIMS) improved from a baseline score of 15.8 to final score of 5 (paired *t*-test; $P=0.0009$). The severity of psychiatric symptoms remained unchanged. This study supports our hypothesis that clinically stable patients with moderate tardive dyskinesia who are under treatment with other first- or second-generation antipsychotics may benefit from switching to aripiprazole with a reduction of TD symptoms but with out any significant benefit in psychiatric symptoms. The results need to be viewed with caution and not considered as indicative of a viable treatment option for TD as this is an open label study, and a small sample size.

**Key words:** *Schizophrenia, schizoaffective disorder, antipsychotics, tardive dyskinesia, EPS*

## Introduction

Tardive dyskinesia (TD) is a drug-induced movement disorder associated with prolonged administration of conventional dopamine blocking antipsychotics (Casey 1999). Incidence rates of new TD cases approximate 3–5% per year with a cumulative incidence of 53% after 10 years of continuous neuroleptic therapy (Correll and Schenk 2008). TD can be distressing and debilitating to the patients and preclude their successful social integration and rehabilitation. The newer, atypical antipsychotics have a much lower incidence of TD even after long-term use and some of these medications may help reduce TD symptoms possibly due to the different action profile (Kane 2004; Soares-Weiser and Fernandez 2007). Clozapine has been shown to be effective in treating TD and there are reports of improvement in TD with risperidone, olanzapine and quetiapine (Kane 2004). Aripiprazole, the newest atypical antipsychotic (Fleischhacker et al. 2001) has also been associated with a lower incidence of involuntary movements compared to the conventional antipsychotic, haloperidol (Tarsy and Baldessarini 2006; Miller et al. 2007).

TD is hypothesized to result from an antipsychotic-induced hypodopaminergic state leading to dopaminergic supersensitivity (Casey 2004). The reason for lower incidence of TD with newer atypical agents is not well understood. Some of the prominent hypotheses include lesser degree of D2 blockade, 5HT2A receptor antagonism, fast dissociation and sparing of the nigrostriatal dopamine pathway. Aripiprazole is a unique antipsychotic that acts as a weak, partial dopamine agonist and so it can be speculated to mitigate the dopaminergic supersensitivity that may underlie TD. Several case reports have document improvement in TD with Aripiprazole (Sharma et al. 2005; Duggal and Mendhekar 2006; Kantrowitz et al. 2007; Lykouras et al. 2007) but to our knowledge there have been no systematic prospective studies reported. In this open label study we examined the usefulness of short-term (16 weeks) aripiprazole treatment in patients with schizophrenia and/or

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schizoaffective disorder who are suffering from moderate to severe neuroleptic induced TD.

## Subjects

Six individuals with a DSM-1V diagnosis of schizophrenia or schizoaffective disorder were recruited from the outpatient services affiliated to Wayne State University School of Medicine. The Wayne State University Human Investigations committee approved the study. Inclusion criteria were: (a) age between 18 and 55 years; (b) DSM-1V (American Psychiatric Association 1994) diagnosis of schizophrenia or schizoaffective disorder; (c) Research Diagnostic Criteria for TD (Schooler and Kane 1982); The Schooler-Kane criteria require a sum of 4 (a score of $>3$ on any one of the AIMS items 1 through 7, and $>1$ on another categorical item); and (d) a total PANSS score between 60 and 120. We excluded patients with current or past history of neurological illness; current or recent (within the past month) DSM-1V diagnosis of substance abuse or dependence; pregnant or breast-feeding individuals as the physiological changes with pregnancy/lactation may affect TD; and patients who received depot neuroleptics within 3 months of entry.

## Methods

A clinical diagnosis using DSM-IV-R was determined using all available clinical information including chart review and interview. Following informed consent, and baseline screening procedures, participants were scheduled for six follow-up visits at 1, 2, 4, 8 and 16 weeks. At day 0 or baseline visit, the participants signed consent, and data regarding clinical presentation, symptomatology and abnormal movements were collected using Positive and Negative Syndrome Scale (PANSS) and Abnormal Involuntary Movement Scale (AIMS) by a psychiatrist and a senior research nurse. If eligible, aripiprazole was started and their current antipsychotic medication was reduced to 50% at this visit. At visit 1 (baseline $+7$) the participants' prior antipsychotic was decreased by another 25% and aripiprazole was titrated if necessary. At visit 2 (baseline $+14$) the previous antipsychotic was discontinued. Use of concomitant medications and adverse events were addressed during each visit along with PANSS and AIMS. Visits 3 to 6 (baseline $+28$, 64 and 112 days) consisted of the same assessments as visit 2. The patients were not allowed to take anticholinergic medication during the study. The dosage of aripiprazole was adjusted by the physician depending upon the clinical presentation. The mean dose of aripiprazole at the beginning of the study was 9.16 mg and the mean dose of aripiprazole at study end was 20 mg. Following completion of the trial, the patient was either continued on aripiprazole, or switched to another antipsychotic treatment. One participant signed consent and then withdrew consent prior to any assessments. The following two case summaries highlight the key aspects of our observation.

1. BR is a 51-year-old female under treatment for schizophrenia since 1989. Her major symptomatology included auditory hallucinations and paranoid delusions of being poisoned, being watched and being placed under a curse. She was initially treated with fluphenazine deconoate 25 mg, intramuscularly every 4 weeks then with fluphenazine oral pill 5mg twice a day for which she responded well. About 4 years ago, she gradually began to manifest signs of tardive dyskinesia, with oral, buccal, and lingual dystonic movements. At this time her medication was changed to risperidone which helped her with psychiatric symptoms but her TD did not improve. She agreed to participate in this study, and completed the entire study. With aripiprazole treatment her TD symptoms improved (AIMS score 16 to 7) and psychiatric symptoms stayed about the same (PANSS score 78 to 75). She is currently taking aripiprazole 20 mg once a day.
2. SG is a 42-year-old male with more than a 5-year history of schizophrenia. He was clinically stable but exhibited high residual symptoms

Table I. Clinical data

| | | | | AIMS | | PANSS | | |
|---|---|---|---|---|---|---|---|---|
| Subject | Age | Sex | Diagnosis | Begin | End | Begin | End | Completed visits |
| 1 | 46 | M | Schizophrenia | 12 | 5 | 81 | 79 | 6 |
| 2 | 42 | M | Schizophrenia | 21 | 7 | 96 | 112 | 5 |
| 3 | 42 | M | Schizophrenia | 22 | 6 | 88 | 87 | 5 |
| 4 | 42 | M | Schizophrenia | 12 | 5 | 43 | 45 | 4 |
| 5 | 51 | F | Schizoaffective d/o | 12 | 0 | 42 | 34 | 4 |
| 6 | 51 | F | Schizophrenia | 16 | 7 | 78 | 75 | 6 |
| Mean (SD) | | | | 15.8 (4.6) | 5 (2.6) | 71.3 (23.1) | 72 (28.5) | |
| Two-tailed paired *t*-test | | | | 0.0008871 | | 0.849 | | |

with a total PANSS score of 96 and moderate auditory hallucinations. He had severe TD with buccal/oral movements and a total AIMS score of 21. He tolerated aripiprazole well and experienced a noticeable improvement in TD, but immediately after discontinuing haloperidol his psychiatric symptoms worsened with an increase in the severity of hallucinations. However, he discontinued aripiprazole and started back on haloperidol on his own. He discontinued from the study but continues treatment with one of the authors (JD). He is maintained on a combination of haloperidol and aripiprazole with good control of his symptoms and improvement in TD.

## Results

Six participants (four males and two females; mean age was $45.6 \pm 5.6$ years) were included in this study. The mean PANSS score did not change during the study (71.3 at base line and 72 at the end of study). This was not statistically significant but one of the patients became more symptomatic and needed to restart haloperidol (case example 2). The mean total AIMS score at baseline visit was $15.8 \pm 4.7$ which improved at the completion of the study to $5 \pm 2.6$ ($P = 0009$; paired *t*-test). As it is shown in the table, each patient improved to a noticeable degree with their TD symptoms. One patient (subject 2) showed early signs of relapse and discontinued from the study. No major adverse events were noted.

## Discussion

This open label prospective study illustrates that aripiprazole may help reduce the symptoms of TD. One of the major limitations with this study is that this is an open label study and the role of patient and rater bias cannot be ruled out; however, the degree of change in the AIMS score is encouraging and tentatively supports our hypothesis. Another limitation is that the sample size is small and so type I errors can not be ruled out. These findings should therefore be considered as preliminary.

As expected, the patients did not change symptomatically. All of them tolerated the switch except for one patient who had worsening of symptoms subjectively and objectively (case example 2). Although this patient dropped out of the study, he is currently stable symptomatically on a combination of haloperidol and aripiprazole with an improvement in TD. He appears to have benefited from the combination with a reduction in TD symptoms and stability through older antipsychotic. This case might imply that aripiprazole can be used as an add-on medication for TD, but further studies are needed to assert this observation.

Our observation of the improvement in TD with aripiprazole is consistent with several anecdotal reports (Kantrowitz et al. 2007). However, occurrence of TD has also been reported in patients treated with aripiprazole (Abbasian and Power 2008; Lim et al. 2008) and recent studies report higher than expected incidence of TD with atypical antipsychotics, especially when they are administered in higher doses for long periods of time (Correll and Schenk 2008). Yet, one of the major advantages and a popular reason for preference by patients and clinicians of the newer second-generation antipsychotics is the lower incidence of extrapyramidal side effects and TD (Tandon and Jibson 2001). Our observations support the idea that patients and clinicians may prefer to switch to aripiprazole for the benefits of relief in TD symptoms, however this observation can not be considered as evidence that aripiprazole is useful in treating TD as the relief from TD symptoms can be due to the discontinuation of the previous medications. We believe that aripiprazole may be considered as a treatment option for patients who are suffering from tardive dyskinesia, but larger controlled studies are needed to confirm our preliminary observation, before this becomes a recommendation in clinical practice.

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## Statement of Interest

None.

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**ORIGINAL INVESTIGATION**

# Blood pressure changes during clozapine or olanzapine treatment in Korean schizophrenic patients

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**Abstract**

*Background.* Numerous reports have linked atypical antipsychotics, especially clozapine and olanzapine, to the development of cardiovascular risk factors. In this retrospective chart review study, we investigated the blood pressure changes in Korean schizophrenic inpatients treated with clozapine or olanzapine. *Method.* We reviewed the medical record of schizophrenic patients treated with clozapine or olanzapine for 8 weeks. A total of 167 patients were included in the study; 70 patients in clozapine group and 97 patients in olanzapine group. Systolic and diastolic blood pressures prior to medication and at post-treatment (8-week) were assessed, and changes in blood pressure were analyzed. The prevalence of hypertension at the time of study period was assessed and compared between the two groups. *Results.* There was a significant difference in hypertension prevalence in comparisons between the clozapine and olanzapine group. The systolic and diastolic blood pressures in the clozapine group were significantly increased after treatment, but systolic and diastolic blood pressures in olanzapine group did not change significantly. *Conclusion.* Our findings suggest that clozapine treatment may be associated with increased blood pressure and higher prevalence of hypertension, which may have a significant impact on medical morbidity and mortality.

**Key words:** *Clozapine, blood pressure, olanzapine, schizophrenia*

## Introduction

Side effect profiles related to long term antipsychotic treatment have become the focus of attention recently as concern regarding maintenance treatment has increased. Cardiovascular disease is the most frequent cause of death among both men and women, accounting for approximately 30% of all deaths in the United States (Mokdad et al. 2004). Moreover, patients with schizophrenia have a higher prevalence of cardiovascular disease than the general population (Davidson 2002). The use of atypical antipsychotics has been associated with cardiovascular risk factors including weight gain, hyperlipidemia and diabetes mellitus (Ryan and Thakore 2002). While a growing body of evidence related to serum lipid abnormalities, weight gain, obesity and diabetes mellitus is emerging, there are few reports on the prevalence of hypertension in patients treated with atypical antipsychotics.

Clozapine is associated with significant weight gain, increase in serum lipids, and cardiomyopathy; there are some reports that clozapine is related to onset of hypertension (Gupta and Rajaprabhakaran 1994; George and Winther 1996; Henderson et al. 2004). However, the relationship between clozapine treatment and onset of hypertension is controversial. Lund et al. (2001) found no significant differences in patients receiving clozapine versus conventional antipsychotic agents. Only 5.3% of conventional antipsychotic-treated patients and 4.1% of clozapine-treated patients received treatment for hypertension.

Olanzapine is similar to clozapine in structure and receptor profile and is a well-known cause of significant weight gain and dyslipidemia (Beasley et al. 1997; Lambert et al. 2003). Fontaine et al. (2001) estimated the effects of weight gain on the prevalence of hypertension in patients treated with

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antipsychotics using raw data from 5209 respondents from the Framingham Heart Study's (Dawber et al. 1951) public use data set. Hypertension and weight gain were estimated to have a linear effect. However, only a few studies have assessed the risk for hypertension in patients treated with olanzapine (Sachs and Guille 1999; Conley and Meltzer 2000).

Therefore, the objective of this retrospective chart review study was to examine the change in systolic and diastolic blood pressure in inpatients treated with olanzapine or clozapine. In addition, we compared the prevalence of hypertension between the two treated groups.

## Materials and methods

### *Subjects and setting*

We reviewed the medical record of Korean patients who were admitted to St. Mary's Hospital, The Catholic University of Korea and Naju National Hospital from 1 January 2001 to 31 December 2005. Medical record review was performed by a psychiatrist (YSW) not involved in clinical care of the patients and blind to patient identity. Institutional review board (IRB) reviewed and approved the protocol, and the study was conducted in accordance with good clinical practices and the Helsinki Declaration. Our IRB waived patient-specific informed consent for this confidential chart review and anonymous reporting of aggregate data. All patients with a DSM-IV diagnosis of schizophrenia at the time of data collection were evaluated. The researchers (WK and BHY) confirmed the diagnosis of schizophrenia made by the treating clinician according to DSM-IV criteria. We selected the records of patients who had stopped taking medication because of poor insight into illness for more than 1 week before admission and clozapine or olanzapine treatment was started on the day of admission and continued for more than 8 weeks. We excluded patients who were discharged from hospital before the eighth week. Patients treated with mood stabilizers, antidepressants, propranolol and antipsychotics other than clozapine or olanzapine were also excluded.

All measurements were obtained as a part of routine clinical monitoring. At the St. Mary's hospital and Naju national hospital, monitoring of daily vital signs, weekly weight, monthly blood chemistry were routinely performed for hospitalized patients. Systolic and diastolic blood pressure measurements were obtained using a manual sphygmomanometer with a standard cuff. Measurements were obtained with the patients in a sitting position after 5 min of quiet rest every morning at 07:00 h. Two measurements were made everyday and systolic blood pressure and diastolic blood pressure were the average of two readings measured at 5-min intervals. Pre-treatment blood pressure data were determined to be the average of the admission day and the next day to rule out the possible psychological effect of hospitalization on blood pressure. Post-treatment blood pressure data were the average of day 55 and 56. Hypertension was defined according to the classification recommended by the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al. 2003). Patients who met criteria for hypertension at baseline were excluded. Finally, the records of 70 clozapine-treated patients and 97 olanzapine-treated patients were analyzed. Systolic blood pressure, diastolic blood pressure, weight, serum triglyceride, total cholesterol and fasting glucose prior to medication initiation (pre-treatment) and post-treatment (8-week) were examined. Data on age and gender were also collected.

### *Data analysis*

Patients' demographic and baseline clinical characteristics were compared between treatment groups using an independent *t*-test for continuous variables and the chi-square test or Fisher's exact test for discrete variables.

The changes from baseline on systolic and diastolic blood pressure, weight, fasting glucose, triglyceride and total cholesterol were compared using paired *t*-test for each group. We used independent *t*-tests to compare the mean changes in systolic and diastolic blood pressure between clozapine group and olanzapine group. Correlation analysis was used to examine the relationship between changes in blood pressure and body weight. The number of hypertensive patients after 8-week antipsychotics treatment were compared between treatment groups using the Fisher's exact test. All tests were considered significant when $P$ values were less than 0.05 (two-tailed).

## Results

The clozapine and olanzapine groups did not differ significantly in any of the baseline demographic or clinical variables. The mean age at the time of medication initiation was $40.2 \pm 8.5$ years with 36 (51.4%) male and 34 (48.6%) female in the clozapine group and $37.7 \pm 11.6$ years with 51 (52.6%) male and 46 (47.4%) female in the olanzapine group (Table I).

Table I. Comparison of demographic characteristics between clozapine group and olanzapine group (mean ± SD).

| | Clozapine ($N=70$) | Olanzapine ($N=97$) | $P$ value |
|---|---|---|---|
| Age (years) | 40.2 ± 8.5 | 37.7 ± 11.6 | 0.110[a] |
| Gender | | | |
| Male | 36 (51.4%) | 51 (52.6%) | 0.883[b] |
| Female | 34 (48.6%) | 46 (47.4%) | |

[a]$P$ values on independent $t$-test.
[b]$P$ values on chi-square test.

At 8 weeks post-treatment, prevalence of hypertension was significantly higher in the clozapine group. In the clozapine group, 16.7% of patients ($n=11$) fulfilled the criteria of hypertension, whereas 5.4% of patients ($n=5$) fulfilled the criteria for hypertension in the olanzapine group ($P=0.021$).

For the clozapine group, there were significant changes in systolic blood pressure ($P=0.031$) and diastolic pressure ($P=0.001$). Weight ($P=0.003$) and serum triglyceride significantly elevated ($P=0.026$), but not for fasting glucose ($P=0.582$) and total cholesterol ($P=0.515$). For olanzapine-treated patients, there were no significant changes in systolic ($P=0.362$) and diastolic ($P=0.800$) blood pressure. Weight elevated significantly ($P=0.000$), but there was no significant change in fasting glucose ($P=0.332$), total cholesterol ($P=0.184$) and triglyceride ($P=0.066$) (Table II). The changes between the clozapine and olanzapine groups were significantly different for systolic blood pressure ($P=0.017$) and diastolic blood pressure ($P=0.002$) (Table III). In addition, we found no significant correlation between changes of systolic (clozapine group: Rp = 0.080, $P=0.508$; olanzapine group: Rp = 0.109, $P=0.369$) or diastolic blood pressure (clozapine group: Rp = 0.079, $P=0.441$; olanzapine group: Rp = 0.134, $P=0.192$) and changes of body weight.

## Discussion

The average life expectancy in schizophrenic patients is approximately 20% shorter than general population in United States (Harris and Barraclough 1998). The significantly lower life expectancy in schizophrenic patients is caused primarily by coronary heart disease. In a meta-analysis, death rates from coronary heart disease were 90% higher in patients with schizophrenia than among the general population (Harris and Barraclough 1998). Hypertension is one of the chief risk factors for excess coronary heart disease mortality among schizophrenic patients (Hennekens et al. 2005).

When comparing the clozapine and olanzapine groups, the prevalence of hypertension at post-treatment was significantly higher in the clozapine group. Moreover, the change in blood pressure between pre- and post-treatment was significant in the clozapine group. Our data show the effect of clozapine on blood pressure, which is consistent with findings of a previous study (Henderson et al. 2004) that clozapine treatment is associated with increased rates of hypertension. Clozapine-induced hypertension was reported by Ereshefsky et al. (1989). In an open-label study conducted to determine the effectiveness and safety of a method of slow cross-titration from clozapine to olanzapine with 20 patients (Littrell et al. 2000), three cases of clozapine-induced hypertension (15%) were reported. While this rate appears elevated, there was no comparison group available to directly test the effect of clozapine. The present study, however, involved data from 97 patients taking olanzapine. We were able to demonstrate significantly high prevalence of hypertension and increased systolic and diastolic blood pressure in the clozapine group compared to the olanzapine group.

There are possible explanations for the influence of clozapine on blood pressure. The increase of blood pressure might be influenced by lowered

Table II. Change of blood pressure and related factors from baseline to endpoint (week 8).

| | Clozapine ($N=70$) | | | Olanzapine ($N=97$) | | |
|---|---|---|---|---|---|---|
| | Baseline | End-point | $P$ value | Baseline | End-point | $P$ value |
| Blood pressure (mmHg) | | | | | | |
| Systolic | 115.68 ± 8.64 | 118.64 ± 11.65 | 0.031* | 116.06 ± 9.03 | 115.23 ± 8.84 | 0.362 |
| Diastolic | 75.64 ± 6.52 | 79.36 ± 8.68 | 0.001* | 75.28 ± 7.6 | 75.08 ± 7.58 | 0.800 |
| Weight (kg) | 65.09 ± 13.48 | 66.56 ± 13.71 | 0.003* | 63.19 ± 12.21 | 65.44 ± 12.29 | 0.000* |
| Fasting glucose (mg/dl) | 88.59 ± 22.46 | 90.47 ± 13.98 | 0.582 | 93.32 ± 31.19 | 89.91 ± 18.03 | 0.332 |
| Total cholesterol (mg/dl) | 211.97 ± 44.11 | 208.43 ± 41.56 | 0.515 | 208.34 ± 38.09 | 216.08 ± 36.65 | 0.184 |
| Triglyceride (mg/dl) | 155.88 ± 54.08 | 173.09 ± 54.39 | 0.026* | 149.53 ± 56.34 | 167.91 ± 55.05 | 0.066 |

Values are presented as mean ± standard deviation.
[a]$P$ values on paired $t$-test, *$P<0.05$ .

Table III. Comparisons of pre- and post-treatment blood pressure mean change between clozapine and olanzapine treatment group.

| | Clozapine | | | Olanzapine | | | |
|---|---|---|---|---|---|---|---|
| Blood pressure (mmHg) | Pre- | Post- | Mean change | Pre- | Post- | Mean change | *P* value |
| Systolic | 115.68±8.64 | 118.64±11.65 | 3.18±11.25 | 116.06±9.03 | 115.23±8.84 | −0.68±8.79 | 0.017 |
| Diastolic | 75.64±6.52 | 79.36±8.68 | 4.24±8.46 | 75.28±7.60 | 75.08±7.58 | 0.16±7.91 | 0.002 |

Mean changes of blood pressure obtained from endpoint values minus baseline values; *P* values are from independent *t*-test of mean blood pressure change for clozapine group versus olanzapine group.

activity due to hospitalization. However, because both groups equally affected by hospitalization, blood pressure change would not be affected by hospitalization.

Many studies confirm that atypical antipsychotics induce substantially more weight gain in comparison to typical antipsychotics (Allison and Casey 2001; Russell and Mackell 2001), and have reported a variability of weight gain among atypical antipsychotics. Long-term data indicate that olanzapine and clozapine are associated with the greatest weight gain (Nemeroff 1997; Casey 1997). Risperidone and quetiapine may produce less weight gain than olanzapine while aripiprazole and ziprasidone are associated with a relatively low risk of weight gain (Haupt 2006).

Weight gain may correlate with increase in systolic blood pressure (Henderson et al. 2004). In addition, a positive linear relationship between BMI and relative risk of developing hypertension has been reported. Community screening in the United States has demonstrated that the prevalence of hypertension in overweight people is twice as high as it is in those of normal weight (Stamler et al. 1978). Approximately 77% of hypertension in obese people is attributable to obesity (Benotti et al. 1992).

However, the rate of clozapine-induced weight gain remains controversial. In a review of over 13,000 patients treated with clozapine worldwide, weight gain was observed in 0.73% of patients (Liebermann et al. 2001). There was a study which noted weight gain in only three patients treated with clozapine in a retrospective review of 503 inpatient medical charts (Naber and Hippius 1990). In another retrospective study of 216 inpatients treated with a mean daily dose of 317mg/day of clozapine for up to 12 years, a weight gain frequency of 11.6% was noted (Povlsen et al. 1985). According to the results of the present study, weight in the clozapine group increased significantly during 8 weeks of treatment. However, the correlation between body weight change and blood pressure change was not significant. Furthermore, weight in the olanzapine group increased but systolic and diastolic blood pressures have not significantly changed.

Clozapine has a unique pharmacological profile, interacting with dopaminergic (D1, D2, D4), serotonergic (5-HT1C, 5-HT2A/C, 5-HT3), muscarinic, histaminergic, and adrenergic (α1, α2) receptors (Meltzer 1995). The typical cardiovascular effects, due to α1-adrenergic and muscarinic antagonism, are hypotension and tachycardia, respectively (Baldessarini and Frankenburg 1991). However, Kane et al. (1988) reported that 12% of patients treated with clozapine exhibited hypertension. Furthermore, sustained hypertension appeared to be blocked by coadministration of propranolol, a nonselective β-blocker. This result suggests the involvement of adrenergic hyperactivity, at least in part, in the mechanism of clozapine-induced hypertension (George and Winther 1996). Moreover, there are a few reports regarding autonomic dysfunction with increased adrenergic activity in patients receiving prolonged clozapine treatment. Patients treated with clozapine exhibited marked differences in autonomic nervous system functioning compared with those treated with olanzapine as shown by increased heart rate and low-frequency components and lower HRV and high-frequency components (Cohen et al. 2001). This reflects a basal autonomic dysfunction with increased sympathetic and decreased parasympathetic tone. And the inspiratory gasp response (IGR) redilatation time was prolonged in clozapine-treated patients compared to control, but not in olanzapine-treated patients (Mueck-Weymann et al. 2001). This finding can be explained by a prolonged release of noradrenaline due to the α2 antagonistic effect of clozapine. These results raise the possibility that the α2 antagonistic effect may play an important role in increasing blood pressure. The rationale for this hypothesis was supported by a report which evaluated binding potencies of antipsychotic drugs (Richelson and Souder 2000). They reported that clozapine had an affinity 18 times higher than olanzapine for the α2 adrenoreceptor, which may

explain the difference in blood pressure change between clozapine and olanzapine group.

The major limitation of the present study is the retrospective design and small numbers of participating subjects. Retrospective chart reviews cannot control for differences in baseline characteristics not captured in the charts. Absence of assessment of a variety of clinical information was another limitation of our study. The relatively short study duration of 8 weeks would be a reasonable criticism of this study and lack of appropriate assessment for psychiatric symptoms such as anxiety or agitation which can affect blood pressure were severely limited the generalizability of our findings. Uncontrolled previous medication, concomitant medication, medication dosage, comorbid medical condition and other clinical variables may affect the conclusions. Moreover, blood pressure can be affected by behavioural pattern including cigarette smoking, alcohol use, physical activity and food intake. However, most psychotropic medications which can affect blood pressure, such as venlafaxine, bupropion, and propranolol, have a half life of less than 1 week. So, the effect of previous medication can be ruled out because we selected patients who had stopped taking medication for more than 1 week and treated with clozapine or olanzapine for more than 8 weeks. Moreover, increase in blood pressure was not associated with clozapine dose (Henderson et al. 2004). Furthermore, because we selected patients who were stayed in hospital during the study period, the effect of behaviour change can be disregarded. Therefore, the results of the present study are a reflection of the "real world" clinical situation. Further data derived from large studies with a longer study period using standardized measurement of psychiatric symptoms are necessary to more fully explore this important issue.

The results of present study raise concerns about the potential deleterious effects of clozapine on cardiovascular health. Therefore, concern for cardiovascular disease in clozapine-treated patients should be considered. Obesity, diabetes mellitus and dyslipidemia are well-known cardiovascular risk factors in clozapine-treated patients and adding hypertension to these adverse events increases the risk of cardiovascular disease. We suggest that patients treated with clozapine undergo routine medical screening for weight gain, dyslipidemia, diabetes mellitus, and especially hypertension on a regular basis. With vigilant monitoring and the institution of appropriate interventions, we may be able to reduce the medical morbidity and mortality in patients treated with clozapine.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# Prediction of symptom remission in schizophrenia during inpatient treatment

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**Abstract**

*Objective:* Standardized consensus criteria for remission in schizophrenia were recently proposed. The present study applied the symptom-severity component of these criteria to a sample of inpatients in order to determine the rates of remission during inpatient treatment and to explore predictors of remission. *Method:* A total of 288 inpatients from a multi-centre follow-up programme who fulfilled ICD-10 criteria for schizophrenia were included in the present analyses. PANSS ratings at admission and at discharge from hospitalization were used to examine remission status. Clinical and sociodemographic variables at admission were tested for their ability to predict remission at discharge. *Results:* In total, 55% of the sample achieved symptom remission during inpatient treatment; 84% percent showed remission with respect to 'reality distortion', 85% with respect to 'disorganization' and only 65% with respect to 'negative symptoms'. Logistic regression analysis revealed that the global functioning (GAF) in the year before admission, the total score of the Strauss–Carpenter Prognostic Scale and the PANSS negative subscore at admission were predictive for symptom remission. The regression model showed a predictive value of about 70% and explained 36% of the observed variance. *Conclusion:* The results highlight the impact of negative symptoms for the course and treatment response of schizophrenic illness.

**Key words:** *Schizophrenia, remission, PANSS, prediction, negative symptoms*

## Introduction

Schizophrenia is a heterogeneous disorder. The identification of subgroups that have a differential course and outcome is an important challenge for psychiatric research (Andreasen 2006). Numerous attempts have been made to identify predictors in schizophrenic disorders, both for the long-term outcome (Möller et al. 1986; Gaebel and Pietzcker 1978; Lenoir et al. 2005) and for the acute treatment response (Möller et al. 1985; Gaebel 1996; Corell et al. 2003). However, the results of those studies depend on the diagnostic concept of schizophrenia applied and the definition of outcome (Lauronen et al. 2007). While operationalized diagnostic criteria were introduced more than 25 years ago, until now standardized outcome criteria have been lacking.

Recently, the Remission in Schizophrenia Working Group proposed consensus criteria for remission in

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schizophrenia (Andreasen et al. 2005). In this consensus, remission was defined by using an absolute threshold of severity of symptoms, rather than percentage improvements from a particular baseline score. The proposed criteria consist of two elements: a symptom-based criterion (low scores in diagnostically relevant symptoms) and a time criterion (duration of 6 months). The items that constitute the symptom-severity component of the remission criteria are represented in the three symptom dimensions 'reality distortion (psychoticism)', 'disorganization' and 'negative symptoms' (Andreasen et al. 2005). This definition of remission was considered as conceptually viable and recommended for implementation in clinical trials, health services research and clinical practice (van Os et al. 2006a). The implementation of standardized remission criteria should be the first step 'that will permit us to base our predictions about course on empirical evidence' (Andreasen 2006).

In the meantime, several studies have applied these new consensus criteria to different samples of patients suffering from schizophrenic disorders (Lasser et al. 2005; Dunayevich et al. 2006; van Os et al. 2006b; Wunderink et al. 2006; Emsley et al. 2007). These studies have demonstrated that the proposed remission criteria correlate with established outcome measures and appear achievable for a significant proportion of patients receiving pharmacological treatment (Nasrallah and Lasser 2006). The generalization of these results, however, is limited because they are mainly derived from selected samples of controlled clinical drug trials (Dunayevich et al. 2006; Lasser et al. 2005; Emsley et al. 2007) or first-episode follow-up programmes (Wunderink et al. 2006).

The evaluation of the proposed remission criteria in a naturalistic and more representative sample could be helpful for the assessment of therapeutic effects in future controlled clinical trials. Furthermore, there is the question whether predictive factors for treatment response that were identified in previous studies (Gaebel and Pietzcker 1978; Möller et al. 1985; Möller et al. 1986; Gaebel 1996; Corell et al. 2003; Lenoir et al. 2005) have the ability to predict symptom remission also according to the new criteria.

On this background, the symptom-severity component of the proposed remission criteria was applied to a large sample of inpatients who received treatment for schizophrenia under naturalistic conditions. The aims of the present study were (i) to examine what proportion of patients achieve symptom remission during inpatient treatment and (ii) to identify predictive factors for symptom remission.

## Subjects and methods

### *Subjects*

The sample stems from a multi-centre follow-up programme (German Research Network on Schizophrenia) (Wölwer et al. 2003) conducted at eleven psychiatric university hospitals (Aachen, Berlin, Bonn, Cologne, Düsseldorf, Essen, Göttingen, Hamburg, Mainz, Munich, Tübingen) and three psychiatric district hospitals (Augsburg, Gabersee, Haar). In this programme, subjects were randomly selected from all patients who were admitted between January 2001 and December 2004 to one of the 14 hospitals mentioned above for treatment of a schizophrenic spectrum disorder (ICD-10: F20, F21, F22, F23, F24, F25, F28). Randomization software was used to select subjects. Subjects were aged between 18 and 65. Exclusion criteria were a major medical illness as possible cause of psychiatric symptoms, head injury and drug or alcohol dependence. All patients had given informed, written consent to participate in the study. The study protocol was approved by the local ethics committees (Jäger et al. 2007).

In total, 474 patients with schizophrenic spectrum disorders were enrolled in the entire multi-centre follow-up programme. Forty-six patients (10%) dropped out for different reasons (e.g. withdrawal of informed consent, retrospective violation of inclusion criteria, incomplete information). A total of 140 patients were excluded from the analysis: 28 (6%) because they were discharged from hospital within 7 days after admission; 77 (16%) because they did not fulfil diagnostic criteria for ICD-10 schizophrenia (F20) but for other psychotic disorders (F21–F28); and 35 (7%) because they already fulfilled remission criteria at the time of admission.

Therefore, the sample available for analysis comprised 288 subjects with ICD-10 schizophrenia of whom 168 (58.3%) were male and 120 (41.7%) female. ICD-10 diagnoses were as follows: paranoid schizophrenia ($n=236$, 82%), hebephrenic schizophrenia ($n=14$, 5%), catatonic schizophrenia ($n=3$, 1%), undifferentiated schizophrenia ($n=10$, 4%), residual schizophrenia ($n=14$, 5%) and other schizophrenia ($n=11$, 4%). 251 subjects (87%) met the criteria for DSM-IV schizophrenia and 37 (13%) those for DSM-IV schizophreniform disorder.

The mean age ($\pm$standard deviation) was 35.4 years ($\pm$11.3) and the mean duration of illness 11.0 years ($\pm$11.2). At admission, the PANSS total score was 75.0 ($\pm$18.2), PANSS positive subscore 20.2 ($\pm$5.9) and PANSS negative subscore 19.0 ($\pm$7.2). The mean duration of inpatient treatment was 57.1 days ($\pm$46.6). Eighty-three patients

(28.8%) were suffering from their first episode of schizophrenia.

Patients were treated under naturalistic conditions: 65% percent of the patients received first-generation antipsychotics, 52% second-generation antipsychotics, 54% tranquilizers and 7% mood stabilizers. Antidepressants were administered to 17% of the sample.

### *Assessments*

Clinical researchers interviewed the patients at admission and diagnosed them according to the ICD-10 research diagnostic criteria (WHO 1993).

Sociodemographic variables (partnership, employment state) and course-related variables such as age at onset, age at first hospitalization, duration of illness, number of previous hospitalizations and episodes of illness or presence of preceding stressors were recorded during interviews with patients, relatives and care providers using a standardized documentation system (Cording 1998). First-episode disorder was defined as the first hospitalization for schizophrenia together with the absence of an earlier episode of schizophrenic disorder. Duration of illness was defined as the time since the first occurrence of any psychopathological symptoms.

Further prognostic factors were assessed with the 21-item version of the Strauss–Carpenter Prognostic Scale (Kokes 1977). In this scale, each item can be graduated on a five-point (0–4) scale, and the total score ranges from 0 to 84. Global functioning (level at admission, highest and lowest level in the year before admission) was recorded using the Global Assessment of Functioning Scale (GAF) (APA 1994). This is an internationally well-known, unidimensional rating scale for the evaluation of the overall functioning of a subject on a continuum from severe psychiatric illness (rated 0) to health (rated 100).

Psychopathological characteristics were assessed with the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) (Kay 1991), and the 17-item version of the Hamilton rating scale for depression (HAM-D) (Hamilton 1960). The PANSS, a widely used 30-item scale for assessing schizophrenic symptoms, is composed of three subscales: positive symptoms (items P1–P7), negative symptoms (items N1–N7) and general psychopathology (items G1–G16). Each item can be graduated on a seven-point scale (1–7) The PANSS total score ranges from 30 to 210, the positive and negative subscores from 7 to 49, and the general psychopathology subscore from 16 to 112.

Ratings were performed within the first 3 days after admission, and at discharge. All raters (research clinicians) had been trained how to use the scales. A high inter-rater reliability was achieved (ANOVA-ICC $>0.8$).

### *Statistical analyses*

Using the symptom-severity component of the recently proposed standardized remission criteria (Andreasen et al. 2005), remission was defined as a PANSS rating of three or less in all of the following items: 'delusions' (P1), 'unusual thought contents' (G9), 'hallucinatory behavior' (P3), 'conceptual disorganization' (P2), 'mannerism/posturing' (G5), 'blunted affect' (N1), 'social withdrawal' (N4) and 'lack of spontaneity' (N6). This definition was applied to the PANSS ratings at admission and discharge. Patients who met the criterion for symptom remission at admission were excluded from the remaining analyses.

In a descriptive analysis, the symptom remission status at discharge was examined, and the frequency of schizophrenic core symptoms (delusions, unusual thought contents, hallucinatory behaviour, conceptual disorganization, mannerism/posturing, blunted affect, social withdrawal, lack of spontaneity) at admission was compared with those at discharge.

The prediction of symptom remission during inpatient treatment was examined with a logistic regression model using the 'split-half technique'. This model is commonly applied when the outcome variable is binary and the independent variables include both numerical and nominal measures. The total sample ($n=288$) was randomized into a definition sample ($n=142$) and a validation sample ($n=146$).

The logistic regression model was developed using the definition sample ($n=142$). In the first step, patients with symptom remission at discharge were compared with those without symptom remission with respect to potential predictor variables (psychopathological symptoms at admission, sociodemographic variables, course-related variables) that were available at admission to hospital. For this purpose, the *t*-test and the $\chi^2$-test were used to perform an exploratory analysis without an adjustment for multiple testing. A $P$ value of $<0.05$ was considered to be statistically significant. In the second step, variables that showed significant differences between both groups in the univariate analyses were included in a multiple logistic stepwise forward regression model in order to identify independent predictor variables for symptom remission. The goodness-of-fit of the model was tested using the Hosmer–Lemeshow test (Hosmer and Lemeshow 2000). The results of the analysis are expressed in terms of Nagelkerke's $R^2$, predictive value, sensitivity,

specificity, regression coefficients (B) and corresponding odds ratios.

In a further step, the model was applied to the validation sample ($n=146$). Predictive value, sensitivity and specificity are presented for the usual cut-off point of 0.5. However, sensitivity and specificity levels depend on the cut-off point of the model. Therefore, the discriminative ability of the regression model was also evaluated using a receiver-operating characteristic (ROC) curve. The area under the curve (AUC) is a measure of the overall discriminative power. A value of 0.5 for the AUC represents no discriminative ability; a value of 1.0 indicates a perfect power. According to Weinstein and Fineberg (1980) a value of 0.7–0.8 is considered as a reasonable, and a value greater than 0.8 as a good discriminative capacity.

All statistical analyses were carried out using the SPSS 12.0 Software for Windows.

## Results

### *Frequency of symptom remission during inpatient treatment*

The frequency of schizophrenic core symptoms (PANSS rating of more than three) at admission and discharge are shown in Figure 1. At admission, the most frequent symptoms were 'delusions' (68%), 'conceptual disorganization' (60%) and 'hallucinatory behaviour' (47%). At discharge, however, the most frequent symptoms were 'blunted affect' (44%), 'social withdrawal' (39%) and 'lack of spontaneity' (25%).

At discharge, 55% of the sample ($n=158$) met symptom-severity criteria for remission. A total of 84% ($n=243$) showed remission with respect to 'reality distortion (psychoticism)' (PANSS rating of 3 or less in the items 'delusions', 'unusual thought contents' and 'hallucinatory behavior'), 85% ($n=245$) with respect to 'disorganization' (PANSS rating of 3 or less in the items 'conceptual disorganization' and 'mannerism/posturing') and only 65% ($n=188$) with respect to 'negative symptoms' (PANSS rating of 3 or less in the items 'blunted affect', 'social withdrawal' and 'lack of spontaneity'). This means that negative symptoms were more stable during inpatient treatment than the dimensions 'reality distortion' and 'disorganization'.

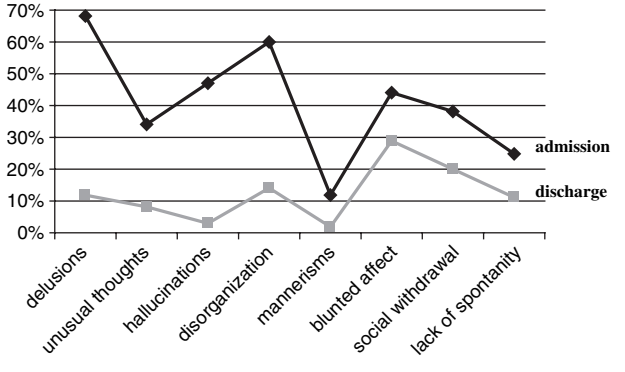


Figure 1. Frequency of core symptoms of schizophrenia (PANSS >3) at admission and discharge.

No statistically significant differences between patients with remission at discharge and those without remission were found in the administration rate of first-generation antipsychotics (66 vs. 65%, $\chi^2=0.46$, $P=0.830$), second-generation antipsychotics (52 vs. 52%, $\chi^2=0.04$, $P=0.951$), tranquilizers (55 vs. 53%, $\chi^2=0.53$, $P=0.819$), mood stabilizers (7 vs. 8%, $\chi^2=0.56$, $P=0.812$) and antidepressants (16 vs. 19%, $\chi^2=0.352$, $P=0.819$). The duration of inpatient treatment also failed to reach statistic significance (52.4 days for patients with remission at discharge vs. 62.8 days for patients without remission, $T=1{,}814$, $P=0.067$).

### *Development of the prediction model*

The prediction model was developed using the definition sample ($n=142$). In the first step, patients with remission during inpatient treatment ($n=79$) were compared to those without ($n=63$). The results of these univariate comparisons are shown in Table I. Significant differences were found with respect to employment status, age at occurrence of first symptoms, age at first hospitalization, duration of illness, duration of previous hospital stays, proportion of first episode disorders, presence of preceding stressors, total score of Strauss–Carpenter Prognostic Scale, the highest and lowest level of global functioning (GAF) in the year before admission, PANSS total score and PANSS negative subscore. The group of patients who achieved remission during inpatient treatment included a higher percentage of first episode disorders, and showed a more favourable social functioning at admission, less pronounced negative symptoms at admission and a better global functioning in the year before admission. These patients were older at onset, and presented more often with preceding stressors. Compared with patients without remission they showed a shorter duration of both illness and previous hospital stays.

When the variables that showed significant differences between patients with and without remission were included in a logistic regression model, the only independent significant predictors were the total score of the Strauss–Carpenter Prognostic Scale, the PANSS negative subscore at admission and the highest level of GAF in the year before admission. This means that higher values in the total score of

Table I. Univariate comparisons between patients with and patients without symptom remission at discharge (definition sample, $n=142$).

| | Patients with remission ($n=79$) | Patients without remission ($n=63$) | $P$ level |
|---|---|---|---|
| Age | 34.3 (±10.5) | 34.3 (±12.11) | $T=-0.010$, $P=0.992$ |
| Gender (female) | 37% | 38% | $\chi^2=1.093$, $P=0.296$ |
| *Social functioning* | | | |
| Stable partnership | 30% | 16% | $\chi^2=3.582$, $P=0.058$ |
| Regular employment | 70% | 36% | $\chi^2=16.636$, $P<0.001$ |
| *Course of illness* | | | |
| Age at first symptoms (years) | 26.2 (±10.9) | 21.7 (±11.0) | $T=2.507$, $P=0.013$ |
| Age at first hospitalization (years) | 28.4 (±10.9 | 23.5 (±10.8) | $T=2.685$, $P=0.008$ |
| Duration of illness (years) | 8.1 (±10.4) | 12.6 (±11.2) | $T=-2.476$, $P=0.014$ |
| Duration of previous hospitalization (weeks) | 12.9 (±27.8) | 37.0 (±63.6) | $T=2.802$, $P=0.006$ |
| First episode disorders | 41% | 19% | $\chi^2=7.547$, $P=0.006$ |
| Acute onset of current symptoms | 54% | 49% | $\chi^2=0.383$, $P=0.536$ |
| Preceding stressors | 40% | 15% | $\chi^2=11.106$, $P=0.001$ |
| Strauss–Carpenter Prognostic Scale (total score) | 53.6 (±11.4) | 41.8 (±9.8) | $T=6.439$, $P<0.001$ |
| *Global Assessment of Functioning (GAF)* | | | |
| Admission | 41.3 (±10.6) | 39.4 (±9.6) | $T=1.143$, $P=0.255$ |
| Highest level in the year before admission | 74.5 (±14.8) | 60.8 (±14.1) | $T=5.586$, $P<0.001$ |
| Lowest level in the year before admission | 41.2 (±15.0) | 35.0 (±9.1) | $T=3.028$, $P=0.003$ |
| *Psychopathological symptoms (admission)* | | | |
| PANSS total score | 71.1 (±17.1) | 77.7 (±17.8) | $T=-2.243$, $P=0.026$ |
| PANSS positive subscore | 20.0 (±5.4) | 19.1 (±6.1) | $T=0.994$, $P=0.322$ |
| PANSS negative subscore | 16.6 (±6.5) | 21.7 (±6.7) | $T=-4.575$, $P<0.001$ |
| PANSS general psychopathology subscore | 24.4 (±10.1) | 36.9 (±10.2) | $T=-1.446$, $P=0.150$ |
| Hamilton Depression Scale (total score) | 13.2 (±8.4) | 13.6 (±9.1) | $T=-0.285$, $P=0.776$ |

Values are presented as means (±standard deviation).

the Strauss–Carpenter Prognostic Scale, higher levels of global functioning in the year before admission and lower levels in the PANSS negative subscore at admission predict symptom remission during inpatient treatment. The model is presented in Table II. The Hosmer–Lemeshow statistic ($\chi^2=7.554$, df = 8, $P=0.478$) indicated an adequate goodness-of-fit, and Nagelkerke's $R^2$ suggested that 36% of the variance could be explained with the model. Using a cut-off-point of 0.5 the overall predictive validity was 73%, the sensitivity (correct identification of patients with remission) 75% and the specificity (correct identification of patients without remission) 69%.

### *Validation of the prediction model*

When this logistic regression model was applied to the validation sample ($n=146$), a predictive validity of 71%, sensitivity of 71% and specificity of 70% were found using a cut-off point of 0.5. Variations of the cut-off point, however, change sensitivity and specificity of the model. These relationships are presented in a receiver-operating characteristic (ROC) (Figure 2). The AUC was 0.77 (SE 0.04, $P<0.001$, 95% CI 0.69–0.85), indicating that the model has a reasonable discriminative power.

## Discussion

### *Rates of symptom remission*

The aim of the present study was to examine the rate of and predictive factors for symptom remission during inpatient treatment. The symptom-severity component of the consensus criteria proposed by the Remission in Schizophrenia Working Group (Andreasen et al. 2005) was applied to a sample of inpatients treated under naturalistic conditions for schizophrenia diagnosed according to ICD-10

Table II. Logistic regression model for the prediction of symptom remission at discharge (definition sample, $n=142$).

| | Coefficient (β) | SE | $P$ level | OR | 95% CI |
|---|---|---|---|---|---|
| Strauss–Carpenter Prognostic Scale (total score) | 0.055 | 0.024 | =0.022 | 1.057 | 1.008–1.108 |
| PANSS negative subscore at admission | −0.068 | 0.032 | =0.035 | 0.934 | 0.876–0.995 |
| Highest level of GAF (year before admission) | 0.035 | 0.017 | =0.042 | 1.036 | 1.001–1.071 |

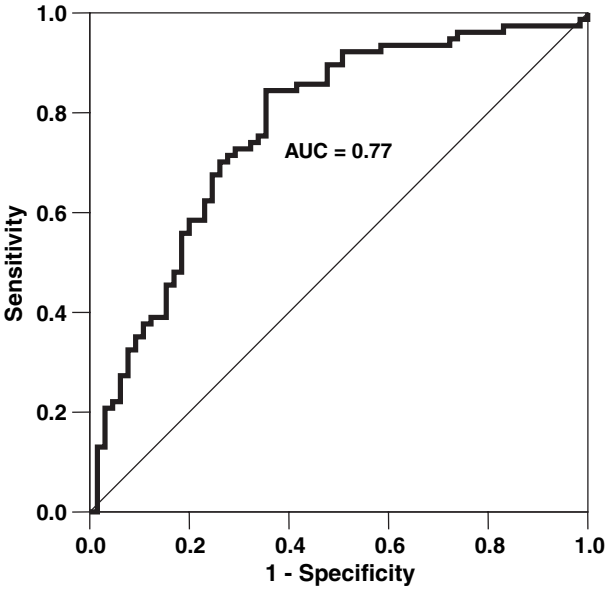


Figure 2. ROC curve for prediction of symptom remission at discharge using logistic regression (validation sample, $n = 146$).

criteria. The analyses revealed that 55% of the sample showed symptom remission at discharge from hospital. This means that 45% of the patients were discharged in a non-remitted state. Lack of remission was mainly because of persisting negative symptoms.

The results are comparable with those of van Os et al. (2006b) and Wunderink et al. (2006), both of whom reported similar rates for cross-sectional symptom remission (46 and 48%, respectively). Lasser et al. (2005), however, found that only 32% of 'clinically stable' patients met the symptom-severity component of the proposed criteria. In this context, one has to mention the differences in the inclusion criteria of these studies (e.g., diagnostic criteria). Taken together, the reported cross-sectional remission rates in patients who are considered to be 'responsive to antipsychotic treatment' (Wunderink et al. 2006) or 'clinically stable' (Lasser et al. 2005) or who were discharged from hospital range from 32 to 55%. This raises the question whether or not the threshold of the proposed criteria is appropriate. Further studies should examine the predictive validity of the proposed remission criteria.

### *Discriminative power of prediction models in schizophrenia*

The logistic regression model showed a predictive validity of about 70%. The AUC of 0.77 indicated a reasonable discriminative capacity. It was possible to confirm the model by cross-validation using the split-half technique. However, only 36% of the variance could be explained with this model. Although differences in many variables were found between patients with and those without remission, the logistic regression model identified only three factors as independent predictors for symptom remission: Strauss–Carpenter Prognostic Scale, PANSS negative subscore at admission and global functioning in the year before admission.

The results are in line with previous studies that found a similar amount of explained variance (Gaebel 1978; Kay and Murill 1990), even if additional variables such as self-rating scales (Möller et al. 1985) or neuropsychological and radiological parameters (Robinson et al. 2004) were included in the prediction models. The crucial question whether a combination of clinical, neuropsychological and biological variables will increase the predictive power of prediction models should be answered by further studies.

### *The impact of the Strauss–Carpenter Prognostic Scale and Global Assessment of Functioning (GAF)*

In keeping with previous studies that reported a high predictive value of the Strauss–Carpenter Prognostic Scale for both the acute treatment response (Möller et al. 1985) and the long-term outcome (Gaebel and Pietzcker 1978; Möller et al. 1986; Lenoir et al. 2005), this scale was identified as a predictor for symptom remission during inpatient treatment.

The Strauss–Carpenter Prognostic Scale comprises several areas of functioning, e.g. employment state, social class, family history of psychiatric hospitalization, age at onset and psychopathological symptoms. However, it has been criticized for making its prediction on the basis of items that reflect chronicity of the illness, so that its results are more a conclusion from the former course of the illness than a real prediction (Bland et al. 1978). Although the validity of the scale was also demonstrated for patients in the early course of schizophrenic illness (Gaebel and Pietzcker 1978; Lenoir et al. 2005), this criticism is supported by empirical findings about the factor structure of the scale. Händel et al. (1996) reported that the items which are strongly associated with the social functioning in the previous 12 months are especially valuable for prognostic aspects.

The present study also identified the level of global functioning in the year before admission as an independent predictor for symptom remission during inpatient treatment. This factor also reflects a pre-existing chronicity of schizophrenic illness.

*The impact of negative symptoms*

The results of the present study are in line with those of several studies which showed that pronounced negative symptoms are predictive of an unfavorable treatment outcome (Wieselgren et al. 1995; Ho et al. 1998; Alptekin et al. 2005). The findings can be explained by the fact that positive symptoms ('reality distortion') such as hallucinations or delusions and symptoms of disorganization were frequent at admission, i.e. in the acute stage of illness, but showed a good remission during inpatient treatment. In contrast, negative symptoms such as blunted affect or social withdrawal were less frequent at admission, but more stable and less responsive to treatment. These findings are not surprising because a number of previous studies have shown similar results (Jonstone et al. 1987; Phillips et al. 1991; Arndt et al. 1995; Szymanski et al. 1996).

Conflicting findings were reported about the predictive validity of positive symptoms and symptoms of conceptual disorganization for the acute treatment response (Mauri et al. 1994) and the long-term outcome (Harrow and Marengo 1986; Kay and Murill 1990). However, the findings of Mauri et al. (1994) that more pronounced positive symptoms in an acute stage of schizophrenic illness are associated with a higher amelioration of psychopathological symptoms do not challenge the results of the present study, but rather confirm the assumption that positive symptoms are often transient and responsive to antipsychotic treatment. On the other hand, the findings of Harrow and Marengo (1986) and Kay and Murill (1990) that positive symptoms are predictive of a more unfavourable long-term outcome can be explained by the fact that these studies assessed positive symptoms not in an acute, but in a more chronic stage of schizophrenic illness.

*Identification of homogeneous subgroups in schizophrenia*

Because the ICD-10 and DSM-IV concepts of schizophrenia include both cases with full remission as well as those with a chronic course (Jäger et al. 2004), it is an important challenge for psychiatric research 'to identify subgroups within this heterogeneous disorder that have a differential course and outcome' (Andreasen 2006). The findings of the present study, which highlight the impact of negative symptoms on the course and treatment response of schizophrenic illness, are in line with the classical concept of Crow (1980) who postulated two distinct subtypes of schizophrenia: Type I, with predominantly positive symptoms, was associated with good premorbid functioning, acute onset, good response to treatment and a better long-term outcome, whereas type II, with mainly negative symptoms, was associated with an insidious onset, poor premorbid functioning, a tendency to drug resistance and a poorer long-term outcome. In fact, several studies have found that negative symptoms are associated with a chronic course of illness (Murphy et al. 1994; Bottlender et al. 2001) and with distinct neurobiological alterations (Ho et al. 2003; Liu et al. 2004).

However, this classical dichotomous concept, which considers positive symptoms as transient and negative symptoms as persistent, was criticized because in some cases positive symptoms persist and negative symptoms are transient (Liddle et al. 1989). This fact, which is also supported by the present analysis, may explain the limited discriminative power of predictor models in schizophrenia. In the mean time, Crow's dichotomous concept has been replaced with a multidimensional model of schizophrenia consisting of a negative symptom dimension, a psychotic symptom dimension ('reality distortion') and a disorganization dimension (Liddle et al. 1989; APA 1994). A number of studies even tried to establish models with a five-factor structure of schizophrenic symptoms, but such models are still being discussed controversially (van der Gaag 2006). Nevertheless, as yet, assessment tools for psychopathological symptoms in patients with schizophrenic disorders do not allow a reliable and valid differentiation between transient and persistent symptoms. Such a differentiation, however, would help to identify more homogenous subgroups.

*Limitations*

In contrast to previous studies that examined the recently proposed consensus criteria for remission in schizophrenia (Lasser et al. 2005; Sethuraman et al. 2005; van Os et al. 2006b; Wunderink et al. 2006), patients with schizoaffective disorders were excluded from the present study because the consensus definition can only be applied to patients who have previously been diagnosed using recognized criteria for schizophrenia (Andreasen et al. 2005). However, one has to mention that 28.8% of the sample were first-episode patients. This means that the sample was heterogeneous and included patients at different stages of schizophrenic illness. Remission is expected to be more frequent after the first than after multiple episodes of schizophrenia. Furthermore, the sample only included inpatients and may therefore not be representative for all patients suffering from schizophrenia.

The patients were treated under naturalistic conditions. Such a naturalistic design does not allow the results to be sufficiently controlled for the effect of different pharmacological and psychological

treatments. However, the aim of the present study was to examine an unselected and representative sample of patients routinely treated in a psychiatric hospital. No differences were found between patients with and those without symptom remission with respect to the pharmacological treatment during the inpatient stay (administration rate of antipsychotics, tranquilizers, mood stabilizers and antidepressants).

Furthermore, one should note that the outcome of schizophrenia is multidimensional and that the applied consensus criteria for symptom remission do not address other outcome domains such as cognition, psychosocial functioning or quality of life (Remington and Kapur 2005).

The most serious limitation, however, may be the fact that the present study, like others (Sethuraman et al. 2005), only used the symptom-severity component of the proposed remission criteria without considering the time criterion, which requires an absence of relevant symptoms for at least 6 months (Andreasen et al. 2005). The aim of the present study, however, was to examine the acute treatment response during inpatient treatment in terms of a 'cross-sectional remission'.

*Conclusions*

In accordance with previous findings, the results highlight the impact of negative symptoms on the course and treatment response of schizophrenic illness. However, the results also underline the limited power of predictor models for schizophrenic disorders, not only with respect to the long-term outcome but also with respect to the acute treatment response. Further analyses should examine whether the inclusion of neurobiological and cognitive variables can improve the validity of such predictor models.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# History of early abuse as a predictor of treatment response in patients with fibromyalgia: A post-hoc analysis of a 12-week, randomized, double-blind, placebo-controlled trial of paroxetine controlled release

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**Abstract**

*Objectives.* We conducted a post-hoc analysis to determine whether a history of physical or sexual abuse was associated with response to treatment in a double-blind, randomized, placebo-controlled trial of paroxetine controlled release (CR) in fibromyalgia. *Methods.* A randomized, double-blind, placebo-controlled trial of paroxetine controlled release (CR) (dose 12.5–62.5 mg/day) was conducted in patients with fibromyalgia for 12 weeks. A total of 112 subjects provided complete information on childhood history of abuse that was recorded using the Sexual and Physical Abuse Questionnaire and randomized to treatments. Outcome evaluations in the abuse subgroup were identical to those in the entire sample. Health Status was determined using the 36-Item Short Form Health Survey (SF-36), the Sheehan Disability Scale (SDS), and the Perceived Stress Scale (PSS). Fibromylagia symptom severity was determined using the Fibromyalgia Impact Questionnaire (FIQ) and the Visual Analogue Scale for Pain (VAS). The primary outcome was treatment response defined as $\geq$25% reduction in the FIQ-total score. Secondary outcomes include changes in scores on the Clinical Global Impression-Severity and Improvement (CGI-S and CGI-I respectively) and SF-36. *Results.* The rate of childhood physical and/or sexual abuse was 52.7% ($n=59$). The baseline characteristics (health status, perceived stress, symptom severity) were not associated with abuse history. In logistic regression, the history of abuse did not predict treatment response as measured by $\geq$25% reduction in FIQ-total score (OR = 1.16, 95% CI = 1.18–1.60, $P=0.35$), while the drug status (paroxetine CR) was significantly associated with treatment response (OR = 2.51, 95% CI = 1.12–5.64, $P=0.02$). Abuse history did not predict CGI-I ($P=0.32$) or CGI-S ($P=0.74$) improvements during treatment. After 12 weeks of treatment, subjects with sexual abuse history showed significantly lower mean change in health status (SF-36) than those without sexual abuse history ($P=0.04$). *Conclusions*. Although, a significant proportion of patients with fibromyalgia reported a history of abuse, it does not appear to have any significant clinical correlates at baseline. History of abuse did not predict response to treatment in patients with fibromyalgia participating in a controlled trial of paroxetine controlled release. Prospective, well-designed studies are needed to confirm whether selective serotonin uptake inhibitors are effective in patients with fibromyalgia irrespective of their abuse history.

**Key words:** *Fibromyalgia, abuse, treatment, paroxetine CR, SSRI*

## Introduction

A number of studies have reported high rates of childhood trauma in fibromyalgia patients, ranging from 32 to 64% (Goldberg et al. 1999; Imbierowicz and Egle 2003). Although not uniformly consistent, the evidence suggests that the prevalence of childhood trauma is higher in fibromyalgia compared to other chronic pain disorders (Alexander et al. 1998; Van Houdenhove et al. 2001; Imbierowicz and Egle 2003). Elevated rates of both lifetime sexual and physical abuse (Boisset-Pioro et al. 1995; Walker et al. 1997; Imbierowicz and Egle 2003), and lifetime emotional abuse and neglect have been reported in these patients (Walker et al. 1997; Van Houdenhove et al. 2001). Further evidence supporting the link between abuse and fibromyalgia comes

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from a community study that found women reporting rape were 3 times more likely to have fibromylagia compared to women who did not report rape. However, there was no evidence of increased childhood abuse in the fibromyalgia group (Ciccone et al. 2005). History of abuse seems to be associated with symptom severity (Walker et al. 1997; McBeth et al. 1999). Evidence suggests that patients reporting abuse experience greater psychological distress, have more severe physical symptoms, greater functional disability, poorer psychological adjustment (Taylor et al. 1995; Walen et al. 2001) and utilize more health care services (Alexander et al. 1998). In addition several physiological and psychological mechanisms might explain how early abuse could influence the onset, clinical expression, and perpetuation of fibromyalgia symptoms. Finally a recent study suggested that childhood sexual or physical abuse history may cause neuro-endocrine alteration in adulthood among fibromyalgia patients, which should be associated with differential effects of pharmacological treatment in patients with fibromyalgia (Weissbecker et al. 2006). Hence trauma history should be evaluated as early as possible in clinical practice and this factor may be potentially involved in components of response to treatment and clinical course in patients with fibromyalgia (Weissbecker et al. 2006).

Meanwhile, antidepressant medications are widely used by clinicians to treatment symptoms of fibromyalgia, despite lack of Food and Drug Administration (FDA) approved indication. Although not uniformly consistent, data from randomized controlled trials indicate that tricyclic antidepressants (amitriptyline), selective serotonin uptake inhibitors (fluoxetine and paroxetine controlled release), and dual norepinephrine and serotonin uptake inhibitors (duloxetine) may be beneficial in the treatment of fibromyalgia (Arnold et al. 2000, 2002; O'Malley et al. 2000; Patkar et al. 2007).

There have been few systematic studies examining clinical predictors of response to antidepressants in patients with fibromyalgia despite suggestions that identification of subgroups based on specific clinical characteristics may be useful for maximizing treatment efficacy (Turk et al. 1998; King et al. 2002). In this context there has been limited information on whether fibromyalgia patients with a history of abuse will do as well as those with no such history following antidepressant treatment. This issue is clinically important because of the possible need for additional or alternative interventions for fibromyalgia patients which comorbid trauma.

We had previously investigated the efficacy and safety of paroxetine controlled release (CR) in a 12-week, RCT of paroxetine controlled release in 124 subjects with fibromyalgia (Patkar et al. 2007). This post-hoc analyses was to determine whether a history of physical and sexual abuse was associated with the response to treatment in a 12-week, RCT of paroxetine controlled release (CR) in fibromyalgia. We hypothesized that the fibromyalgia patients with childhood abuse history is related with poorer response to the pharmacological treatment since the aforementioned findings (Taylor et al. 1995; Walker et al. 1997; Turk et al. 1998; McBeth et al. 1999; Walen et al. 2001; King et al. 2002; Weissbecker et al. 2006) and several preliminary studies proposed that differential antidepressant treatment effects may exist by presence/absence of early abuse history (Nemeroff et al. 2003).

## Methods

### *Design*

This was a randomized, double-blind, placebo-controlled trial of paroxetine controlled release (CR) (dose 12.5–62.5 mg/day) in fibromyalgia. Detailed methodology is available elsewhere for which we describe briefly the methods (Patkar et al. 2007).

### *Subjects and medication*

Subject selection was independent of an early abuse history. Eligible subjects included men and women, 18–65 years of age, who fulfilled American College of Rheumatology (ACR) diagnostic criteria for fibromyalgia (Wolfe et al. 1990). Those with history of paroxetine CR treatment for any disease were excluded. Other detailed description refers to our original paper (Patkar et al. 2007).

### *Assessment*

A history of childhood physical and sexual abuse was recorded using the Sexual and Physical Abuse Questionnaire developed by Leserman and colleagues (Leserman et al. 1995; Kooiman et al. 2002). The questionnaire captures responses on sexual abuse, physical abuse and both sexual and physical abuse during childhood ($\leq$13 years) and adulthood. Assessments also included the Fibromyalgia Impact Questionnaire (FIQ) (Burckhardt et al. 1991), the 36-Item Short Form Health Survey (SF-36) (Stansfeld et al. 1997), Clinical Global Impression-Severity and Improvement (CGI-S and CGI-I, respectively), the Sheehan Disability Scale (SDS) (Sheehan 1983), the Visual Analogue Scale for pain (VAS-P), the Beck Depression Inventory (BD)I (Beck et al. 1961), Beck Anxiety Inventory (BAI) (Beck et al. 1988), and Perceived Stress Score (PSS) (Cohen

et al. 1983). All assessments were measured at the baseline and at each week of the study, with the exception of PSS (at baseline only). Treatment response was defined as $\geq$25% reduction in FIQ-total score from randomization to the end of treatment.

### *Data analysis*

Chi-square analysis was used for all categorical associations and analysis of variance (ANOVA) or *t*-tests and paired *t*-tests were used to examine differences for all continuous variables. The results of the primary efficacy analyses have been published elsewhere (Patkar et al 2007). In multivariate logistic regression, we determined if a history of early abuse was an independent predictor of response to treatment on primary and secondary endpoints. All statistical significance was two-tailed and set at $P<0.05$. Statistical analysis was done using the SPSS 10.0 for Windows program (SPSS Inc., Chicago, USA).

## Results

### *Rate of abuse*

One hundred and twelve subjects ($n=112/124$, 90.3%) participating in the trial responded to the abuse questionnaire. One hundred and six (94.6%) of the subjects were women. Childhood physical abuse was reported by 13 subjects (11.6%), sexual abuse by 31 subjects (27.7%) and 15 participants (13.4%) indicated having experienced both physical and sexual abuse. 53 (47.3%) reported experiencing no physical and sexual abuse.

For the purpose of analysis, we divided the subjects into two groups: Those who had a history of physical and/or sexual abuse ($n=59$, 52.7%) and those with no abuse history ($n=53$, 47.3%). Comparing the baseline characteristics, we found that a greater proportion of Caucasians reported abuse history compared to African-Americans ($P=0.006$). There were no significant differences between the two groups in FIQ-total (FIQ), health (SF-36), disability (SDS), anxiety (BAI), depressive symptoms (BDI), perceived stress and pain (VAS-P) scores at baseline. Table I summarizes the baseline comparisons between the two groups.

### *Abuse history as a predictor of response*

*Primary outcome.* There was no significant difference in the number of responders defined as $\geq$25% reduction in FIQ-total score over the 12-week trial between subjects with ($n=22$, 37.2%) or without history of abuse ($n=26$, 49.1%) (Fisher's exact test, $P=0.49$). We also examined the proportion of responders in the drug and the placebo groups separately and found no significant differences in the proportion of responders with or without history of abuse in the paroxetine CR (abuse history $n=16$, 53.3%; no abuse history $n=14$, 46.7%, Fisher's exact test, $P=0.48$) or in the placebo groups (abuse history $n=7$, 38.9%; no abuse history $n=11$, 61.1%, Fisher's exact test, $P=0.16$). Figure 1 summarizes the distribution of abuse history among responders and non-responders in the paroxetine CR group. On the primary outcome, multivariate logistic regression showed that history of abuse did not predict a treatment response (OR = 1.16, 95% CI = 1.18–1.60, $P=0.35$), while the drug status (paroxetine CR) was significantly associated with treatment response (OR = 2.51, CI = 1.12–5.64, $P=0.02$). Results of the completer analyses ($n=82$) were consistent with ITT with LOCF analyses.

### *Secondary outcomes*

Similarly the responder rate did not differ between subjects with ($n=19$, 54.3%) or without history of abuse ($n=16$, 45.7%; overall Fisher's exact test, $P=0.468$) when response was defined as a CGI-I score of 1 or 2 at the end of treatment. In a multivariate logistic regression analysis, a history of abuse did not predict a treatment response based on CGI-S score (OR = 1.282, 95% CI = 0.288–5.731, $P=0.74$), or CGI-I scores (OR = 1.526, 95% CI = 0.663–3.516, $P=0.32$). On other secondary outcomes, after 12 weeks of treatment, subjects with abuse history showed significantly lower mean change in health status (SF-36) than those without abuse history, although it was marginal significance ($P=0.04$) (Table II). Other outcome measures showed no difference between randomization and end of treatment. Table II summarizes changes in primary and secondary outcomes between subjects with or without abuse history.

We also analyzed the data separately for subjects with history of sexual abuse ($n=46$) and those with history of physical abuse without any sexual abuse ($n=13$) to determine if there was a differential effect of type of abuse on treatment response. The direction and strength of the results were consistent with the original analysis combining the physical and sexual abuse groups. Logistic regression showed that neither a history of sexual (OR = 0.803, 95% CI = 0.343–1.878, $P=0.61$) nor physical abuse (OR = 2.261, 95% CI = 0.892–5.734, $P=0.08$) did not predict response based on the primary outcome measure of $\geq$25% reduction in FIQ-total score.

There were no significant differences between our previous findings (Patkar et al 2007) and results

Table I. Baseline characteristics of fibromylagia subjects with or without a history of abuse.

| | Without abuse history ($N=53$) | With abuse history ($N=59$) |
|---|---|---|
| Demographic variables | | |
| Male | 5 (9.4%) | 1 (1.7%) |
| Female | 48 (90.6%) | 58 (98.3%) |
| Age | 47.0 (10.9) | 48.6 (10.0) |
| Race* | | |
| Caucasian | 44 (83.0) | 38 (63.3) |
| African-American | 9 (17.0) | 21 (36.7) |
| Employment | | |
| Full-time | 26 (50.0) | 29 (48.3) |
| Part-time | 11 (21.2) | 7 (11.7) |
| Unemployed | 15 (28.8) | 23 (40.0) |
| Marital Status | | |
| Married | 31 (59.6) | 35 (59.3) |
| Single | 21 (40.4) | 24 (40.7) |
| Education (years) | | |
| $\leq 12$ | 20 (39.2) | 23 (41.8) |
| $>12$ | 31 (60.8) | 36 (58.2) |
| Duration of Illness (mean in years) | 3.36 (0.9) | 3.13 (1.1) |
| Clinical variables | | |
| FIQ total score | 51.3 (8.7) | 40.5 (8.9) |
| Health (SF-36) | 91.4 (13.8) | 88.2 (14.4) |
| Disability (SDS) | 14.15 (7.5) | 16.67 (7.2) |
| BAI score | 9.72 (7.9) | 11.71 (10.2) |
| BDI score | 9.11 (5.8) | 9.5 (7.1) |
| Perceived Stress Score | 14.84 (6.0) | 16.40 (9.7) |
| Pain (VAS-P) | 7.25 (1.6) | 7.5 (1.9) |
| Depression history** | | |
| No | 31 (60) | 24 (41) |
| Yes | 21 (40) | 34 (59) |

Data represent number (%) or mean (standard deviation). *$P=0.006$, **Fisher's exact $=0.043$ otherwise not significant between subjects with or without abuse history. FIQ, Fibromyalgia Impact Questionnaire; SF-36, 36-Item Short Form Health Survey; SDS, Sheehan Disability Scale; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; VAS-P, Visual Analogue Scale-Pain.

from this analysis. The mean dose of paroxetine CR in the abuse group (39.7 mg/day) was comparable to the entire sample (39.1 mg/day). The dropout rate was 26.8% ($n=30$) in the study.

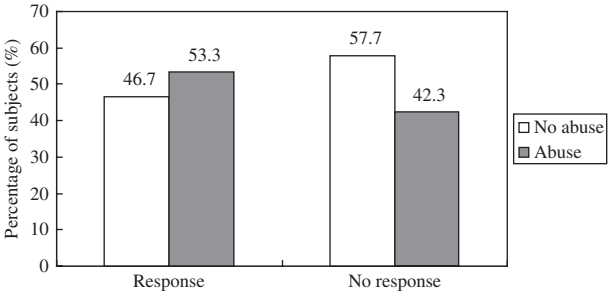


Figure 1. The distribution of abuse history among responders and non-responders in the paroxetine CR group ($n=56$). Treatment response was defined as $\geq 25\%$ reduction in Fibromyalgia Impact Questionnaire (FIQ)-total score from randomization to the end of treatment. Fisher's Exact test, $P=0.480$.

## Discussion

To the best of our knowledge this is the first study examining the relationship between abuse history and response to treatment in fibromyalgia. The post hoc analysis was conducted after the initial randomized controlled trial showed a beneficial effect of paroxetine CR in fibromyalgia. The results failed to demonstrate a significant effect for abuse history to predict treatment response based on the primary and most of the secondary outcome measures. Subjects with or without abuse history were equally likely to respond to paroxetine CR. There could be several explanations for the findings. First, early abuse may not contribute to any specific symptoms profiles in adult fibromyalgia patients that are associated with response to paroxetine CR. Second, our sample was not a chronic, severely ill population. Most of our patients were ill for less than 5 years and over two-thirds were employed. It is possible that effects of abuse may be relevant in a sicker population, as it is

Table II. Mean changes in clinical variables from baseline to the end of treatment (week 12) in fibromyalgia subjects with and without a history of abuse

| | Without abuse history ($N=53$) | With abuse history ($N=59$) |
|---|---|---|
| FIQ total | −6.8 (4.2)[a] | −6.1 (4.7)[a] |
| Health (SF-36) | −10.85 (16.6)[a] | −7.73 (14.39)[a] |
| Disability (SDS) | −9.92 (8.39)[a] | −5.19 (8.15)[a] |
| BDI score | −2.13 (5.66)[a] | −2.95 (5.83)[a] |
| BAI | −1.64 (6.67) | −3.83 (8.51)[a] |
| Pain (VAS-P) | −3.11 (3.22)[a] | −14.93 (16.19)[a] |
| CGI-Severity* | −0.02 | −0.15 |
| CGI-Improvement* | −2.90[b] | −2.81[b] |

Data represent mean (standard deviation). Paired *t*-test was performed to determine mean changes. *Wilcoxon ranked test performed. FIQ, Fibromyalgia Impact Questionnaire; SF-36, 36-Item Short Form Health Survey; SDS, Sheehan Disability Scale; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; VAS-P, Visual Analogue Scale-Pain. [a]All significant changes at $P<0.03$. [b]$Z=-5.489$, $P=0.000$.

often seen with patients with chronic pelvic pain and PTSD (Meltzer-Brody et al. 2007). Third paroxetine has been shown to effect on a wide range of psychiatric disorders whose symptoms overlap with fibromyalgia. These include PTSD (Marshall et al. 2001), generalized anxiety disorder (Pollack et al. 2001), panic disorder (Ballenger et al. 1998) and major depression (Golden et al. 2002). Early abuse may be broadly associated with several of these symptom domains that are common in most patients with fibromyalgia lending categorization of subgroups based on a single variable (abuse) inappropriate. Fourth fibromyalgia is a complex disease and several factors – neuroendocrine, behavioural, psychological, physiological, and neuroanatomic – have been implicated (Goldenberg et al. 2004). It is likely in a multifactorial disease, several variables – apart from trauma – may influence treatment response and a very large sample size may be required to determine these effects. It is also interesting to note that most fibromyalgia studies have found that predictors only explained a small proportion of variance in treatment success ranging between 13 and 15% (King et al. 2002; Ciccone et al. 2005). Finally, the trial was 12 weeks in duration and it is possible that the predictive influence of trauma becomes more apparent in a longer trial.

There are other findings that deserve comments. Consistent with several other studies (Walker et al. 1997; Goldberg et al. 1999; Imbierowicz and Egle 2003). We found approximately 30% of subjects had history of childhood sexual abuse and 50% of subjects reported both sexual and physical abuse during childhood. This lends further support to the evidence that childhood trauma is common among fibromyalgia sufferers. We did not find any association of abuse with severity of symptoms or poor health, unlike some other studies (Taylor et al. 1995; Walen et al. 2001). One possible explanation could be that the most harmful abuse appears to be that involving penetration and multiple incidents (Leserman 2005); our study had few subjects with such severe childhood trauma. Alternatively abuse may have a stronger effect on health care utilization and disability (Alexander et al. 1998) and these relationships may be more evident in a naturalistic study in clinical settings. Interestingly recent studies have shown that the prevalence rate of fibromyalgia in African-American women may be slightly higher than Caucasian Women (Gansky and Plesh 2007). There has been little research on African-American fibromyalgia patients and this group deserves more attention. Finally, the history of depression and/or anxiety did not predict treatment response as measured by $\geq 25\%$ reduction in Fibromyalgia Impact Questionnaire (FIQ) score.

There are some relatively straightforward clinical implications of the findings. Obtaining a history of sexual and physical abuse should be a part of the evaluation of fibromyalgia. Unfortunately, trauma history often remains hidden from health care practitioners and there are excellent reviews on how to obtain such a history in a sensitive manner in medical settings (Leserman 2005). Given our previous findings (Patkar et al. 2007) and the accumulating evidence (Arnold et al 2002), it appears that SSRIs and SNRIs may have a beneficial role in fibromyalgia, although they do not have FDA approval for the treatment of this condition. In this context it is worth noting that the FDA recently approved pregabalin, an alpha-2 delta ligand that acts on voltage gated calcium channels, as the first drug to treat fibromyalgia. Until more evidence is available; it seems prudent to suggest that a history of abuse should not be an important factor in a physician's judgment about whether antidepressants would potentially benefit an individual patient with fibromyalgia uncomplicated by co-morbid depression or anxiety disorders. However, our results should not be interpreted to conclude that behavioural interventions have no role in patients with fibromyalgia with history of abuse. For those with history of abuse, a referral to a psychiatric provider may be necessary both to screen for comorbid disorders such as PTSD and to decide about appropriate behavioural interventions that have been shown to be effective in PTSD (Harvey et al. 2003). In fact, we should acknowledge that biological treatment is only one of options for successful management of fibromyalgia. More scientifically designed integrated treatment plan such as a combination of pharmacological treatment with proper patient education, aerobic exercise, and/or cognitive-behavioural therapy may lead to the most best effective

treatment outcomes in patients with fibromyalgia (Goldenberg 2008).

The data should be interpreted in the context of certain limitations inherent in the design. Because this study was based on self-report of retrospective events, and background variables such as socioeconomic status were not controlled, the relationship between childhood abuse and adult symptoms of fibromyalgia cannot be assumed to be causal. Second subjects with current major depression or anxiety disorders were excluded from the trial; it is possible that these subjects may have had a higher preponderance of abuse and therefore biased the results. Third, the relatively small number of subjects in the abuse categories and the modest sample size of the overall trial could have underpowered the trial. Finally, the trial was relatively brief in duration (12 weeks) and did not have any behavioural interventions specific for abuse. Therefore it was beyond the scope of the study to address whether behavioural interventions could have had an added benefit.

In conclusion, the findings suggest that a significant proportion of patients with fibromyalgia have a childhood history of abuse. However, childhood abuse failed to predict response to treatment with paroxetine CR or placebo. Research to determine what mechanisms might account for the association of abuse with health status in fibromyalgia and what treatments may benefit fibromyalgia patients with abuse history may help to clarify the role of abuse in fibromyalgia.

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## Statement of interest

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Dr Marks is a consultant for Bristol Myers Squibb, and is on the speaker's bureau of Eli Lilly and Company and Pfizer. He has received research support from Merck and Johnson & Johnson.

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Dr Patkar is a Consultant for Bristol-Myers Squibb, GlaxoSmithKline, and Reckitt Benckiser; is on the speaker's bureau and received honoraria from Bristol-Myers Squibb, Cephalon, Pfizer, and Reckitt Benckiser; has received grant support from National Institutes of Health, AstraZeneca, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Janssen, Jazz Pharmaceuticals Lundbeck, McNeil Consumer & Specialty Inc, Organon, and Pfizer.

Drs Peindl and Mannelli have no financial interest.

Mr Krulewicz is an employee of GlaxoSmithKline.

Dr Masand is a consultant for Bristol-Myers Squibb Company, Eli Lilly and Company, I3CME, Janssen Pharmaceutica, Organon, Pfizer; is on the speaker's bureaus of Bristol-Myers Squibb Company, GlaxoSmithKline, Janssen Pharmaceutica, Pfizer Inc., Eli Lilly and Company.

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**ORIGINAL INVESTIGATION**

# Cognitive impairment of executive function as a core symptom of schizophrenia

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**Abstract**

Cognitive dysfunction is a common finding in schizophrenia. Nevertheless the specific pattern of neuropsychological impairment in schizophrenia compared to other severe mental illnesses has not been intensively studied. Twenty-four patients with schizophrenia belonging to different stages of the disease (11 first-episode patients, 13 patients with multiple episodes), 18 patients with bipolar disorder and 23 healthy control subjects underwent standardized neuropsychological assessment. Statistical analysis of covariance (ANCOVA) demonstrated that, compared to control subjects, patients with schizophrenia performed significantly worse in the trail-making test ($P=0.012$), verbal fluency (category letter, $P=0.004$), verbal learning/memory ($P=0.005$), and the Wisconsin Card Sorting Test (WCST) ($P=0.004$ for administered trials; $P=0.025$ for perseverative responses, $T$ value) indicating significant deficits in attention and psychomotor performance, and in particular in verbal working memory and cognitive flexibility for schizophrenic patients. A significant difference between schizophrenic and bipolar patients was found only in the WCST. Schizophrenic patients made significantly more perseverative responses ($P=0.002$, ANCOVA), indicating a more pronounced and specific deficit in cognitive flexibility and frontally based executive function. In conclusion, these results may suggest a cognitive endophenotype in schizophrenia and underline the role of the prefrontal cortex in schizophrenic pathophysiology.

**Key words:** *Schizophrenia, bipolar disorder, cognitive dysfunction, endophenotype, first-episode psychosis*

## Introduction

Schizophrenia is a complex illness with an estimated lifetime morbidity risk of 1% (Goldner et al. 2002). People suffering from schizophrenia experience abnormalities in many different kinds of mental activities, suggesting that more than one distinct brain region may be involved in pathophysiology. Cognitive dysfunction in schizophrenia has been documented in numerous studies in particular (e.g., Heinrich and Zakzanis 1998). Some studies have found rather widespread cognitive dysfunction (e.g., Bozikas et al. 2006), whereas others have provided evidence for more selective cognitive dysfunction (Saykin et al. 1991; Saykin et al. 1994). Cognitive domains especially of interest in schizophrenia are attention, memory, and executive functions. These domains are subserved by neural networks linking frontal and temporal-limbic regions of the brain (Aggleton and Brown 1999; Hopfinger et al. 2000; Gaffan, 2005), thus, the pattern of cognitive dysfunction specifically points to fronto-temporal networks being primarily affected (Hoff and Kremen 2002). In agreement with patterns of neuropsychological dysfunction, changes of brain morphology were observed particularly in these areas by volumetric magnetic resonance imaging (MRI) (e.g., Shenton et al. 2001; Wright et al. 2000). Brain energy metabolism has also been shown to be differently effected in gray and white matter of the fronto-temporal-striatal region in schizophrenic patients (Jensen et al. 2006).

Impairments in neuropsychological function have been demonstrated in patients with schizophrenia already at first presentation and even in their unaffected relatives (e.g., Heinrichs and Zakzanis 1998). Cognitive impairment is also found in patients with bipolar disorder (e.g., Quraishi and Frangou 2002). A study comparing schizophrenia

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patients with bipolar patients and controls found an impairment on seven out of eight cognitive domains (verbal, visual-spatial, abstraction-executive, verbal declarative memory, executive-motor, perceptual-motor, mental control, and sustained attention/vigilance) in schizophrenic patients, whereas bipolar patients were impaired on measures of verbal declarative memory. The pattern of deficits was similar for bipolar and schizophrenia patients, but schizophrenia patients were more significantly impaired than bipolar patients on abstraction, perceptual-motor, executive-motor, and vigilance measures (Seidman et al. 2002). A recent study compared neuropsychological disturbances in schizophrenia with bipolar patients, unaffected relatives of both diagnostic groups, and control participants (McIntosh et al. 2005). Current, verbal, and premorbid IQ were reduced in patients with schizophrenia and their close relatives, memory was impaired in all patient and relative groups, and psychomotor performance and performance IQ were impaired in both patient groups. In conclusion, there was no specific impairment in bipolar disorder, but a pattern of cognitive dysfunction related to genetic liability for schizophrenia.

Given phenomenological similarities between schizophrenia and frontal dysfunction syndromes, executive functions have been suggested to be especially affected in schizophrenics. This assumption has been corroborated by behavioral (e.g., Chan et al. 2006), functional imaging (e.g., Holmes et al. 2005) and psychophysiological (e.g., Weisbrod et al. 2000) research. Executive functions encompass a number of abilities, including but not limited to the ability to initiate, plan, and sequence behaviors, the ability to abstract a principle or problem-solving strategy, and the ability to be cognitively flexible (i.e. switch cognitive sets). Although executive functions are largely subserved by the frontal cortex, they are also related to other parts of the brain that have strong connections to the frontal cortex such as the temporal-limbic complex (Gruber and Goschke 2004). Schizophrenia patients had been noted to have a loss of abstract attitude and to exhibit concrete thinking deficits as early as the 1950s. A recent meta-analysis of 71 studies demonstrated an overall effect size of $-1.45$ for schizophrenia patients relative to controls on measures of executive functioning. This is a substantial effect, suggesting that patients with schizophrenia are significantly impaired on these measures as compared with other psychiatric patients (effect size $-0.40$).

With regard to the cited literature there is some aspect of specificity in the degree and type of cognitive dysfunction of schizophrenia patients. To explore the specificity issue further, we administered a battery of neuropsychological tests to patients with schizophrenia, bipolar disorder, and healthy controls. Tests were organized into cognitive domains, assessing a wide spectrum of cognitive functions, including those reported in the literature to be sensitive to schizophrenia. That is, the potential functional consequences of the described disturbed communication between frontotemporal and frontothalamic structures – deficits in verbal fluency, attention, memory, and executive functioning in particular – were assessed. These deficits may be more related to structural and functional neuroimaging findings than psychopathological symptoms, which may result in part from these cognitive disturbances.

## Method

### *Patients and control subjects*

The present analysis includes the data of 65 persons recruited from the Department of Psychiatry and Psychotherapy, Saarland University, between 2003 and 2005. Diagnostically, the sample consisted of 24 patients with schizophrenia (ICD-10: F20, 11 patients with first-episode schizophrenia, 13 schizophrenic patients with multiple episodes), 18 euthymic patients with bipolar disorder (ICD-10: F31) and 23 healthy control subjects. All schizophrenic and bipolar patients were treated with psychotropic drugs. Subjects suffering from dementia, neurological illnesses, severe brain injuries, or brain tumours at the time of examination were excluded from the sample. Demographic statistics by diagnostic group are given in Table I.

This study, which is in accordance with the Declaration of Helsinki, was approved by the local ethic committee of the Saarland. After a complete description of the study, written informed consent was obtained from each participating subject.

### *Diagnostic procedures*

The following standardized examinations were performed on each subject: a detailed biographic interview and a consensus diagnosis based on SCID I and II interviews (Wittchen et al. 1991) of two independent psychiatrists. The status of healthy controls was confirmed using SCID I and II.

### *Neuropsychological assessment*

For neuropsychological assessment, we used tests in paper-pencil form and computerized versions (e.g., the Wiener Test System, WTS, and the CANTAB, Cambridge Neuropsychological Test Automated Battery). Premorbid intellectual function was

Table I. Sociodemographic characteristics in diagnostic groups.

| | SZ-FE ($N=11$) | SZ-Chron ($N=13$) | Bipolar ($N=18$) | Controls ($N=23$) | $F$ | $P$ |
|---|---|---|---|---|---|---|
| Age in years, mean (SD) | 29.8 (7.4) | 34.8 (10.4) | 42.1 (12.2) | 30.13 (8.7) | 7.91[a] | 0.001 |
| Gender; male/female ($N$) | 8/3 | 10/3 | 7/11 | 10/13 | 5.90[b] | 0.052 |
| Education in years, $<9/10–12/13/>13$ ($N$) | 0/6/5/0 | 2/7/4/0 | 2/10/3/3 | 1/7/11/4 | 9.45 [b] | 0.15 |

*N*, number of subjects; SZ, schizophrenia; FE, first-episode; Chron, chronic disease; Bipolar, patients with bipolar disorder; *P*, probability; SD, standard deviation; *F*, F statistic.
[a]One-way analysis of variance.
[b]$\chi^2$-test.

estimated by a test of verbal intelligence ('Wortschatztest', WST, Schmidt and Metzler 1992). For detecting deficits in attention, speed processing, and psychomotor performance, reaction times were measured using simple and associative perception tasks ('Reaktionstests', RT1 – single stimulus and RT3 – compound stimulus, subtests of the Wiener Test System, WTS, Schufried/Potsdam), and a divided attention task ('Geteilte Aufmerksamkeit', DA, a subtest of the test battery 'Aufmerksamkeitsprüfung', TAP, Zimmermann, Fimm/PSYTEST). For assessment of psychomotor performance and speed, we used the 'Zahlenverbindungstest' (ZVT; Oswald and Roth 1987), a test similar to the trail-making test (TMT, part A). Attention and working memory were appraised with a digit span task ('Zahlennachsprechen', a subtest of the HAWIE, Tewes 1991) and the working memory test 'Arbeitsgedächtnis' (subtest of the TAP), and visuospatial working memory was measured with the Corsi Block-Tapping Test (of WTS battery) and the test of Spatial Recognition Memory (CANTAB). Additionally, a verbal learning and memory test similar to the Auditory or California Verbal Learning Test ('Verbaler Lern- und Merkfähigkeitstest' VLMT; Helmstaedter et al. 2001) was administered, measuring the course of encoding capacity together with both direct and delayed recall and episodic recognition abilities. Nonverbal learning capacity was examined by the 'Nonverbaler Lerntest' (NVLT, subtest of WTS) (Sturm and Willmes 1999). Verbal fluency (VF) was measured by the 'Regensburger Wortflüssigkeits-Test' (RWT; Aschenbrenner et al. 2000). For the detection of deficits in concept formation, cognitive flexibility, and executive functions, the Wisconsin Card Sorting Test (WCST; Heaton et al. 1993), and the Tower of London test (TOL; Shallice 1982) were used. Additionally, a test tapping the controlled integration of information from different sensory channels, that is, intermodal comparison (IC, 'Intermodaler Vergleich', subtest of the Attention Test Battery, TAP) was conducted. Interference was tested by the 'Stroop' task (subtest of the WTS), including the word (Stroop-LI) and color (Stroop-BI) interference conditions.

### *Statistics*

In order to reduce the large number of variables available, from each test a small number of variables was selected for further inspection, based on the literature, theoretical considerations and exploratory discriminant function analysis. This resulted in the selection of 20 variables potentially discriminating between groups. Statistical analyses were then performed with SPSS 10.0 for Windows (Norusis 2002). Domains of neuropsychological function (e.g., executive function and psychomotor performance) were compared between groups using analyses of covariance (ANCOVAs). Thus, analyses were controlled for age, gender, and education, including these potentially intervening variables as covariates. If the assumption of normal distribution was violated as indexed by Kolmogorov–Smirnov testing, non-parametric Kruskal–Wallis tests were performed. In the following, after reviewing demographic group differences, the results of the three group analysis (schizophrenic patients, bipolar patients, healthy control subjects) will be reported, followed by post-hoc group comparisons, applying the Bonferroni correction where applicable.

## Results

For demographic variables, there was a significant difference in age between groups: patients with bipolar disorder were older than schizophrenic patients and control subjects (see Table I). In addition, the portion of males was not significantly higher in the group of schizophrenic patients, and control subjects tended to have higher education as compared to the other groups. In Table II, the results of the neuropsychological assessment, reporting means and standard deviations, are presented.

From the ANCOVA adjusted for gender, age and duration of education comparing controls, bipolar patients and schizophrenic patients, seven variables were capable of detecting significant across groups

Table II. Neurocognitive measures in diagnostic groups (mean, SD).

| | SZ (FE) ($N=11$) | SZ (chron) ($N=13$) | Bipolar ($N=18$) | Controls ($N=23$) |
|---|---|---|---|---|
| ZVT (*T* value) | 39.27 (15.90) | 34.73 (8.79) | 43.94 (10.31) | 52.36 (9.22) |
| Digit Span (value points) | 10.18 (3.28) | 11.45 (3.11) | 11.43 (2.68) | 12.14 (3.24) |
| RWT, prenames, (sum 1st and 2nd minute) | 29.08 (6.65) | 24.55 (8.34) | 31.50 (11.78) | 36.00 (7.81) |
| RWT, s-words (sum 1st and 2nd minute) | 17.33 (6.31) | 13.09 (4.46) | 19.88 (8.25) | 23.50 (5.57) |
| VLMT 1–5 (sum of trials 1–5) | 51.55 (8.99) | 41.50 (12.60) | 50.07 (8.24) | 60.05 (6.13) |
| VLMT-diff (difference between trial 5 and 7) | 2.27 (2.24) | 3.25 (1.54) | 2.40 (2.53) | 1.27 (1.42) |
| WST (*T* value) | 53.55 (8.49) | 47.69 (9.51) | 52.06 (7.23) | 58.78 (6.23) |
| NVLT (sum score, difference between right and false answers) | 42.18 (13.27) | 45.77 (12.81) | 40.65 (7.09) | 51.22 (10.00) |
| Corsi | 5.55 (1.37) | 5.46 (1.13) | 5.29 (1.21) | 6.00 (1.09) |
| Stroop-Reading (s) | 0.23 (0.13) | 0.26 (0.25) | 0.24 (0.19) | 0.17 (0.15) |
| Stroop-Naming (s) | 0.21 (0.26) | 0.15 (0.24) | 0.27 (0.21) | 0.09 (0.10) |
| RT1 (s) | 54.45 (9.53) | 50.83 (7.09) | 54.39 (8.40) | 59.17 (6.15) |
| RT3 (s) | 47.91 (9.12) | 46.50 (7.17) | 43.00 (11.00) | 51.43 (9.02) |
| WCST-ta (trials administered) | 110.91 (18.56) | 104.09 (18.42) | 103.25 (20.92) | 88.45 (16.77) |
| WCST-pr (% of perse-verative responses, *T* value) | 52.82 (13.77) | 52.55 (13.25) | 63.69 (15.43) | 62.00 (12.92) |
| TOL (sum score) | 93.73 (11.36) | 92.25 (9.36) | 95.12 (9.92) | 101.91 (8.43) |
| SRM (% of correct standard score) | −0.99 (1.71) | −1.37 (1.28) | −1.44 (1.15) | −0.56 (1.20) |
| TAP-WM (s) | 724.5 (134.0) | 856.3 (155.6) | 739.9 (213.5) | 625.0 (146.6) |
| TAP-DA (s) | 777.5 (59.6) | 758.4 (72.2) | 784.7 (87.0) | 695.4 (83.4) |
| TAP-IC (s) | 545.7 (41.4) | 576.8 (95.7) | 549.8 (114.7) | 437.5 (68.1) |

SD, standard deviation; *N*, number of subjects; SZ, schizophrenia; FE, first-episode; Chron, chronic disease; Bipolar, patients with bipolar disorder; ZVT, Zahlenverbindungstest (similar to Trail Making Test A); RWT, Regensburger Wortflüssigkeitstest (Verbal Fluency); VLMT, Verbaler Lern- und Merkfähigkeitstest (similar to the Auditory Verbal Learning Test); WST, Wortschatztest (comparable to the Boston Naming Test); NVLT, Nonverbaler Lerntest (Nonverbal Learning Test); Corsi, Corsi Block Tapping; Stroop, Stroop Test; RT1, Reaction Test (of the Wiener Test System), single stimulus; RT3, Reaction Test (of the Wiener Test System), compound stimulus; WCST, Wisconsin Card Sorting Test; TOL, Tower of London Test; SRM, Spatial Recognition Memory; TAP, Testbatterie zur Aufmerksamkeitsprüfung (Attention Test Battery); WM, Working Memory; DA, Divided Attention; IC, Intermodal Comparison.

differences in cognitive function (see Table III for details): performance in the trail making test (ZVT) was significantly different across groups, pointing to differences in attention and speeded psychomotor processing. Significant group differences were also found in lexical word fluency (RWT 's'-words), verbal learning and immediate recall capacities (VLMT, sum of trials 1–5), verbal episodic recognition performance (VLMT, difference between trial 5 and 7), premorbid verbal intelligence (WST), executive function (WCST), and crossmodal binding (TAP-IC).

*Schizophrenia patients versus controls*

Compared to control subjects, patients with schizophrenia performed significantly worse in the trail-making test (ZVT), the verbal fluency test (RWT 's'-words) (see Figure 1), verbal learning and memory test (immediate list recall of VLMT), and the Wisconsin Card Sorting Test (sum of administered trials, and perseverative responses). This cognitive pattern indicates significant deficits in attention and psychomotor performance, and in particular in verbal working memory and cognitive flexibility for schizophrenic patients. Thus, high order cognitive functions were most affected in schizophrenics. Interestingly, there was evidence for significantly reduced premorbid intellectual function (lower WST score) only in chronic schizophrenic patients with multiple episodes, but not in first-episode patients, compared to control subjects. Nevertheless, first-episode patients performed worse in the WCST (sum of administered trials), suggesting cognitive dysfunction even at the beginning of the disease.

*Schizophrenia versus bipolar patients*

A significant difference between schizophrenic and bipolar patients was found only in the WCST. Schizophrenic patients made significantly more perseverative responses, indicating a more pronounced and specific deficit in cognitive flexibility and executive function (see Table III and Figure 2).

*Correlations with other parameters*

For sake of completeness, the influences of intervening demographic variables were tested by correlation analysis. Sex influenced performance as expected; females performed better in verbally based tests (RWT, VLMT, Stroop-LI) and the working memory test requiring cross-modal integration, whereas males fared better on the spatial Corsi test, corroborating a well-established pattern of

Table III. Diagnostic group comparisons.

| | ANCOVA[a] Factor diagnostic group | | | SZ (total) vs. Controls | SZ (total) vs. Bipolar | SZ-FE vs. Controls | SZ-Chron vs. Controls |
|---|---|---|---|---|---|---|---|
| | df | *F* | *P* | *P* | *P* | *P* | *P* |
| ZVT (*T* value) | 2, 51 | 3.84 | **0.028** | **0.012** | 0.17 | **0.041** | **<0.0005** |
| Digit span (value points) | 2, 50 | 1.00 | 0.38 | | | | |
| RWT, prenames (sum 1st and 2nd minute) | 2, 51 | 0.42 | 0.66 | | | | |
| RWT, s-words (sum 1st and 2nd minute) | 2, 51 | 3.84 | **0.028** | **0.004** | 0.12 | **0.035** | **<0.0005** |
| VLMT 1–5 (sum of trials 1–5) | 2, 52 | 5.81 | **0.005** | **0.005** | 0.12 | **0.013** | **<0.0005** |
| VLMT-diff (difference between trial 5 and 7) | 2, 52 | 1.80 | 0.18 | | | | |
| WST (*T* value) | 2, 56 | 3.45 | **0.039** | **0.047** | 0.92 | 0.31 | **0.003** |
| NVLT (sum score, difference between right and false answers) | 2, 56 | 2.70 | 0.076 | | | | |
| Corsi (total) | 2, 56 | 0.97 | 0.39 | | | | |
| Stroop-Reading (s) | 2, 56 | 0.69 | 0.50 | | | | |
| Stroop-Naming (s)⋆ | 2⋆ | 2.32 | 0.31 | | | | |
| RT1 (s) | 2, 56 | 2.87 | 0.065 | | | | |
| RT3 (s) | 2, 56 | 1.62 | 0.21 | | | | |
| WCST-ta (administered trials) | 2, 52 | 3.51 | **0.037** | **0.004** | 0.44 | **0.015** | **0.038** |
| WCST-pr (% of perseverative responses; *T* value) | 2, 52 | 5.31 | **0.008** | **0.026** | **0.002** | 0.069 | 0.13 |
| TOL (sum score) | 2, 55 | 2.07 | 0.14 | | | | |
| SRM (% of correct standard score) | 2, 48 | 1.83 | 0.17 | | | | |
| TAP-WM (s) | 2, 50 | 1.99 | 0.15 | | | | |
| TAP-DA (s) | 2, 48 | 2.85 | 0.067 | | | | |
| TAP-IC (s) | 2, 47 | 5.66 | **0.006** | **0.002** | 0.54 | **<0.0005** | **0.001** |

[a]ANCOVA (factors diagnosis, gender; covariates age, education).

⋆Values were not normal distributed. In consequence non-parametric Kruskal–Wallis Test was performed, and $?^2$ instead of *F* are shown in the table.

N, number of subjects; SZ, schizophrenia; FE, first-episode; Chron, chronic disease; Bipolar, patients with bipolar disorder; df, degrees of freedom; *F*, *F* statistic; *P*, probability; ZVT, Zahlenverbindungstest (similar to Trail Making Test A); RWT, Regensburger Wortflüssigkeitstest (Verbal Fluency); VLMT, Verbaler Lern- und Merkfähigkeitstest (similar to the Auditory Verbal Learning Test); WST, Wortschatztest (comparable to the Boston Naming Test); NVLT, Nonverbaler Lerntest (Nonverbal Learning Test); Corsi, Corsi Block Tapping; Stroop, Stroop Test; RT1, Reaction Test (of the Wiener Test System), single stimulus; RT3, Reaction Test (of the Wiener Test System), compound stimulus; WCST, Wisconsin Card Sorting Test; TOL, Tower of London Test; SRM, Spatial Recognition Memory; TAP, Testbatterie zur Aufmerksamkeitsprüfung (Attention Test Battery); WM, Working Memory; DA, Divided Attention; IC, Intermodal Comparison.

cognitive sex differences. The influence of age was also as expected. Looking only at the directions of correlations, age correlated negatively with performance in every single index (correlation in the Stroop-LI test being the only exception), although of course many of these correlations were non-significant. Significant coherence, however, was found for memory measures in particular, including immediate recall and episodic recognition of VLMT, nonverbal learning performance (NVLT), and spatial and basic working memory (Corsi, TAP-WM), but also in tests tapping capacities to divide attention (TAP-DA) and react to compound stimuli (RT3). Although these results seemingly contradict findings of more specific age-related deficits especially with regard to memory (e.g., Cabeza 2006), age was already confounded with diagnostic group (i.e., older subjects were more likely to suffer from bipolar disorder). Finally, education had a large influence on many tests, as well, including but not limited to measures of verbal and nonverbal learning (VLMT, NVLT), working memory (ZNS, Corsi), and intelligence (WST). Most importantly for present concerns, there was no influence of these demographic variables on the WCST index selectively impaired in the schizophrenic group of patients.

## Discussion

In our study, overall, psychiatric patients exhibited poorer performance on many variables of neurocognitive assessment, although it should be highlighted that about half of the variables did not vary significantly with subjects' status. Although variables were selected in order to mirror group effects, many cognitive functions seem to be rather unimpaired in schizophrenia (and bipolar disorder). For example, patients performed quite well in pure working memory tasks (digit span, Corsi, TAP-WM) and did not show dramatically increased susceptibility to basic interference (Stroop). Differences in ZVT (trail making) might also be due to differences in

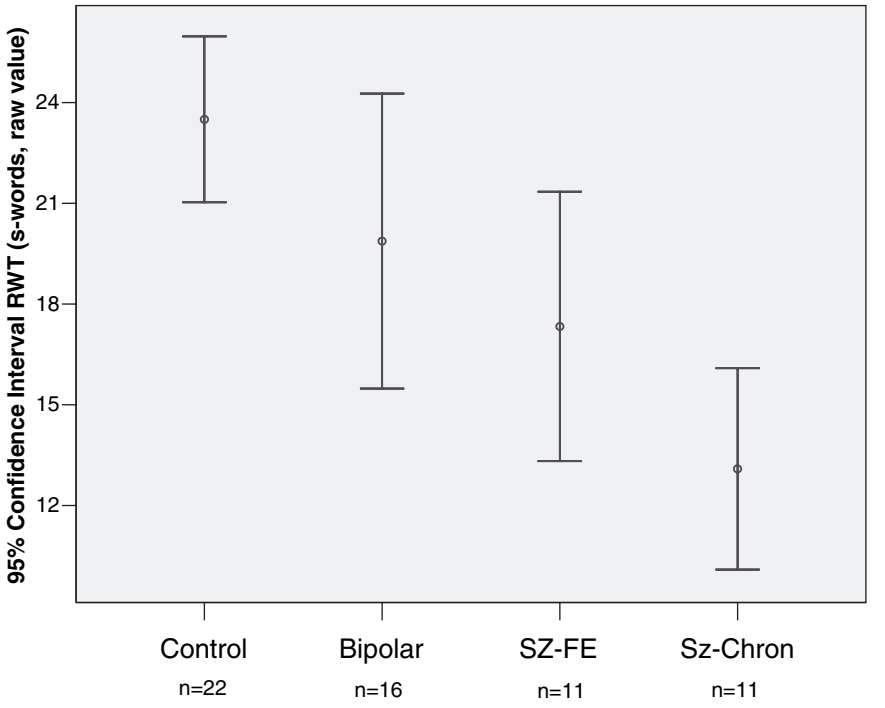


Figure 1. Verbal fluency (RWT, s-words) in diagnostic groups. Comparison of the performance in the verbal fluency task (RWT), letter category, words beginning with the letter 's' (s-words), sum of raw values in first and second minute. ANCOVA (factors diagnosis, gender; covariates age, education), $F=3.84$; df $=2,51$; $P=0.028$, for factor diagnosis. Analysis of diagnostic subgroup: SZ-FE vs. Controls: $P=0.035$; SZ-Chron vs. Controls: $P<0.0005$. RWT, Regensburger Wortflüssigkeits-Test; ANCOVA, analysis of covariance; df, degree of freedom; *P*, probability; SZ, schizophrenia; FE, first-episode; Chron, chronic disease; Controls, healthy controls; Bipolar, bipolar patients.

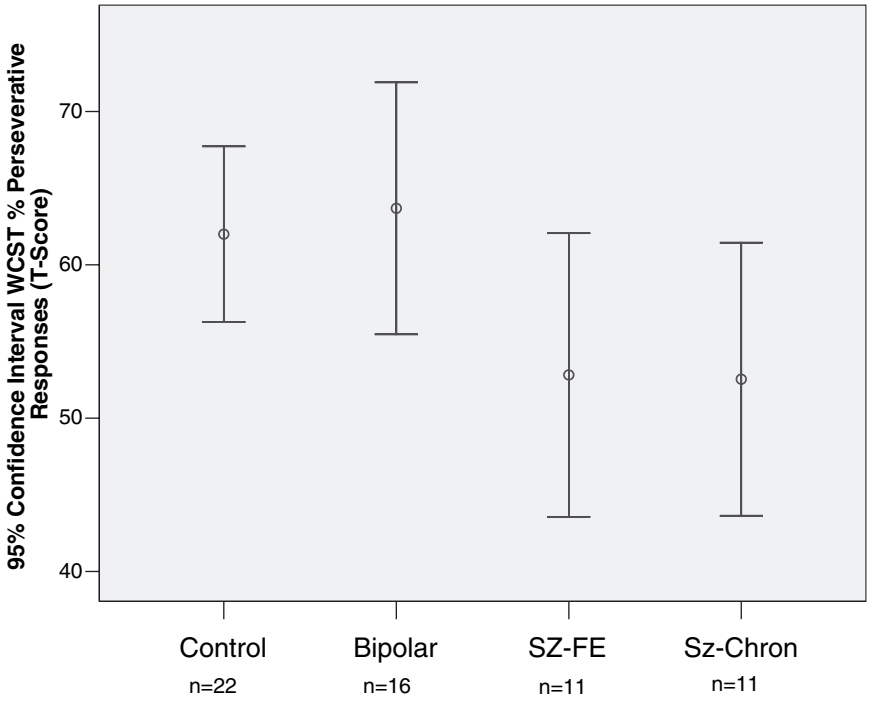


Figure 2. WCST (Perseverative Responses) in diagnostic groups. Comparison of the performance in the WCST, percent (%) of perseverative responses, *T* score, in diagnostic groups. ANCOVA (factors diagnosis, gender; covariates age, education ), $F=5.31$; df $=2,52$; $P=0.008$, for factor diagnosis. Analysis of diagnostic subgroup: SZ (total) vs. Controls: $P=0.026$; SZ (total ) vs. Bipolar: $P=0.002$. WCST, Wisconsin Card Sorting Test; ANCOVA, analysis of covariance; df, degrees of freedom; *P*, probability; SZ, schizophrenia; FE, first-episode; Chron, chronic disease; Controls, healthy controls; Bipolar, bipolar patients.

assessment-related arousal, as basic reaction time distributions did not differ across groups, speaking against a pure 'speed of processing' account of deficits. Overall results did not indicate a simplified 'task difficulty' account of deficits, as there were no significant group differences in the most challenging tasks (TOL, Stroop, TAP-WM), whereas easier tasks did show group differences (e.g., the VLMT recognition task, in which controls performed near ceiling, or the TAP-IC test).

Taken together, the results point to general higher-level cognitive functioning being primarily impaired in the patient groups. For instance, basic (pure) working memory tests were not sensitive to group differences (despite numerical tendencies); however, groups differed in the immediate recall of categorized word lists. Even though the latter also heavily relies on working memory processing (and working memory processing per se comprises control processes), performance in this task strongly benefits from additional strategic processing (clustering the groups, generating associations or images, and other strategies). A similar picture arises when comparing performance in the two word fluency tests. In the semantic version (i.e., list all first names you can think of), there is less need for generation of retrieval cues (letters of the alphabet are easily available cues) and strategy switches (subjects would typically follow the alphabet or go through their friend/family circle) as compared to the lexical version (i.e., list all words you can think of commencing with an 's'), requiring flexible generation of retrieval cues and strategy adjustments. Summarising our findings, schizophrenic patients showed deficits as compared to healthy controls in a number of tasks that seem to tap into higher-order cognitive processes, thus requiring multiple brain regions to act in concert to achieve high levels of performance.

The comparison with the bipolar group demonstrates, however, that these deficits might be of a rather general nature, not specific to schizophrenia. In contrast, they could be based on factors in common to both types of psychotic disorders (i.e. bipolar disorder and schizophrenia (Häfner et al. 2005)). Other related causes may be the intake of psychotropic drugs in both groups, a lower premorbid level of functioning, the influence of hospitalization, motivational factors, or an interaction of these and other conceivable factors.

More importantly, however, this study revealed a very specific deficit more peculiar to schizophrenia: schizophrenic patients (both first episode and chronic patients) produced significantly higher levels of perseverative responding in the Wisconsin Card Sorting Test (WCST), demonstrating inability to shift from an incorrect response set, compared to bipolar patients. Although the WCST has been subject to some criticism – mainly due to initial attempts to treat it as a 'pure' measure of frontal cortex function – it is not coincidentally one of the most administered neuropsychological tests worldwide, contributing uniquely to neuropsychological assessment (e.g., Greve et al. 1998). The present pattern points to a specific deficit in the ability of schizophrenic patients to flexibly adapt to the environment – a behaviour requiring high levels of action–effect monitoring and strategic control. This deficit is typically demonstrated by patients suffering from frontal lobe lesions. It indicates that frontally based executive functions are more disturbed in schizophrenia than in bipolar disorder. Yet, other functions also require a certain amount of frontal control, those subserving pure working memory tasks for example, and these were not strongly affected by group status in this study. Thus, the index of perseverative responses may be a rather pure measure of the psychological function needed to solve the task (strategy adjustment), but this function is most likely subserved by a distributed neural network rather than a single region. Apparently, this network is specifically disturbed in schizophrenia, and equally so across successive stages of the disease.

There are several limitations of this study. We did not control for the possible influence of psychotropic drugs on cognitive dysfunction. In this study, all of the schizophrenic subjects patients were treated with antipsychotic agents, in nearly all cases with second-generation antipsychotics. Bipolar patients received mainly mood stabilizers and to some extent second generation antipsychotics. Both patient groups were not treated with anticholinergic agents (like biperiden), or benzodiazepines at the time of neuropsychological assessment.

However, the influence of antipsychotics on neuropsychological performance should not be overrated, because a recent study (Rémillard et al. 2005) suggests that antipsychotics (risperidone, haloperidol) do not substantially or differentially affect executive functioning as measured by the WCST. Although several methods were applied intending to reduce the number of dependent variables, there still remained the large number of 20 variables. This raises the question of false positive findings caused by multiple testing. However, findings for the different neuropsychological tests were consistent, therefore there is evidence that the results may be interpreted as outlined above.

Notably, our results confirm some findings in the literature. For instance, it was reported that schizophrenia patients perform worse than normal controls on the WCST, with patient siblings showing

intermediate performance (El Hamaoui et al. 2006). This is in line with our assumption that frontal lobe/executive dysfunction marks a cognitive endophenotype in schizophrenia. In a study including both schizophrenic and depressed patient groups, executive dysfunction was found specifically in schizophrenic patients, which was associated with abnormal right frontal cortex activity (Holmes et al. 2005). Another study reported similar results, also showing that executive function (WCST, number of categories) was significantly more impaired in schizophrenic than in bipolar patients (Martinez-Aran et al. 2002).

Other studies have reported more global differences between the two patient groups. For instance, a study with large patient samples revealed evidence for more severe global and specific neuropsychological deficits in schizophrenia compared to psychotic affective disorders (Mojtabai et al. 2000), and another trial demonstrated similar patterns of cognitive dysfunction across groups, but the deficits of schizophrenic patients were more severe (Dickerson et al. 2004).

Nevertheless our results stand in contrast to other studies comparing performance of schizophrenic patients and controls. In one study the greatest deficits of schizophrenics were reported in visuomotor and attentional functions, whereas only intermediate deficits in cognitive flexibility could be demonstrated (Albus et al. 1996). Our study suggests that the former deficit is probably not peculiar to schizophrenia, whereas the latter may in fact be. Also, other research groups comparing neuropsychological performance between the two clinical groups have reported other result patterns, for instance, comparable performance on WCST accompanied by a selective deficit in verbal fluency tests shown by schizophrenia patients (Frangou et al. 2006). Patients suffering from schizophrenia have been reported to show a significant decrease in memory test performance, compared with both normal controls and affectively disturbed patients (Landrø et al. 1993), and these deficits did not correlate with performance on the WCST. A comparison between unaffected siblings of these two patient groups suggested a common impairment in verbal recall and a more pronounced deficit of sensory-perceptual analysis and spatial working memory in the unaffected siblings of schizophrenic patients (Keri et al. 2001). Finally, a study comparing neuropsychological performance between schizophrenic and bipolar patients over a 3-year period found similar results in both groups and stability of cognitive dysfunction over time also in bipolar disorder (Balanza-Martinez et al. 2005).

We propose that a key factor in the resolution of this rather ambiguous state of affairs will be concerned with the ascertainment of more homogeneous clinical subgroups. Interestingly, when bipolar patients with manic or depressive features were compared with schizophrenic patients with disorganized or predominantly negative symptoms, it was observed that executive dysfunction was more related to the symptom profile than to the diagnosis itself (Kravariti et al. 2005). An investigation similar to our study demonstrated a more generalized cognitive impairment and greater degree of impairment in schizophrenia compared with euthymic bipolar subjects (Altshuler et al. 2004). Compared to healthy controls, subjects with bipolar disorder were impaired in two specific domains (verbal memory, measured by CVLT, and executive function, measured by WCST). In this study the bipolar group consisted of a subset with relatively normal executive functioning and a subset with significant impairment, indicating that this patient group is not homogenous.

Further research also including modern imaging methods is necessary in order to confirm the suggestion that impairment of executive function or frontal lobe dysfunction describes a cognitive endophenotype in schizophrenia. Although meta-analyses have concluded that WCST performance is not specifically affected in schizophrenia in general (Laws 1999), there is some evidence of impaired executive functioning as indexed by WCST in patients exhibiting predominantly negative symptoms (Nieuwenstein et al. 2001). Thus, applying multi-method approaches and taking individual pathology patterns into account (e.g., Donohoe et al. 2006; Kurtz and Wexler 2006; Zalla et al. 2006) seems to be the most promising agenda for future research.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# Cognitive profiles of healthy siblings of schizophrenia patients: Application of the cognitive domains of the MATRICS consensus battery

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**Abstract**

Even though a large body of data suggests the presence of various types of cognitive deficits in the unaffected relatives of schizophrenia patients, more study is needed to clarify the comparative sensitivities of specific cognitive measures for relative-control differences. In this study, the authors compared the cognitive profiles of unaffected siblings of schizophrenia patients and those of patients and normal controls, and attempted to identify cognitive markers that might be associated with genetic liability to schizophrenia. Eighty-eight clinically stable schizophrenia patients, 44 healthy patient siblings, and 100 normal controls were evaluated using comprehensive neuropsychological tests. The domain structure of the MATRICS consensus cognitive battery was adopted, and both domain scores and individual test scores were used in the analysis. Performances of the sibling group were intermediate between those of patients and controls on most measures. A significant difference between the sibling and control groups was observed only in the Category Fluency Test. This cognitive deficit might be caused by familial predisposition to schizophrenia and could be a candidate of endophenotype for schizophrenia.

**Key words:** *Cognitive deficit, Endophenotype, the Category Fluency Test*

## Introduction

It has been well established that cognitive deficits are pervasive in schizophrenia over a wide range of domains (Nuechterlein and Dawson 1984; Cannon et al. 1994). Certain portions of cognitive impairments appear to be related to the clinical progress of the disease and show partial responses to antipsychotic treatments (Lee et al. 1999; Bilder et al. 2002), whereas some other portions may index genetic liability to schizophrenia (Bredgaard and Glenthoj 2000; Cannon et al. 2001; Erlenmeyer-Kimling 2001; Gottesman and Gould 2003). The latter expectation originates from a large body of findings which indicate that unaffected relatives of patients, who are at greater risk of developing the disease than the general population, also show cognitive task impairments (Cannon et al. 2000; Faraone et al. 2000; Egan et al. 2001; Keri et al. 2001; Sitskoorn et al. 2004; Snitz et al. 2006; Trandafir et al. 2006; Woodward et al. 2007; Groom et al. 2008). Cognitive declines consistently exhibited in the relatives of patients could be candidate endophenotypes that may yield greater statistical power in genetic studies (Myles-Worsley et al. 1999; Gasperoni et al. 2003; Gottesman and Gould 2003; Golimbet et al. 2006).

In terms of the detailed profiles of cognitive deficits in the relatives of schizophrenia patients, previous studies have reported diverse results. Pronounced deficits have been reported in the domains of verbal memory, visual memory, working memory, executive functioning, psychomotor speed, and attention by studies that have evaluated single or

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multiple cognitive areas (Sitskoorn et al. 2004; Snitz et al. 2006) However, it remains to be determined which specific cognitive measures are more sensitive to relative-control group differences. Because previous studies have been heterogeneous in terms of their designs, e.g., types of relatives, psychopathological statuses of relatives, covariates controlled for in the analysis, and neuropsychological tests applied, it is difficult to draw conclusions on the relative effect sizes of individual tasks using meta-analytic approaches. Thus, further individual studies using comprehensive test batteries are warranted to compare multiple cognitive tasks in terms of their abilities to discriminate patients' relatives from controls.

Assumptions concerning cognitive domains raise an important issue that should be resolved to identify valid cognitive markers. Because performance on any one measure reflects multiple cognitive processes, it is difficult to isolate specific mechanisms responsible for deficient performance (Harris et al. 1996). Thus, grouping measures according to a predominant common factor could strengthen conclusions on specific defect profiles (Harris et al. 1996; Genderson et al. 2007). On the other hand, some significant information is likely to be lost by collapsing individual scores into a broader test category (Snitz et al. 2006). Therefore, comparative examinations using both approaches in same studies are required to clarify this issue. Detailed domain structures, i.e. the way in which cognitive domains have been divided and individual tests selected for each domain, also vary between studies. The National Institute of Mental Health (NIMH) of the United States recently developed the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) cognitive battery (Green and Nuechterlein 2004; Green et al. 2004; Nuechterlein et al. 2004), which is the first standard instrument to have been developed via an extensive consensus process between schizophrenia research experts. MATRICS adopts seven separable cognitive domains, namely, speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition. These cognitive dimensions are of relevance to a wide range of studies, including the exploration of cognitive deficits related to liability to schizophrenia. Moreover, it is apparent that the application of a common standard cognitive structure in independent studies provides a basis for inter-study comparisons.

The control of the psychopathologies of relatives is another issue that should be considered in research on the identification of cognitive markers in the relatives of schizophrenia patients. The majority of studies carried out to date have applied stricter psychopathological exclusion criteria to controls (excluding all axis I disorders) than to relatives (excluding only psychotic disorders) (Tuulio-Henriksson et al. 2002, 2003; Davalos et al. 2004; Snitz et al. 2006). Certain cognitive deficits might be influenced by the manifestation of sub-psychotic levels of schizophrenia-related symptoms or psychopathologies in general (Nuechterlein et al. 2002; Johnson et al. 2003; Thompson et al. 2005; Warnick and Allen 2005; Snitz et al. 2006). Therefore, so as not to overestimate deficits due to the nonspecific effects of psychiatric symptoms (including prodromal symptoms) in relatives, controlling for index relatives' psychopathology is required during subject-screening or data analysis (Hoff et al. 2005).

The present study was performed to delineate the cognitive profiles of unaffected siblings of schizophrenia patients, and to identify specific cognitive markers that might be used as endophenotypes in future genetic studies. The cognitive domain structure of the MATRICS consensus cognitive battery was applied, and both domain scores and individual test scores were analyzed. To identify cognitive deficits associated with liability to disease, we used the same exclusion criteria for psychopathologies in siblings and controls.

## Methods

### *Subjects*

Patients who met the diagnostic criteria of DSM-IV schizophrenia were recruited from outpatient clinics of the Samsung Medical Center and of Yong-In Mental Hospital. Clinical interviews were performed by psychiatrists using the Korean Version of the Diagnostic Interview for Genetic Studies (DIGS-K) (Joo et al. 2004). To minimize performance variations due to clinical state during cognitive testing, patients were required to have been clinically stable, i.e. no exacerbation of psychotic symptoms and no change in general clinical state and medication, for at least 3 months prior to the time of assessment. We excluded patients with a concurrent axis I diagnosis of DSM-IV, a current or past central nervous system disease, a medical disease with likely significant central nervous system effects, or a physical problem that would render cognitive measures difficult to administer or interpret.

In the sibling group, full siblings of patients available for study were recruited. The normal control subjects consisted of volunteers from the community. A trained research nurse with more than 7 years of clinical psychiatric experience performed screening interviews (based on DIGS-K) on both

siblings and controls. These subjects were free of any history of psychiatric symptoms of clinical significance. The same exclusion criteria applied to the patients were used with respect to medical, neurological, and physical conditions. Only one sibling per patient was included in the sibling group. In cases of families in which more than one sibling meet the inclusion criteria, only one sibling whose age was closest to that of the proband's was selected. Controls were also required to have no first- or second-degree relatives with a psychotic illness. As an additional controlling process for current psychopathology, the Korean version of the Personality Assessment Inventory (PAI: Hakjisa Press, Seoul, Korea) (Morey 1991; Kim et al. 2001), a self-report measure of personality and psychopathology, was administered to siblings and controls. *T* scores of the following subscales were examined, i.e. Inconsistency, Infrequency, Negative Impression, Positive Impression, Somatic Complaints, Anxiety, Anxiety Related Disorders, Depression, Mania, Paranoia, Schizophrenia, Borderline Features, Antisocial Features, Alcohol Problems, Drug Problems, Aggression, Suicide Ideation, Stress, Non-support, Treatment Rejection, Dominance, and Warmth.

Eighty-eight patients, 44 healthy siblings, and 100 normal control subjects were finally included in the analysis. Demographic characteristics of the 232 study subjects (age range 16–50 years) are summarized in Table I. Written informed consent was obtained from all subjects after a complete explanation of the study. The study was approved by the institutional review boards (IRBs) of Samsung Medical Center and Yong-In Mental Hospital.

### *Neuropsychological assessment*

A comprehensive neuropsychological test battery was administered. Table II provides a description of the measures and corresponding cognitive domains assessed. Two trained clinical psychologists administered the cognitive tests. All subjects received the same battery of tests in a fixed order. Total time for the test battery was about 2 h, but subjects were allowed to take breaks as needed. Examiners attempted to obtain maximal performance at all times. Most of the tests applied were reduced to the six cognitive domains (Table II) of the MATRICS consensus battery (Green and Nuechterlein 2004; Green et al. 2004; Nuechterlein et al. 2004). Social cognition, which was added to the battery due to recent interest in this area, was not included in the present study. Scores of the Vocabulary subtest of the Korean version of the Wechsler Adult Intelligence Scale (K-WAIS: Korea Guidance, Inc., Seoul, Korea) (Yeom et al. 1992) were used to predict patients" premorbid functioning and subjects" general ability.

### *Statistical analyses*

One-way analysis of variance (ANOVA) and the $\chi^2$-test were conducted to compare demographic variables between groups. For all neuropsychological measures, raw test scores were transformed to standardized $z$ scores, using control group means and standard deviations.

Group (patients, siblings, and controls) cognitive performance differences were tested by ANCOVA analyses of covariance) model with age, sex, education, and K-WAIS Vocabulary subtest scores that were selected using the likelihood ratio test (LRT) and Akaike information criterion (AIC) (Akaike 1974) as covariates. Since related siblings and patients are not independent, a familial correlation was incorporated into the model. In addition, multiple comparison tests were applied to all pair-wise group comparisons, i.e. patients versus controls, siblings versus controls, and patients versus siblings.

We considered six cognitive domains of the MATRICS consensus battery. Factor analysis followed by principal components was separately performed for each cognitive domain. We examined if the main factor of each domain representing the general characteristics of that domain sufficiently explained variances. In addition, we explored the

Table I. Subject demographic characteristics.

| Variable | Patients ($N=88$) | Siblings ($N=44$) | Controls ($N=100$) | Test statistics | |
|---|---|---|---|---|---|
| Age (mean years ± SD, range) | 28.7 ± 6.0 (18–47) | 28.8 ± 7.3 (16–50) | 27.2 ± 4.0 (20–43) | $F=2.41$ | NS |
| Sex (% female) | 48.9 | 52.3 | 45.0 | $\chi^2=0.65$ | NS |
| Vocabulary score of K-WAIS[a] | 10.8 ± 2.7 | 11.6 ± 2.3 | 13.1 ± 1.9 | $F=23.94$ | $P<0.0001$[b] |
| Education | | | | | |
| 9–12 years | 13 | 6 | 3 | $\chi^2=9.503$ | $P=0.008$[c] |
| 13 < years | 75 | 38 | 97 | | |

[a]Korean version of the Wechsler Adult Intelligence Scale.
[b]Patient vs. Sibling $P=0.049$, Patient vs. Control $P<0.0001$, Sibling vs. Control $P=0.001$ (by post-hoc test using LSD).
[c]Patient vs. Sibling $P=0.861$, Patient vs. Control $P=0.007$, Sibling vs. Control $P=0.024$ (by Fisher's exact test).
NS, not significant.

Table II. Cognitive domains and tests used.

| Cognitive Domain | Test | Recorded test variable |
|---|---|---|
| Speed of processing[a] | Digit Symbol subtest of K-WAIS [b] | |
| | Word Fluency[c] | Letter fluency (three Korean consonant alphabets)<br>Category fluency (animal, items in supermarket) |
| | Trail Making Test[d]: Part A | Time of part A |
| | Trail Making Test[d]: Part B | Time of part B |
| Working memory[a] | Digit Span subtest of K-WAIS [b] | Digit span backward |
| | N-back Test[e] | 1-back,<br>2-back |
| Verbal learning and memory[a] | Rey-Kim Auditory Verbal Learning Test[f] | Immediate recall (sum of 5 trials)<br>20-min delayed recall recognition |
| Visual learning and memory[a] | Rey-Kim Complex Figure Test[g] | Copy immediate recall 20-min delayed recall |
| Attention/vigilance[a] | DS-CPT[h] | Sensitivity *d'* value |
| | Span of Apprehension[i] | % correct of 3-letter array and 12-letter array |
| Reasoning and problem solving[a] | Picture Arrangement subtest of K-WAIS [b] | |
| | Block Design subtest of K-WAIS [b] | |
| Not assigned to domains | Digit Span subtest of K-WAIS [b] | Digit span forward |
| | N-back Test[e] | 0-back |
| | Finger Tapping Test[j] | Scores of dominant hand and non-dominant hand |

[a]Cognitive domains of the MATRICS consensus cognitive battery.
[b]The Korean version of the Wechsler Adult Intelligence Scale (Korea Guidance, Inc., Seoul, Korea) (Yeom et al. 1992).
[c]The Korean version of Controlled Oral Word Association Test (COWAT) including the categories of "animal" and "supermarket list" and the letters of three Korean consonant alphabets (Kang et al. 2000).
[d]The Trail Making Test materials and guidelines used in this study are parts of the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Neuropsychological Assessment Battery (Lee et al. 2002).
[e]Working Memory Task V1.06 (Richard Coppola, CBDB, NIMH Neuroscience Center, Washington, DC, USA).
[f]Korean version (Kim 2001) of the Rey Auditory Verbal Learning Test (Rey 1941, 1964) (Neuropsychology Press, Daegu, Korea).
[g]Korean version (Kim 2001) of the Rey Complex Figure Test (Rey 1941, 1964) (Neuropsychology Press, Daegu, Korea).
[h]Degraded Stimulus-Continuous Performance Test (DS-CPT; UCLA CPT version 8.12, Nuechterlein and Asarnow 1999).
[i]Span of apprehension (Span; UCLA SPAN version 5.3, Asarnow and Nuechterlein 1994).
[j]Finger Tapper (Psychological Assessment Resources, Inc., Florida, USA).

component structures of the domains. Factors with eigenvalues of greater than 1 were retained as significant sub-categories of domains. The ANCOVA model, used to analyze individual tests scores, was also applied to detect group domain (factor) score differences.

Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). In comparisons of cognitive performances, the Bonferroni correction using significance values derived by dividing $P$ values by the total number of statistical comparisons (23 for individual tests, and 6 for domains) were applied to control for the experiment-wise type I error. Therefore, only $P$ value of $<0.0022$ for individual tests, and of $<0.0083$ for domains were considered significant. $P$ values higher than these and lower than 0.05 were considered to indicate trends.

## Results

### *Subject characteristics*

No differences were observed between the three groups in terms of age or sex (Table I). The patient and sibling groups had significantly lower K-WAIS Vocabulary subtest scores and educational levels than the control group (Table I). There was no significant difference between the patient and sibling groups in educational level. The mean duration of illness for patients was $6.6 \pm 5.2$ years. All of the patients were taking second-generation antipsychotics at the time of assessment except for one patient receiving haloperidol. Seventy-three (82%) of the patients were receiving concomitant medications including benzodiazepines, propranolol, anticholinergics, mood stabilizers, and/or antidepressants. In terms of psychopathology evaluation using PAI, siblings and controls did not differ on any PAI subscale, except for "Suicide Ideation", and for this subscale, the mean of the control group ($51.3 \pm 14.0$) was significantly higher than the mean of the sibling group ($45.8 \pm 7.7$) with $t = 3.0$ and $P = 0.03$.

### *Group differences in individual neuropsychological test scores*

The $z$ score profile of all study subjects is shown in Figure 1. Results of comparisons between the three groups after adjusting for age, sex, education, and K-WAIS Vocabulary subtest score are given in Table III.

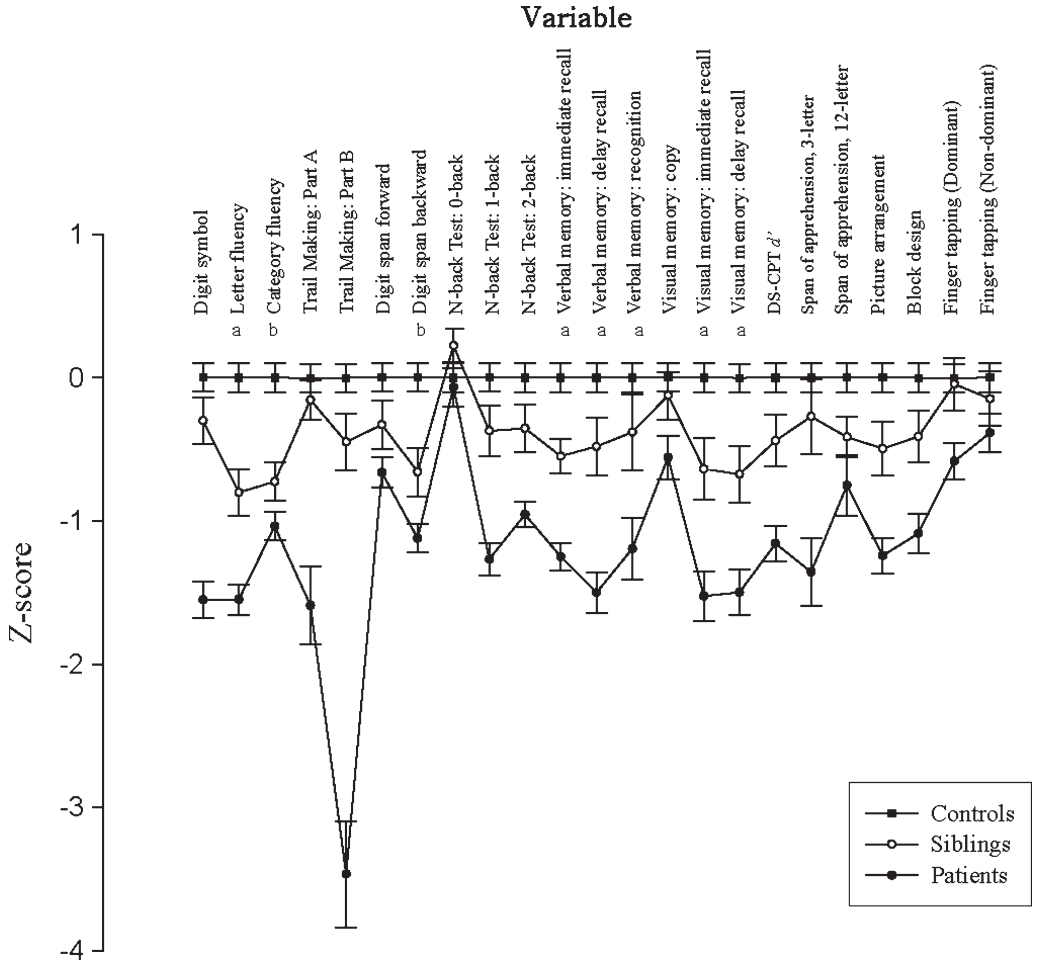


Figure 1. Performances of schizophrenia patients, siblings and controls on neuropsychological tests. [a]Siblings differed significantly from patients and controls (patients <siblings <controls). [b]Siblings differed significantly from controls (patients =siblings <controls). Error bars represent ±1standard error.

Effect sizes were computed by dividing the difference between mean scores by standard deviation (Cohen's *d*). A positive effect size for comparison means that the level of performance followed the order; patients <controls, siblings <controls, or patients <siblings.

Most of the tests indicated a significant overall group effect. With the exception of the Digit Span forward test, the 0-back of N-back test, copy of the Rey-Kim Complex Figure test, the picture arrangement subtest of the K-WAIS, and the Finger Tapping test patients obtained significantly lower scores than normal controls for all measures.

Siblings performed poorer than control subjects with *P* values that attained conventional levels of significance ($P = 0.05$) on the Category and Letter Fluency tests, immediate and delayed recalls of the Rey-Kim Complex Figure Test, immediate (sum of five trials) and delayed recalls, and recognition of the Rey-Kim Auditory Verbal Learning Test, and the Digit Span backward test. Using the Bonferroni correction for multiple comparisons, only the Category Fluency Test remained significant ($P = 0.0002$). The effect sizes of sibling-control differences ranged from −0.28 (0-back of the N-back test) to 0.78 (the Category Fluency Test).

*Group differences in the factor scores of cognitive domains*

According to factor analysis followed by principal component, each cognitive domain of the MATRICS battery generated only one factor with an eigenvalue greater than 1.0. For each cognitive domain, 53–76% of variance was explained by this single main factor (Table IV). The results of comparisons of factor scores for the three groups after adjusting for age, sex, education, and the K-WAIS Vocabulary subtest score are presented in Table IV. Patients showed significantly lower performance for all six cognitive domains than siblings or controls.

For sibling versus control comparisons, siblings tended to have poorer performance for domains of "verbal learning and memory" ($d = 0.57$) and "visual learning and memory" ($d = 0.56$). However,

Table III. Comparison of individual neuropsychological test scores for the patient, sibling, and control groups.

| | | | Pair-wise test | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Patients vs. Control | | Sibling vs. Control | | Patient vs. Sibling | |
| Variables | $F$(df1, df2) | $P$ value | $P$ value | ES[c] | $P$ value | ES[c] | $P$ value | ES[c] |
| Digit symbol | $F(2,186) = 39.48$ | <0.0001 | <0.0001[a] | 1.41 | NS | 0.27 | <0.0001[a] | 1.12 |
| Letter fluency | $F(2,186) = 29.06$ | <0.0001 | <0.0001[a] | 1.56 | 0.0058[b] | 0.76 | 0.0001[a] | 0.76 |
| Category fluency | $F(2,186) = 16.98$ | <0.0001 | <0.0001[a] | 1.08 | 0.0002[a] | 0.78 | NS | 0.33 |
| Trails Making: Part A | $F(2,186) = 19.80$ | <0.0001 | <0.0001[a] | 0.81 | NS | 0.13 | <0.0001[a] | 0.76 |
| Trails Making: Part B | $F(2,185) = 42.41$ | <0.0001 | <0.0001[a] | 1.35 | NS | 0.39 | <0.0001[a] | 1.13 |
| Digit span forward | $F(2,186) = 1.77$ | NS | NS | 0.66 | NS | 0.33 | NS | 0.30 |
| Digit span backward | $F(2,186) = 11.89$ | <0.0001 | <0.0001[a] | 1.16 | 0.0302[b] | 0.61 | NS | 0.46 |
| N-back Test: 0-back | $F(2,180) = 3.75$ | 0.0254 | NS | 0.05 | 0.0096[b] | −0.28 | NS | 0.30 |
| N-back Test:1-back | $F(2,180) = 23.06$ | <0.0001 | <0.0001[a] | 1.28 | NS | 0.34 | <0.0001[a] | 0.90 |
| N-back Test:2-back | $F(2,180) = 15.45$ | <0.0001 | <0.0001[a] | 1.08 | NS | 0.34 | 0.0008[a] | 0.70 |
| Learning Verbal memory: immediate recall | $F(2,186) = 27.13$ | <0.0001 | <0.0001[a] | 1.35 | 0.003[b] | 0.61 | <0.0001[a] | 0.86 |
| Verbal memory: delayed recall | $F(2,186) = 30.84$ | <0.0001 | <0.0001[a] | 1.28 | 0.0392[b] | 0.41 | <0.0001[a] | 0.78 |
| Verbal memory: recognition | $F(2,186) = 15.41$ | <0.0001 | <0.0001[a] | 0.77 | 0.0455[b] | 0.26 | 0.0166[b] | 0.45 |
| Visual memory: copy | $F(2,186) = 2.37$ | NS | NS | 0.47 | NS | 0.15 | NS | 0.33 |
| Visual memory: immediate recall | $F(2,186) = 21.71$ | <0.0001 | <0.0001[a] | 1.14 | 0.0218[b] | 0.53 | 0.0015[a] | 0.60 |
| Visual memory: delayed recall | $F(2,186) = 24.33$ | <0.0001 | <0.0001[a] | 1.19 | 0.0111[b] | 0.58 | 0.0014[a] | 0.61 |
| DS-CPT $d'$ | $F(2,184) = 13.89$ | <0.0001 | <0.0001[a] | 1.05 | NS | 0.40 | 0.0012[a] | 0.45 |
| Span of apprehension, 3-letter | $F(2,186) = 13.02$ | <0.0001 | <0.0001[a] | 0.81 | NS | 0.20 | 0.0002[a] | 0.57 |
| Span of apprehension, 12-letter | $F(2,186) = 5.12$ | 0.0069 | 0.0016[a] | 0.48 | NS | 0.40 | NS | 0.24 |
| Picture arrangement | $F(2,186) = 3.71$ | 0.0263 | 0.0110[b] | 0.57 | NS | 0.15 | NS | 0.45 |
| Block design | $F(2,186) = 10.37$ | <0.0001 | <0.0001[a] | 0.95 | NS | 0.36 | 0.0026[a] | 0.56 |
| Finger tapping (Dominant) | $F(2,184) = 4.26$ | 0.0155 | 0.0434[b] | 0.53 | NS | 0.03 | 0.0095[a] | 0.46 |
| Finger tapping (Non-dominant) | $F(2,184) = 0.51$ | NS | NS | 0.33 | NS | 0.12 | NS | 0.20 |

ANCOVA was conducted with age, sex, education, and Vocabulary subtest score of the Korean version of the Wechsler Adult Intelligence Scale.
(K-WAIS) as covariates. DS-CPT, Degraded Stimulus-Continuous Performance Test.
[a]Significantly different after applying the Bonferroni correction.
[b]Difference vanishes after applying the Bonferroni correction.
[c]Effect sizes: Cohen's $d$.
NS, not significant.

after applying the Bonferroni correction, none of these domains reached the level of significance.

## Discussion

It is likely that the clinical phenotype of schizophrenia is not consistently expressed among those who carry susceptibility genes. Thus, investigations on neurobiological traits that may reflect an underlying genetic liability are essential to facilitate future genetic studies. The purpose of the present study was to delineate the cognitive profiles of the healthy siblings of schizophrenia patients, and to identify possible cognitive markers that might be associated with genetic liability to schizophrenia. Analyses were performed using both individual test scores and factor scores based on the cognitive domains of the MATRICS consensus battery.

During factor analysis, we could extract only one major factor for each cognitive domain, and the rate of variances explained by this factor was higher than 50%. This result indicates that the assignment of individual tests to cognitive domains in the present study was acceptable. Patients were found to perform markedly worse than control subjects for all six cognitive domains, and for all individual tests, except for a few concentration, visuo-spatial construction, and motor speed tasks. More pronounced cognitive dysfunctions found in patients compared to siblings and controls could be partially explained by side effects of psychotropic medications that can exert significant effects on cognitive functions (Keefe et al. 2007; Mortimer et al. 2007; Voruganti et al. 2007). Siblings performed worse than the control group with $P$ values indicating a trend towards statistical significance for a number of cognitive variables. These included domains of "verbal learning and memory" ($d = 0.57$) and "visual learning and memory" ($d = 0.56$), and most of individual tests assigned to these domains ($d = 0.26$–$0.61$). Additionally, the Digit Span backward test and the Category and Letter Fluency tests also showed a trend toward a significant sibling-control difference with moderate to large effect sizes ($d = 0.61$–$0.78$). However,

Table IV. Comparisons of the cognitive domain scores of the patient, sibling, and control groups.

| Cognitive domain[a] | Variance[b] explained | $F$(df1, df2) | $P$ value | Patients vs. Control | | Pair-wise test Sibling vs. Control | | Patient vs. Sibling | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $P$ value | ES[e] | $P$ value | ES[e] | $P$ value | ES[e] |
| Speed of processing | 53 | $F(2,185) = 42.01$ | <0.0001 | <0.0001[c] | 1.16 | NS | −0.04 | <0.0001[c] | 1.20 |
| Working memory | 64 | $F(2,180) = 18.79$ | <0.0001 | <0.0001[c] | 1.12 | NS | 0.26 | <0.0001[c] | 0.80 |
| Verbal learning & memory | 76 | $F(2,186) = 22.90$ | <0.0001 | <0.0001[c] | 1.28 | 0.0138 [d] | 0.57 | <0.0001[c] | 0.82 |
| Visual learning & memory | 75 | $F(2,186) = 23.37$ | <0.0001 | <0.0001[c] | 1.16 | 0.0134 [d] | 0.56 | 0.0018[c] | 0.57 |
| Attention/vigilance | 55 | $F(2,184) = 7.20$ | 0.0010 | 0.0004[c] | 0.62 | NS | 0.27 | 0.0181[d] | 0.39 |
| Reasoning & problem solving | 72 | $F(2,186) = 7.67$ | 0.0006 | 0.0003[c] | 0.87 | NS | 0.34 | 0.0124[d] | 0.49 |

ANCOVA was conducted with age, sex, education, and vocabulary subtest score of the Korean version of the Wechsler Adult Intelligence Scale (K-WAIS) as covariates.
[a]Cognitive domains identified by the MATRICS consensus process.
[b]% variance explained by the single main domain factor.
[c]Significantly different after applying the Bonferroni correction.
[d]Difference vanishes after applying the Bonferroni correction.
[e]Effect sizes: Cohen's *d*.
NS, not significant.

among those cognitive measures, only the Category Fluency Test showed a statistically significant group difference after applying the Bonferroni correction.

Abnormalities in word fluency tests have consistently been found in relatives of schizophrenia patients (Keefe et al. 1994; Laurent et al. 1999; Chen et al. 2000; Laurent et al. 2000; Dollfus et al. 2002; Appels et al. 2003). Even though negative findings have also been reported by studies on discordant twin samples (Goldberg et al. 1995; Cannon et al. 2000) and mixed first-degree relatives (Krabbendam et al. 2001), a recent meta-analysis (Snitz et al. 2006) demonstrated that among cognitive tests the Category Fluency test shows largest relative-control differences.

Almost all studies, including the present study, that have explored cognitive trait markers in the relatives of schizophrenia patients have shown consistent results in terms that the performances of relatives lie between those of patients and normal controls. However, the relative-control discriminatory powers of specific cognitive measures were variable. Cognitive measures which have shown significant relative-control differences in more than one previous study, e.g., verbal and spatial working memory tasks, verbal memory tasks, the Continuous Performance Test, and the Trail Making B Test (Sitskoorn et al. 2004; Snitz et al. 2006) showed only a trend of difference or did not discriminate between sibling and control groups in the present study. Several factors may account for discrepancies between studies, including differences in sample sizes, types of family members and the control subjects enrolled, types of neuropsychological tests applied, and others. The sizes of differences between siblings and controls found in the present study can be considered conservative, because levels of education and general ability (vocabulary subtest of K-WAIS), which were adjusted for during the analysis, are likely to have been influenced by a predisposition to schizophrenia. In addition, we applied the same clinical exclusion criteria to siblings and control, and these groups were not different in terms of psychopathologies as measured by PAI. Thus, the possibility that the subclinical psychopathologies of siblings might have inflated effect sizes also appears low. Our interpretation of data might also be conservative, as we applied the Bonferroni correction to comparisons of cognitive variables that were not completely independent and which correlated with at least one other measure.

The methodological limitations of the present study need to be considered. First, although we used a broad neuropsychological test battery, we did not test all aspects of cognitive functioning, for example, the social cognition domain of the MATRICS cognitive battery was not included, and for verbal and visual memory domains, we did not include simple memory tests with lower degrees of difficulty. Second, it was difficult to blind assessors to the group identities of subjects. The third limitation is that we could not trace all siblings of patients, and those who participated in this study may have differed from those who did not in terms of cognitive functioning or psychopathology.

Our findings suggest that healthy siblings of schizophrenia patients are significantly impaired on the verbal fluency. Within the limitations discussed before, this cognitive deficit might be associated with genetic liability to schizophrenia. In addition, more subtle deficits in verbal working memory (by the Digit Span backward test), and verbal and visual

memories showing trends with respect to sibling-control difference are also worth pursuing. To test the possibility as endophenotype of these cognitive variables, future studies on heritabilities are required to determine whether the normal variation of these cognitive variables is familial.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**ORIGINAL INVESTIGATION**

# Interleukin-10 gene promoter polymorphism in patients with schizophrenia in a region of East Turkey

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**Abstract**

Schizophrenia is one of the most severe psychiatric disorders, with a worldwide incidence of 1%. Immunological abnormalities have been found to be associated with schizophrenia for decades. Cytokines are key proteins involved in the immune system activation. Interleukin-10 (IL-10), an important immunoregulatory cytokine, is located on chromosome 1q31–32, a region previously reported to be linked to schizophrenia in genetic studies. In the present study it was aimed to examine the IL-10 gene promoter region's polymorphic variants in patients with schizophrenia in a population of the Elazig Region of East Anatolia, Turkey. Polymorphisms at position *−1082, −819 and −592 in the IL-10 promoter region were determined in 171 Turkish patients who were diagnosed with schizophrenia, based on the DSM-IV, and 168 healthy controls, by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). We analyzed allele, genotype, and haplotype distributions using a case–control association study. Genotyping was performed by RFLP. Statistically significant differences were observed in both allelic and genotypic frequencies of the −592A/C polymorphism (Allele,* $P = 0.034$, OR = 1.26, 95% CI 1.02–1.56; Genotype, $P = 0.048$), while the other two polymorphisms in distribution of the alleles and genotypes in patients with schizophrenia were not significantly different from those of controls ($P > 0.05$). Our results show a significant increase of GTA homozygotes (the high IL-10-producing haplotype) in schizophrenic patients compared to control subjects ($P = 0.0001$). These data suggest that the IL-10 gene promoter polymorphism may be one of the susceptibility factors to develop schizophrenia in the Turkish population, and apparently in all humans.

**Key words:** *Biological psychiatry, cytokines, genetics, polymorphism, schizophrenia*

## Introduction

Schizophrenia (MIM 181500) is a complex disorder characterized by disturbed patterns of thought, feelings and behavior. It affects nearly 1% of the population throughout the world (He et al. 2006). Epidemiological studies of schizophrenia indicate that both, genetic and environmental factors are likely to be involved in pathogenesis (Shirts et al. 2006; Ozbey et al. 2008). Several studies show a possible role of the immune system in the genesis of schizophrenia, and thereby support the hypothesis that non-specified infections and associated immune response may lead to schizophrenia (Cazzullo et al. 2003**;** Schosser et al. 2007). Schizophrenia-associated immune alterations include decreased mitogen-induced lymphocyte proliferation, increased numbers of total T or T-helper cells, and the presence of antibrain antibodies in serum. Changes in cytokines, cytokine receptors, and cytokine activity modifiers have been reported in the serum and cerebrospinal fluid of schizophrenic patients (Zhang et al. 2002; Müller et al. 2000).

Cytokines have been associated with various brain activities including immunological, neurochemical, neuroendocrine and stress-related behavioural activities, which have been suggested as the possible mechanisms in the pathogenesis of schizophrenia (Kronfol et al. 2000; Ozbey et al. 2008). Cytokines can also play a role in neurodevelopment, and can have a neuroprotective or neurodegenerative effect, depending on the cellular receptors and intracellular environment (Cazzullo et al. 2003; Shirts et al. 2006). Several groups have reported evidence of cytokine imbalance in schizophrenia (Müller et al.

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2000; Kim et al. 2004). Recently, there has been renewed interest in cytokine genes because of more consistent findings of elevated interleukin-10 (IL-10), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) with decreased interleukin-2 (IL-2) and interferon-γ, indicating predominance of cytokines stimulating the T-helper 2 (Th2) response over T-helper 1 (Th1) cytokines among schizophrenia patients (Kowalski et al. 2000; Müller et al. 2000; He et al. 2006; Shirts et al. 2006). These observations suggest that the Th1/Th2 imbalance is associated with schizophrenia, and that IL-10, the product of Th2, which has been demonstrated to selectively inhibit cytokine production by Th1 cells as IL-2, interferon (IFN)-γ, might be involved in the aetiology of schizophrenia (Kowalski et al. 2000; Yu et al. 2004; Ozbey et al. 2008).

In addition, IL-10 is also a cytokine mainly produced by immune cells (monocytes/macrophages, T- and B-lymphocytes) and exhibits diverse activities in various organs. It has been originally described as "cytokine synthesis inhibitory factor", having regulatory function during inflammation and is thought to counteract pro-inflammatory cytokines such as TNF or IL-1 (Schosser et al. 2007). In the recent study, it has been indicated that there might be a potential role for IL-10 in susceptibility to schizophrenia, though frequencies of allele and haplotype showed differences in different ethnic populations (Yu et al. 2004).

The IL-10 gene has been mapped to the chromosome 1q31–32 region, which is suggested to be linked to schizophrenia (Gurling et al. 2001). Genome scan metaanalysis of schizophrenia also implicated that the region may be a susceptibility locus (He at al. 2006). IL-10 gene is structured by five exons and four introns encoding for a 160-amino acid protein. Twin and family studies have suggested that ~75% of the variation in IL-10 production is genetically determined, and IL-10 production appears to be controlled at the transcriptional level. IL-10 5' flanking region contains numerous polymorphisms that directly influence the expression of the protein (Chiavetto et al. 2002). Three biallelic polymorphisms (−1082G/A, −819T/C, −592C/A) may have a functional effect on IL-10 transcription activation and cytokine production (Yu et al. 2004). These polymorphisms have been found to be in strong linkage disequilibrium in Caucasians, and a significant increase of −1082G allele and the haplotype GCC in schizophrenia was identified in Caucasians (Chiavetto et al. 2002) although not in the Korean population (Jun et al. 2003).

With these point of view, in the present study, the relationship of IL-10 gene promotor polymorphism at position −1082, −819 and −592 for schizophrenia was investigated by using restriction fragment length polymorphism (RFLP) in the Turkish population.

## Material and methods

### *Subjects*

In our hospital-based case-control study, 171 schizophrenic patients (94 males and 77 females) and 168 controls (92 males and 76 females) were recruited. The schizophrenia patient group was consisted of Turkish schizophrenia patients diagnosed by a psychiatrist using a semi-structured interview tool (SCID-I) according to schizophrenia criteria defined in Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association 1994). The mean age of the patients was 37.6 ± 10.8 years (mean ± SD) and the controls' mean age was 35.6 ± 14.8 years (mean ± SD).

Both, patients and controls consisted of entirely native unrelated Turkish population and were also recruited from the same geographic area, Elazig region, which is located in the east of Turkey. Written informed consent was obtained from all subjects after full description of the objectives and procedures of the present study.

From the original group, 27 subjects were excluded from the study after exclusion criteria were carried out. 15 subjects were excluded from the schizophrenia patient group and 12 were excluded from the control group. Before sampling, subjects were interviewed by a senior psychiatrist using a semi-structured scale (SCID- I). With this interview current psychiatric problems or past history of psychiatric disorders, including schizophrenia and schizoaffective disorders and other psychotic, affective disorders and substance abuse were excluded.

The exclusion criteria were as follows for the patient group: in addition to the psychiatric diseases mentioned above, mental illnesses, systemic diseases such as neurological illnesses, endocrine disorders, acute or chronic inflammatory infections and autoimmune diseases.

The control group consisted of unrelated healthy volunteers free from present, past, and family history (first-degree relatives) of psychiatric illness or substance abuse and the exclusion criteria for the patients. The group consisted of medical students and hospital personnels of Firat University Medical School. Subjects with the above-mentioned concomitant diseases were excluded from the study after relevant laboratory tests and consultations with appropriate physicians.

The present study was approved by the Ethical Committee of Firat University Medical School.

*DNA extraction*

Genomic DNA was extracted from whole blood by proteinase K using a method described elsewhere (Miller et al. 1988). The DNA samples were then stored at 4°C until used as a template DNA in polymerase chain reaction (PCR).

*Determination of IL-10 genotype*

A 1017-base pair long fragment from the IL-10 promoter −1082 A/G, −819 T/C and −592 A/C genotypes were determined by using a polymerase chain reaction (PCR)-restriction fragment length polymorphism, the PCR primers were designed based on described previously (Roh et al. 2002). Sequences of primers, restriction enzyme and sizes of resultant PCR products specific for IL-10 −1082, IL-10 −819 and IL-10 −592 are summarized in Table I. PCR reactions were carried out in a total volume of 25 μl, using approximately 100 ng DNA, 2.5 mmol/l $MgCl_2$, 200 mol/l dNTPs, 12.5 ng of each primer, and 0.5 units of Taq DNA polymerase (Promega, Madison, WI) in the PCR buffer provided by the manufacturer (10 mmol/l Tris–HCl, pH 9.0, and 50 mmol/l KCl). The PCR procedure was as follows: an initial denaturation step at 95°C for 5 min, and then amplified for 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, followed by a final extension step at 72°C for 10 min using the Gene Amp PCR System 9700 thermocycler (PE Applied BioSystems).

The genotype was determined using RFLP analysis. The IL-10 promoter polymorphisms were at positions −1082A/G (139 bp), −592A/C (412 bp) and −819T/C (209 bp) (He et al. 2006). Ten percent of PCR products were digested with *Mnl*I (New England Biolabs, Hitchin, UK) at 60°C for 2 h, with *Ras*I (New England Bio Labs) at 37°C for 3 h, with *Mae*III (Roche Diagnostics Gmbh, Mannheim, Germany) at 55°C for 2 h, respectively. Digests were separated on a 3% agarose gel with ethidium bromide. The electrophoresis was run for 2.5 h at 150 V. The ethidium bromide staining DNA fragments were analyzed on UV source using the image analysis system Kodak EDAS 120.

## Statistical analysis

Comparisons for sex, genotype, allele and haplotype frequencies for schizophrenic patients and healthy controls were performed using the $\chi^2$-test and Fisher's exact test when appropriate and odds ratios (OR) and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a particular allele and genotype. Data are presented as mean ± SD. Hardy–Weinberg equilibrium was tested with a goodness of fit $\chi^2$-test with 1 degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. Significance was accepted at $P<0.05$. All calculations were performed with the SPSS 15.0 statistical package.

## Results

The demographics of the cases and controls enrolled in this study are shown in Table II. There were no significant differences between the cases and controls for the mean age or gender distribution and this suggested that the matching based on these two variables was adequate.

Identification of the two alleles at each polymorphic site was performed by incubating PCR products with a restriction enzyme chosen to cut 1 of the 2 alleles (Table I), followed by electrophoresis on agarose gel (3%) (Figures 1–3). The IL-10 −1082 genotypes were named according to the presence or absence of the enzyme restriction sites. *Mnl*I AA, AG and GG are homozygous for the absence of the site

Table I. Primer sequences and reaction conditions for genotyping interleukin-10 polymorphisms

| Polymorphism | Primer sequence | No. of bases | Annealing temperature (°C) | Restriction enzyme | Product size (bp) | Genotype |
|---|---|---|---|---|---|---|
| −1082 (G/A) | F:5′ CTC GCT GCA ACC CAA CTG GC 3′ | 20 | 60 | *Mnl*I | 139 | AA |
| | R:5′ TCT TAC CTA TCC CTA CTT CC 3′ | 20 | | | 139,106 | AG |
| | | | | | 106 | GG |
| −819 (T/C) | F:5′ TCA TTC TAT GTG CTG GAG ATG G 3′ | 22 | 58 | *Mae*III | 209 | TT |
| | R:5′ TGG GGG AAG TGG GTA AGA GT 3′ | 20 | | | 209, 125, 84 | TC |
| | | | | | 125, 84 | CC |
| −592 (A/C) | F:5′ CCT AGG TCA CAG TGA CGT GG 3′ | 20 | 59 | *Rsa*I | 412, 236 | CC |
| | R:5′ GGT GAG CAC TAC CTG ACT AGC 3′ | 21 | | | 412, 236, 176 | CA |
| | | | | | 176 | AA |

Table II. Characteristics of the study populations.

| Variable | Shizophrenia patients ($n=171$) | Controls (n=168) |
|---|---|---|
| Age (years) | 37.6±10.8 | 35.6±14.8 |
| Sex | | |
| Male | 94 | 92 |
| Female | 77 | 76 |

(139 bp), heterozygous for the presence of the site (139/106/33 bp) and homozygous for the presence of the site (106/33 bp), respectively (Figure 1).

The IL-10 –819 genotypes were named according to the presence or absence of the enzyme restriction sites. *Mae*III CC, TC, and TT are homozygous for the presence of the site (125/84 bp), heterozygous for the presence of the site (209/125/84 bp), and homozygous for absence of the site (209 bp), respectively (Figure 2).

Similarly, the IL-10 –592 genotypes were named according to the presence or absence of the enzyme restriction sites. *Ras*I AA, AC, and CC are homozygous for the presence of the site (236/176 bp), heterozygous for the presence of the site (412/236/176 bp) and homozygous for absence of the site (412 bp), respectively (Figure 3).

We analysed −1082G/A, −819T/C, and −592A/C polymorphisms of the IL-10 gene promoter in 171 unrelated patients with schizophrenia and 168 healthy controls. All the markers of the IL-10 gene in our study were in Hardy–Weinberg equilibrium ($P>0.05$). We identified three biallelic polymorphisms at positions −1082, −819 and −592. The first polymorphism at position −1082 is a G to A substitution, the second at position −812 is C to T substitution and the third at position −592 a A to C substitution.

The frequencies of the AA, AG and GG genotypes of −1082 G/A were 85.6, 14.3 and 0.3% in controls and were 85.914.03 and 0% in cases, respectively. The frequencies of the A and G alleles of −1082 were 92.7 and 7.44% in controls and 92.9 and 7.01% in cases, respectively. The frequencies of the TT, TC and CC genotypes of −819 T/C were 42.8, 39.28 and 18.45% in controls and 45.6, 37.42% and 16.95% in cases, respectively. The frequencies of the T and C alleles of −819 were 62.44% and 38.09%

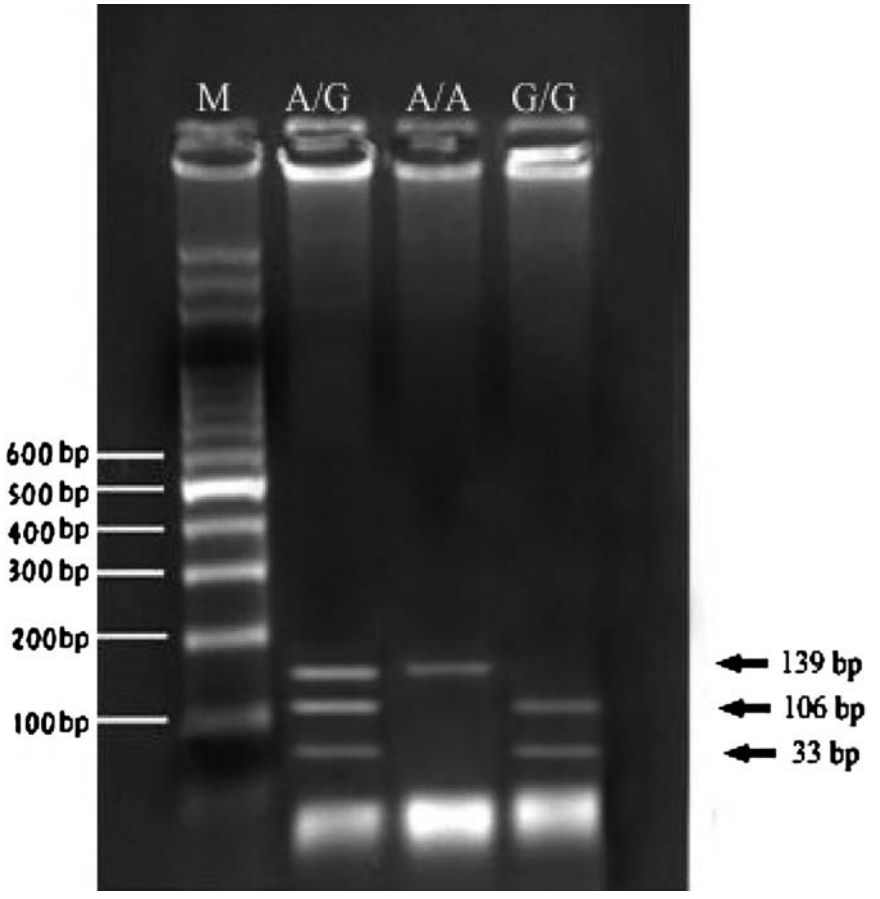


Figure 1. Representative agarose gel electrophoresis illustrating PCR products for the IL-10 promoter polymorphisms. IL-10 −1082G/A polymorphism: lane 1, M was loaded with appropriate molecular markers; lane 2, heterozygous subject, G allele cut with *Mnl*I generating 106- and 33-bp fragments, A allele does not cut; lane 3, homozygous AA subject; lane 4, homozygous GG subject.

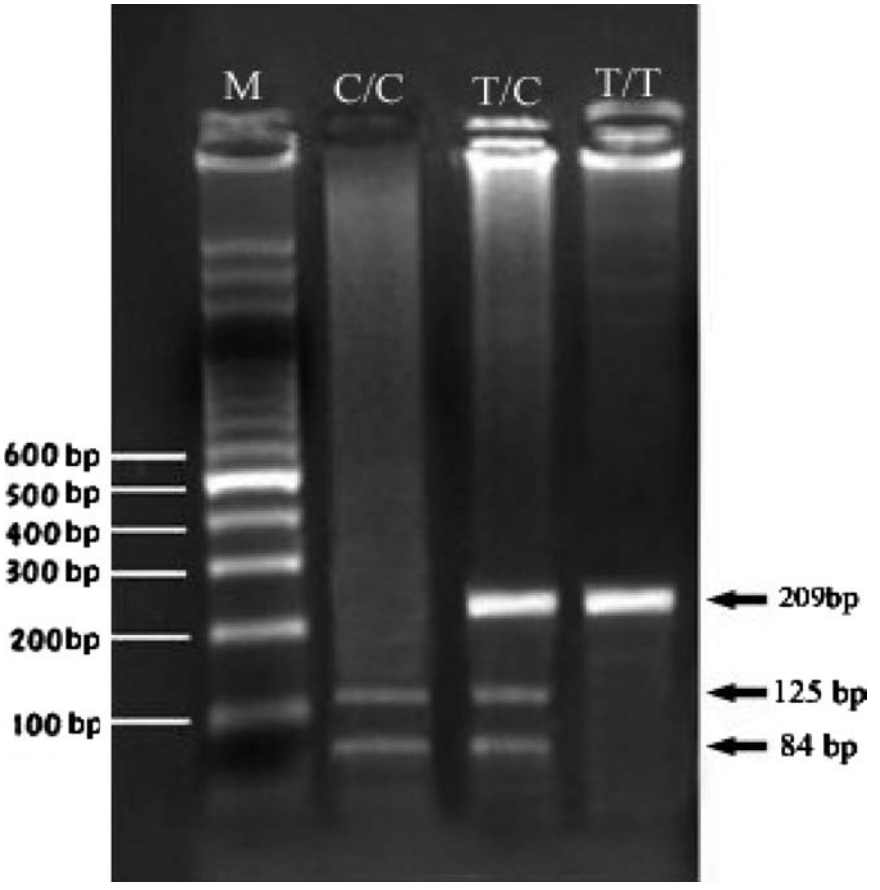


Figure 2. Representative agarose gel electrophoresis illustrating PCR products for the IL-10 promoter polymorphisms. IL-10 −819T/C polymorphism: lane 1, M was loaded with appropriate molecular markers; lane 2, homozygous CC subject, C allele cut with *Mae*III generating 125- and 84-bp fragments; lane 3, heterozygous subject; lane 4, homozygous TT subject, T allele does not cut.

in controls and were 64.31% and 35.66% in cases, respectively. The distribution of the genotype at the position −1082 and −819 between patients and controls was not significantly different as shown in Table III ($\chi^2 = 1.04$, df = 2, $P = 0.599$; $\chi^2 = 0.74$, df = 2, $P = 0.698$, respectively). No significant difference in the alleles at each position was found as shown in Table IV ($\chi^2 = 0.3$, df = 1, $P = 0.865$; $\chi^2 = 0.74$, df = 1, $P = 0.662$, respectively).

A modest increase in frequency of the −592 A allele in the patients (56.1%) compared to the control group (50.86%) was found ($\chi^2 = 4.44$, df = 1, $P = 0.034$; OR = 1.26, 95% CI 1.02–1.56). Although the genotype and allele frequencies of the IL-10 promoter −1082G/A and −819T/C polymorphisms in schizophrenia patients were not significantly different than those in controls ($P > 0.05$), the distribution of genotypes and allele frequencies

Table III. Frequencies of genotypes of the IL-10 gene promoter in patients with schizophrenia and in controls.

| | | | | Genotype | | |
|---|---|---|---|---|---|---|
| Polymorphisms | Genotype counts frequency | | | df | $\chi^2$ | *P* value |
| −1082G/A | AA | AG | GG | | | |
| Control 168 | 143(85.6) | 24(14.3) | 1 (0.3) | | | |
| Schizophrenia 171 | 147(85.9) | 24(14.03) | 0 (0.0) | 2 | 1.04 | 0.599 |
| −819T/C | TT | TC | CC | | | |
| Control 168 | 72(42.8) | 66(39.28) | 31 (18.45) | | | |
| Schizophrenia 171 | 78(45.6) | 64(37.42) | 29 (16.95) | 2 | 0.74 | 0.698 |
| −592A/C | AA | AC | CC | | | |
| Control 168 | 36(21.4) | 99(58.92) | 33 (20) | | | |
| Schizophrenia 171 | 52(30.4) | 88(51.4) | 31 (18.1) | 2 | 6.20 | 0.048 |

IL, interleukin.

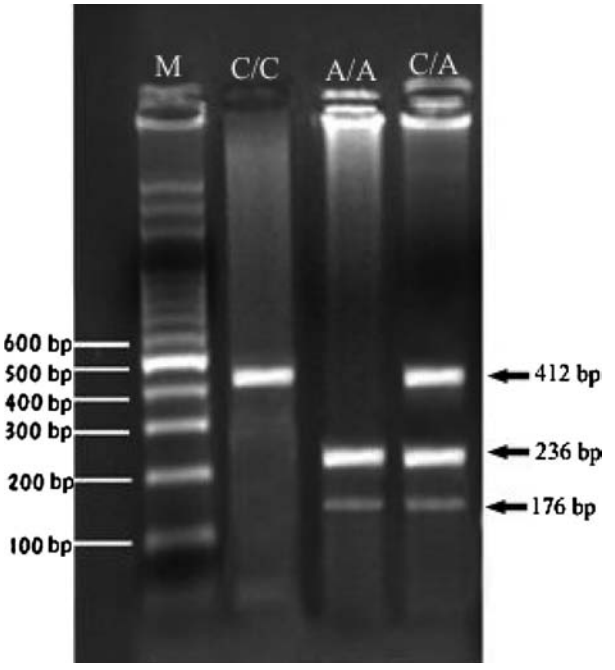


Figure 3. Representative agarose gel electrophoresis illustrating PCR products for the IL-10 promoter polymorphisms. IL-10 −592A/C polymorphism**:** lane 1, M was loaded with appropriate molecular markers; lane 2, homozygous CC subject, C allele does not cut with *Rsa*I; lane 3, homozygous AA subject, A allele cuts with *Rsa*I to generate 176- and 236-bp fragments; lane 4, heterozygous subject.

of the IL-10 promoter −592A/C polymorphism was different between the two groups ($\chi^2 = 6.20$, df = 2, $P = 0.048$).

In the analysis of haplotype distributions, we observed seven haplotypes with a frequency greater than 1%. When the haplotypes had been taken into account, we did find that the distribution of the haplotype defined by −1082G/A, −819T/C and −592A/C differed significantly between the patients with schizophrenia and control groups in our sample ($\chi^2 = 24.551$, df = 8, $P = 0.0009$) (Table V). We also found that the frequency of haplotype GTA was much higher in cases than in controls ($P = 0.0001$).

## Discussion

Previous genetic association studies of schizophrenia and IL-10 generally focused on single polymorphisms (Chiavetto et al. 2002; Jun et al. 2003; Yu et al. 2004; Shirts et al. 2006). In this study, three single nucleotide polymorphisms in the promoter region of the IL-10 gene were investigated to establish its role in susceptibility to schizophrenia in the Turkish population.

In their latest meta-analsis, Potvin et al. (2008) speculated that Th2 polarisation was dominant in schizophrenia and that there was an immunological association between schizophrenia and the Th2 cells. In other words, it has been declared that the physiopathology of schizophrenia or even its aetiology may be associated with an imbalance in inflammatory cytokines, leading to a decrease in Th1 and an increase in Th2 cytokine secretion (Müller et al. 2000). For this point of view, for schizophrenia pathogenesis, IL-10 is also an important anti-inflammatory cytokine that contributes to the dampening of the immune and inflammatory response (Potvin et al. 2008).

A previous study using Caucasian samples demonstrated that the −1082G was strongly associated with schizophrenia (Lazarus et al. 2002). In addition, in the previous results, it was hypothesized that an individual carrying the GA (−1082G and −592A alleles) may also have increased IL-10 expression for susceptibility to schizophrenia (Yu et al. 2004). According to our results, the IL-10 promoter polymorphism at position −1082G/A, −819T/C in patients with schizophrenia was not different from that of controls. However, in agreement with the findings of Yu et al. (2004), the −592A/C in the promoter region of the IL-10 gene displayed a significant association with schizophrenia ($P = 0.034$). This study gives more evidence to the possible involvement of the immune system in the pathogenesis of schizophrenia; however, it is more clear that immune system modulation is not the

Table IV. Frequencies of the alleles of the IL-10 gene promoter in patients with schizophrenia and in controls.

| | | | Allele | | |
|---|---|---|---|---|---|
| Polymorphisms | Allele counts frequency | | df | $\chi^2$ | *P* value |
| −1082G/A | A | G | | | |
| Control 168 | 92.7 | 7.44 | | | |
| Schizophrenia 171 | 92.9 | 7.01 | 1 | 0.3 | 0.865 |
| −819T/C | T | C | | | |
| Control 168 | 62.44 | 38.09 | | | |
| Schizophrenia 171 | 64.31 | 35.66 | 1 | 0.74 | 0.662 |
| −592A/C | A | C | | | |
| Control 168 | 50.86 | 49.46 | | | |
| Schizophrenia 171 | 56.1 | 43.8 | 1 | 4.44 | 0.034 |

IL, interleukin.

Table V. Estimated haplotype frequencies of interleukin-10 polymorphisms.

| Haplotype | −1082G/A | −819T/C | −592A/C | Schizophrenia ($N=171$) | Control ($N=168$) |
|---|---|---|---|---|---|
| 1 | A | C | C | 0.061 | 0.065 |
| 2 | A | T | A | 0.119 | 0.113 |
| 3 | A | T | C | 0.036 | 0.041 |
| 4 | A | C | A | 0.015 | 0.012 |
| 5 | G | C | C | 0.012 | 0.015 |
| 6 | G | T | C | 0.0002 | 0.003 |
| 7 | G | T | A | 0.005 | 0 |

$P=0.0009$; df $=8$; $\chi^2=24.551$.

result of the action of a single cytokine or cytokine gene but, rather, there is a complex interplay of many different molecules and genes.

When the haplotypes had been taken into account, in schizophrenia, it was suggested that the IL-10 gene promoter polymorphism displays ethnic differences (Lazarus et al. 2002). The three common haplotypes ATA, ACC and GCC were also the dominant ones in Caucasian populations (Rood et al. 1999). The commonest haplotype was ATA in Chinese, whereas in Caucasians it was GCC (Chiavetto et al. 2002; He at al. 2006). In addition, Jun et al. (2003) postulated that the GT haplotype was not observed in the Korean and Japanese samples, while the Chinese population was chacterized by the GT haplotype. We did also find that the distribution of the haplotype defined by −1082G/A, −819T/C and −592A/C differed significantly between the patients with schizophrenia and control groups (Table V). The haplotype GTA (−1082G, −819T and −592A alleles) revealed the greatest contribution to schizophrenia, which is in consistent with the hypothesis of Yu et al. (2004) that the haplotype containing −592A and −1082G may indicate susceptibility to schizophrenia. In the other words, the frequency of the −1082G, −819T and −592A alleles in the Chinese population (Jun et al. 2003; Yu et al. 2004), and the −1082G alleles in the Caucasian population (Chiavetto et al. 2002; He et al. 2006) showed similarity with our patient population. The GTA haplotype was the prevalent haplotype in our patient population. The explanation of the haplotype variation in different populations seems to be the ethnic background. We may say that our results also support the different genotypes in the IL-10 promoter region in diverse anthropological origins.

Because we had no facility to mesure any plasma interleukin level in our hospital, and because our aim was not to determine the association of the pathogenesis with the phenotypic characteristic of the Th2 cytokine IL-10, which is dominant in schizophrenia, and because typical and atypical antipsychotic drugs may modulate the production of cytokines (Yu et al. 2004), we have no results about the plasma IL-10 cytokine levels, which, beyond doubt, would make our study more valuable. Instead, we genotyped the IL-10 gene for determining its association with schizophrenia.

Our results suggest an important genetic risk due to polymorphisms in the promoter region of IL-10. Future replicate studies are needed to confirm this risk, and additional cytokine expression studies may benefit from verification of genetic effects. We found no other study referring to IL-10 at the molecular level in schizophrenia patients in Turkey. In our opinion, the results of Turkish schizophrenia patient's genotypes could help elucidate the characterization of the diversity between ethnic origins worldwide. Further, we believe that this study may provide important support for other molecular–genetic studies relating to genotypic variations of schizophrenia. Future independent studies are necessary to confirm these findings. We would like to encourage other researchers to carry out similar future studies of larger patient groups from different areas of Turkey, which may provide the genotypic variation of susceptible IL-10 genes for schizophrenia in the Turkish population.

Additionally, it may also provide a contribution to the basic knowledge of this issue and may help to create specific criteria for early diagnosis to generate information for genetic epidemiological research and even attempts at gene therapy in the future.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**ORIGINAL INVESTIGATION**

# Lack of association of three GRIN2B polymorphisms with bipolar disorder

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**Abstract**

We investigated three polymorphisms in the NMDA receptor 2B subunit gene (*GRIN2B*), involved in glutamatergic neurotransmission, as a candidate gene for bipolar disorder. In the study we included 419 patients with bipolar disorder. Consensus diagnosis by at least two psychiatrists was made, according to DSM-IV criteria, using SCID. The control group consisted of 487 healthy subjects. Genotypes for −200G/T, 366C/G and rs890G/T of *GRIN2B* polymorphisms were established by PCR-RFLP method. Linkage disequilibrium analysis was done with Haploview. Genotype distributions were in Hardy–Weinberg equilibrium for the three polymorphisms in the group of patients and control subjects. No association was found between the three polymorphisms and bipolar disorder. In linkage disequilibrium analysis we did not find linkage between the three polymorphisms of *GRIN2B* gene. The polymorphisms of *GRIN2B* gene analysed in this study are not likely to be associated with bipolar disorder.

**Key words:** *Association, polymorphism, GRIN2B gene, bipolar disorder*

## Introduction

In the aetiology of bipolar disorder, several receptor systems have been implicated. One of them is the glutamatergic receptor system, in particular the *N*-methyl-D-aspartate (NMDA) receptor complex. The neurotransmitter L-glutamate is the mediator of excitatory neurotransmission in the central nervous system and acts via activation of ionotropic ligand gated ion-channels and metabotropic G protein-coupled receptors (Hollmann et al. 1994; Nakanishi et al. 1998; Dingledine et al. 1999; Lerma et al. 2001). Its receptors are expressed throughout the nervous system, localized pre- and post-synaptically. They mediate neurotransmission at central synapses and are involved in the structural and functional plasticity of the synapse, including learning and memory processes (Scannevin and Huganir 2000). Depending on their pharmacological properties, ionotropic receptors subunits were grouped into three families according to different selectivity for the agonists: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-propionate (AMPA), and kainate (Schiffer 2002). The NMDA receptor is heteromeric ligand-gated ion channel that mediates synaptic function like long-term potentation and long-term depression (Ishii et al. 1993; Laurie et al. 1997; Lijam et al. 1997; Cull-Candy et al. 2001). There are two major families of NMDA receptor subunits: NR1 that is necessary for functional receptor formation and NR2 that consists of four subunits (A–D) and determines physiological and pharmacological specificity (Monyer et al. 1992). The NR2B subunit is essential for NMDA receptor structure and function (Krupp et al. 1996; Laube et al. 1997; Vicini et al. 1998).

The gene encoding NR2B subunit (GRIN2B) is localized on the short arm of chromosome 12

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(12p12) (Mandich et al. 1994; Schito et al. 1997), which is a chromosomal region linked to bipolar disorder (Faraone et al. 2004) and was identified as a candidate gene for both schizophrenia and bipolar disorder (Fallin et al. 2005). GRIN2B is expressed predominantly in forebrain structures, i.e. hippocampus, striatum, thalamus and olfactory bulb (Laurie et al. 1997). Its altered expression was found previously in bipolar disorder (Benes et al. 2001; Law and Deakin 2001; Scarr et al. 2003); however, this observation was not confirmed by the recent study (Martucci et al. 2006).

In the gene structure several polymorphisms were identified and analysed in psychiatric disorders such as schizophrenia, bipolar disorder and alcoholism. Ohtsuki et al. (2001) performed thorough mutation analysis of the gene region and showed higher frequency of G allele of 366C/G polymorphism in exon 2 in schizophrenic patients in comparison to the control group. Another study, performed by Martucci et al. (2006) in schizophrenic and bipolar patients, described three polymorphisms: −200T/G located in the 5′-UTR region and A5806C and T5988C, both localized within the 3′-UTR of gene. They found that the G allele of −200T/G polymorphism was associated with schizophrenia but not with bipolar disorder, whereas the C allele of T5988C polymorphism was transmitted more frequently in the bipolar patient sample. The A5806C polymorphism was not associated in any of the groups analysed. For bipolar disorder, there was one large study performed in the population of 337 Ashkenazi Jewish case–parent trios presenting the suggestive evidence for linkage of *GRIN2B* SNPs and haplotypes with this disease (Fallin et al. 2005), which was recently confirmed to be a candidate gene for bipolar disorder in the second-stage follow-up study in the same population (Avramopoulos et al. 2007). Moreover, in alcohol dependence, which is a frequent comorbid disorder in bipolar disease, two *GRIN2B* gene polymorphisms (G2108A and C2664T) were found to be associated with ethanol dependence (Wernicke et al. 2003).

We hypothesized that polymorphisms in the *GRIN2B* gene might lead to a dysfunction of the glutamate receptor system and alter the risk of developing bipolar disorder. To the best of our knowledge, there were only a few studies of *GRIN2B* polymorphisms with bipolar disorder. Therefore, we aimed to analyse the three polymorphisms of the *GRIN2B* gene, analysed previously in schizophrenia with positive results, in association with bipolar disorder.

## Subjects and methods

### *Patients*

The study was performed on 419 patients with bipolar disorder (176 males, with a mean age 43.5 years, SD = 13.5; 243 females, with a mean age of 46 years, SD = 13.5). Patients were recruited from inpatients from Wielkopolska region, considered as ethnically homogeneous (Cavalli-Sforza 1994), treated at the Department of Psychiatry, University of Medical Sciences in Poznan. Consensus diagnosis by at least two psychiatrists was made for each patient, according to DSM-IV and ICD-10 criteria (SCID) (First 1996).

### *Control group*

The control group consisted of 487 subjects (180 males, with a mean age 47 years, SD = 9.6 and 307 females, with a mean age 41 years, SD = 11.4). Control subjects were recruited from the group of healthy volunteers, blood donors, hospital staff and students of University of Medical Sciences in Poznan and Clinical Neuropsychology Unit, Collegium Medicum Bydgoszcz. They were not psychiatrically screened, which is the main limitation of this paper. The local ethics committee accepted the project.

After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the Local Bioethics Committee. The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

### *Genotyping*

DNA was extracted from 10 ml of EDTA anticoagulated whole blood using the salting out method (Miller et al. 1988). The SNP selection was based on their functionality (−200G/T) (Miyatake et al. 2002), and the fact that two other polymorphisms (rs7301328 and rs890) showed positive association with schizophrenia (Ohtsuki et al. 2001; Di Maria et al. 2004) which is suspected to have a common genetic background with bipolar disorder.

The three *GRIN2B* polymorphisms: 366C/G (Pro122) (rs7301328), rs890G/T and −200T/G (rs1019358) were analysed using PCR-RFLP analysis according to Ohtsuki et al. (2001) with minor modifications.

PCR was performed in PTC-200 (MJ Research) thermal cycler. A 15-μl amplification mixture for each polymorphism contained 250 ng of genomic DNA, 0.45 μM of each primer, 0.17 mM of each dNTP, 1.5 mM $MgCl_2$, 75 mM Tris–HCl, 20 mM $(NH_4)_2SO_4$, 0.01% Tween and 0.5 U of Taq DNA

polymerase (MBI Fermentas). Cycling conditions were: initial denaturation at 95°C for 2 min, followed by 30 cycles, with a profile of 94°C for 30 s, 56°C (366C/G polymorphism), 58°C (rs890G/T polymorphism) or 64°C (−200T/G) for 30 s, 72°C for 30 s, and final elongation at 72°C for 5 min. A volume of 5 μl of each PCR product (112 bp for 366C/G; 196 bp for rs890 G/T and 159 bp for −200T/G) was then digested overnight in a total volume of 10 μl at 37°C with 0.5 U of appropriate restriction endonuclease (*Taq*I for 366C/G polymorphism, *Psu*I for rs890G/T polymorphism and *Hpa*II for −200T/G polymorphism). After RFLP analysis, the following alleles were observed: for 366C/G polymorphism, allele C (uncut PCR product, 112 bp) and allele G (93 and 12 bp); for rs890G/T polymorphism, allele T (uncut PCR product, 196 bp) and allele C (136 and 60 bp); for −200T/G polymorphism, allele G (101, 37 and 21 bp) and allele T (122 and 37 bp). The uncut PCR products for 366C/G and rs890G/T were digested twice to confirm the results. The control of RFLP analysis was also performed (25% of randomly chosen samples from both groups).

### *Statistical analysis*

The two-tailed Pearson's chi-square ($\chi^2$) test and Fisher's exact test were used to test differences in the genotypic and allelic (respectively) distribution between the groups of bipolar patients and control subjects. Two-tailed power analysis was also performed. Calculations were performed using the computer programme Statistica, version 7.1. For polymorphisms containing <less than five observations per cell we performed the Freeman–Halton exact test using StatsDirect statistical software, v.2.6.2 (trial). Odds ratios were calculated using a demo of the GraphPad InStat 3 programme. We also performed linkage disequilibrium analysis of the analysed polymorphisms of *GRIN2B* gene using free online software Haploview version 3.2 from the website: http://www.broad.mit.edu/mpg/haploview/index.php (Barrett et al. 2005).

## Results

All analysed polymorphisms (366C/G, rs890G/T and −200G/T) were in Hardy–Weinberg equilibrium for all studied groups: $P=0.979$, $P=0.809$ and $P=0.473$, respectively in the control group; $P=0.680$, $P=0.568$ and $P=0.656$, respectively in the bipolar patients group.

For 366C/G we have not observed any significant differences in genotype distribution nor allele frequencies between the bipolar patients and the control subjects. For rs890G/T we also did not observe any significant differences between patients and the control group. For −200G/T we did not find any differences either for genotype or allele frequencies. The data are presented in Table I.

On the basis of the obtained results, we also performed linkage disequilibrium analysis of analysed polymorphisms of *GRIN2B* gene comparing the group of patients with the control subjects. However, we have not observed any linkage between the analysed polymorphisms (for C/G and G/T, $D'=0.11$, $r^2=0.007$; for T/G and C/G, $D'=0.084$, $r^2=0.004$; for T/G and G/T, $D'=0.002$, $r^2=0.000$), so no haplotype blocks could be created.

The power to detect an association for odds ratio that in our sample was evaluated for 1.0 was about 10% for each SNP.

## Discussion

In recent years, we have analysed the association of the polymorphisms of such genes as brain derived neurotrophic factor (BDNF) and glycogen synthase kinase-3 (GSK-3) with bipolar disorder. As to Val66Met *BDNF* polymorphism, we did not find an association with bipolar disorder (Skibinska et al. 2004). In addition, we observed an association between T-50C polymorphism of GSK-3 and bipolar disorder (Szczepankiewicz et al. 2006).

Table I. Genotype distributions and allele frequencies of the three analysed polymorphisms of *GRIN2B* gene for bipolar patients versus control group (figures in parentheses indicate percentages).

| Polymorphism | Bipolar patients | Control group | *P* value |
|---|---|---|---|
| 366C/G | | | |
| Genotypes | | | |
| CC | 141 (33.25) | 162 (33.26) | |
| CG | 210 (49.53) | 238 (48.87) | 0.736 |
| GG | 72 (16.98) | 87 (17.86) | |
| Alelles | | | |
| C | 492 (58.16) | 562 (57.70) | 0.849 |
| G | 354 (41.84) | 412 (42.30) | |
| rs890G/T | | | |
| Genotypes | | | |
| TT | 124 (31.47) | 148 (30.45) | |
| TG | 199 (50.51) | 238 (48.97) | 0.635 |
| GG | 71 (18.02) | 100(20.58) | |
| Alelles | | | |
| T | 447 (56.73) | 534 (54.94) | 0.469 |
| G | 341 (43.27) | 438 (45.06) | |
| −200T/G | | | |
| Genotypes | | | |
| GG | 129 (30.79) | 161 (33.33) | |
| GT | 211 (50.36) | 229 (47.41) | 0.647 |
| TT | 79 (18.85) | 93 (19.25) | |
| Alelles | | | |
| G | 469 (55.97) | 551 (57.04) | 0.668 |
| T | 369 (44.03) | 415 (42.96) | |

The main finding of the present study is lack of association of the three polymorphisms of *GRN2B* gene (−200G/T, 366C/G and rs890G/T) with bipolar disorder.

Most association studies analysing *GRIN2B* polymorphisms were performed in schizophrenia, whereas for bipolar disorder the data are less abundant. To our knowledge, there was only one study analysing three *GRIN2B* polymorphisms (−200T/G, A5806C, T5988C) in bipolar disorder (Martucci et al. 2006). They performed analysis on 318 nuclear families (comprising father, mother and bipolar proband) and found that the C allele of T5988C polymorphism was overtransmitted in the bipolar sample ($P=0.04$). For the two other polymorphisms ( −200T/G and A5806C) no association was found.

In our study, we have not found an association with any of the three *GRIN2B* polymorphisms analysed. This is consistent with the study by Martucci et al. (2006) as they also did not find an association with −200T/G polymorphism. The other two polymorphisms (366C/G and rs890G/T) were not analysed by them, so we cannot compare our results. However, association of *GRIN2B* gene polymorphisms was found in the Jewish Ashkenazi population and was confirmed recently by the same group. The different outcome may in part result from the fact that this study analysed only three SNPs from the GRIN2B gene, which might not have been sufficient to detect an association with bipolar disorder, but also may be due to the ethnic differences between the two populations (Jewish Ashkenazi and Polish).

In the linkage disequilibrium analysis, the obtained results suggested that *GRIN2B* polymorphisms analysed in this study are not linked as we observed very low $D'$ and $r^2$ values. Therefore, no haplotypes were generated and it seems unlikely that the studied polymorphisms may together affect susceptibility to bipolar disorder. Similar results were obtained in the study by Liou et al. (2007) as they also found only weak LD with the *GRIN2B* polymorphisms they have analysed (−200T/G, 366C/G and 2664C/T) in their sample of schizophrenic patients. However, this was not confirmed by Martucci et al. (2006), who found preferential transmission of haplotype T-C-C (−200T/G, 5806A/C and 5988T/C, respectively) in the bipolar sample of family trios. Also, in the study by Fallin et al. (2005) they found several haplotype blocks in the *GRIN2B* gene based on $D'$ values in the Ashkenazi Jewish trios. The inconsistent results in haplotype analysis may be due to the fact that different polymorphisms from several gene regions as well as different populations (family-based and unrelated case–controls) were analysed by the mentioned studies. One should also consider that three SNPs analysed here did not give the complete information about the linkage within the whole gene region, and therefore might not be the ones associated with bipolar disorder, which is the main limitation of this study. However, inconsistencies may also indicate that family studies are more informative for linkage disequilibrium detection if sample sizes for trios and case–control analyses are comparable.

Of the three analysed polymorphisms, only functionality of −200T/G has been described. It was found to be localized in the putative Sp1 binding site in the 5′ upstream region of the *GRIN2B* gene and different luciferase reporter activity was found for different alleles suggesting that this region is essential for *GRIN2B* regulation (Miyatake et al. 2002). One of the possible explanations of lack of association may be that other functional variants exist in the regulatory or coding region of the *GRIN2B* gene that are involved in the susceptibility to bipolar disorder. However, polymorphism screening over the coding sequence of the *GRIN2B* gene did not identify any functional or non-synonymous SNPs (Ohtsuki et al. 2001). Thus, further genetic and experimental studies with variants encompassing non-coding, untranslated and regulatory regions of *GRIN2B* gene would be required.

The main limitation of the present study is lack of psychiatric screening in the control group that might have yielded false-negative results and inconsistencies with other studies mentioned above. Lack of positive results may be also due to the limited sample size (∼500 cases and ∼500 controls). Although we doubt if further increase of sample size would much affect the results, it would be useful to consider this issue in future studies.

In conclusion, our findings indicate that none of the three studied polymorphisms in the *GRIN2B* gene is involved in the susceptibility to bipolar disorder in our group.

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## Statement of interest

There are no potential conflicts of interest for any of the authors to the subject of the report.

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**ORIGINAL INVESTIGATION**

# Lower rates of comorbidities in euthymic bipolar patients

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**Abstract**

*Objective.* This study assessed the frequency of axis I psychiatric comorbidities in euthymic bipolar patients and the clinical differences between patients with and without comorbidities. *Method.* In this study, 62 euthymic bipolar outpatients assessed using a clinical questionnaire underwent a structured diagnostic interview (SCID/CV – DSM-IV) as well as a symptoms evaluation (YMRS and HAM-D-17). *Results.* The lifetime frequency of patients with comorbidities was 27.4%. The most frequent comorbidities were anxiety disorders (33.7%), and the positive associated variables were more advanced age, the presence of a steady partner, a first episode of the depressive type and lifetime attempted suicide. *Conclusions.* The lower frequency of comorbidities found in our study in comparison with those described in the literature may be due to the evaluation restricted only to euthymic patients. This suggests the importance of assessing psychiatric comorbidity in bipolar individuals while not in acute phases of the disorder.

**Key words:** *Bipolar disorder, mood disorders, comorbidity, outpatients*

## Introduction

Bipolar disorder (BD) represents an important psychiatric disorder with an estimated prevalence ranging between 1 and 1.6% in the general population (Almeida-Filho et al. 1997; Jonas et al. 2003; American Psychiatric Association 2004). The co-occurrence of BD and psychiatric comorbidity is often reported in relation with bipolar patients, with prevalence varying between 50 and 70% in the majority of the studies (Pini et al. 1999; Vieta et al. 2001), but, in some cases, reaching more than 90% (Kessler et al. 1997). The presence of one or more comorbidities has been associated with a worse prognosis, more severe BD subtypes, early onset, mixed mood episodes, lower remission rates, worse functional performance, suicidal behaviour, and lower response to pharmacological treatment in the acute phase of the disease (Vieta et al. 2001; McIntyre and Keck 2006; Goodwin and Jamison 2007). Hence, detecting distinct patterns of comorbidities has an important implication in the treatment therapy and management of these patients (Pini et al. 1999).

However, the identified studies are not unanimous and the heterogeneous aspect of the applied methodologies may make it more difficult to understand these findings. In the literature, some of the most frequent axis I psychiatric comorbidities in bipolar patients are substance abuse/dependence and anxiety disorders (Sanches et al. 2005; Disalver et al. 2006).

A methodological challenge for studies evaluating psychiatric comorbidities in bipolar patients is their extensive array of clinical features, among which are symptoms of depression, mania, anxiety, and cognitive impairment. This issue may lead to overestimation of psychiatric comorbidity prevalence in an acute phase of BD (Vieta et al. 2001; Goodwin and Jamison 2007). However, most studies dealing with bipolar patients have failed to evaluate them in specific, well-defined periods of euthymia, described as the absence of acute episodes during a period of

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time, and low scores on both the Hamilton Depression Rating Scale (HAM-D-17) and the Young Mania Rating Scale (YMRS) (Vieta et al. 2001). Besides that, when euthymic or non-euthymic bipolar patients were compared with healthy control ones, the findings were inconsistent regarding the presence of structural or metabolic differences (Silverstone et al. 2003; Wu et al. 2004; Scherk et al. 2007).

The present study aims to assess the frequency of axis I psychiatric comorbidities in euthymic bipolar outpatients and to evaluate the possible clinical differences between patients with and without psychiatric comorbidities.

## Methods

### *Sample*

All bipolar patients were referred to this study from an outpatient clinic and were seen consecutively through the Affective Disorders Program at the General Hospital of the Federal University of Bahia, Brazil, from March 2005 to March 2007, having diagnostic criteria for BD, according to the DSM-IV. Seventy-one patients were subsequently recruited, nine of whom were excluded for not being in a euthymic phase (characterized by the absence of acute episodes in the previous 2 months and with HAM-D-17 and YMRS scores $<7$) at the time of the assessment (Hamilton 1960; Young et al. 1978).

### *Methods of measurement and data collection*

The study was approved by the local Medical Review Ethics Committee and performed in accordance with the ethical standards set in the 1964 Declaration of Helsinki. Additionally, all patients had provided written informed consent prior to their inclusion in the study.

All patients were evaluated through the Structured Clinical Interview with the DSM-IV axis I (SCID-I) (First et al. 1997), the HAM-D-17 and the YMRS scales. Once both the BD diagnosis and the euthymic state, were confirmed, a questionnaire was submitted in order to gather clinical and sociodemographic characteristics.

The interviews, as well as the scale application, were carried out simultaneously on the day of the patient's consultation. These were done individually, or when necessary, with the presence of family members. The data gathered from the patient's report were complemented, as seen fit, by data contained in the medical chart. The evaluations were carried out by three psychiatrists, trained in using the referred instruments, with more than 5 years of experience in the field.

The descriptive data from the sample, the frequency of psychiatric comorbidities and the most frequent comorbidities types were gathered in a preliminary results analysis. Subsequently, the sample was divided, according to the presence or absence of axis I psychiatric comorbidities, into two groups (17 patients who had presented at least one psychiatric comorbidity throughout his or her life, and 45 who had never been diagnosed with any psychiatric comorbidity).

### *Statistical analysis*

In the statistical analysis, the continued variables were compared using the Student's *t*-test or the Mann–Whitney test. The categorical variables were compared using the chi-squared test or the Fisher's exact test (when necessary) and the forces of association between the independent variables and the studied outcomes (psychiatric comorbidities) evaluated. The data were entered into the Software Statistical Package for Social Sciences (SPSS) version 13.0, and analysed in the EPIDATA version 3.1 R Package program version 2.3.1. Two-tailed values with $P<0.05$ were considered statistically significant.

## Results

A total of 62 patients – most of whom were type I bipolar patients (90.3%) – were included in the study; 13 (21%) were males and 49 (79%) were females. The patients' ages ranged between 19 and 74 years, the average age being 42 (SD = 12.73). Forty-eight of these patients (77.4%) did not have a steady partner and only 16 (25.8%) did any type of remunerated work; 45 of the patients in our sample (72.6%) were Afro-descendents.

The lifetime frequency of patients having axis I psychiatric comorbidities was 27.4% ($n=17$). Of these, 64.7% ($n=11$) presented a single comorbid diagnosis, 23.5% ($n=4$) had two comorbidities and 11.8% ($n=2$) had four other psychiatric diagnoses. The current frequency of psychiatric comorbidities was 24.2% ($n=15$). The most frequent comorbidities observed were anxiety disorders (33.7%), with 14 patients (22.6%) having at least one anxiety disorder (the current and lifetime frequency were the same), followed by substance abuse/dependence (8%), with five patients (8%) having at least one of these disorders (current frequency = 1.6%) and only one patient (1.6%) with a history of eating disorder in the past. The total sum and the percentages of the comorbidities were not equal to the total sum and percentages found in the frequency, as some patients presented more than one comorbidity (Table I).

Table I. Frequency of psychiatric comorbidities in 62 euthymic bipolar patients from a specialized centre in Brazil.

| Psychiatric comorbidities | *N* | % |
|---|---|---|
| Alcohol abuse or dependence | 4 | 6.4 |
| Non-alcoholic substance dependence | 1 | 1.6 |
| Obsessive-compulsive disorder | 1 | 1.6 |
| Pos-traumatic stress disorder | 1 | 1.6 |
| Generalized anxiety disorder | 8 | 12.9 |
| Generalized anxiety disorder – NOS | 2 | 3.2 |
| Hypochondria | 1 | 1.6 |
| Specific phobia | 4 | 6.4 |
| Panic disorder | 2 | 3.2 |
| Social phobia | 2 | 3.2 |
| Periodic compulsive eating disorder | 1 | 1.6 |

Numbers and percentages do not sum to total amounts because some subjects had more then one disorder.
NOS, no other specifications.

The comparison between bipolar patients, with or without comorbidites, did not show any statistically significant differences from the majority of the socio-demographic characteristics (Table II), except for more advanced age ($P=0.03$) and the presence of a steady partner ($P=0.04$), which were associated with the presence of comorbidity.

In the same manner, there were no significant differences between these groups in regards to most of the clinical characteristics (Table II). However, an association between the presence of psychiatric comorbidity with depression being the first episode ($P=0.03$) and a history of suicide attempt throughout life ($P=0.02$) was observed.

## Discussion

The frequency of lifetime axis I psychiatric comorbidity in bipolar patients found in our study (27.4%) was lower than previously described in literature (Kessler et al. 1997; Suppes et al. 2001). This difference may be explained by the fact that most studies included symptomatic bipolar patients, while we evaluated only euthymic patients. The use of such restrictive inclusion criteria, exclusively assessing euthymic patients, aimed to avoid a possible overestimation of comorbid diagnosis, a result of overlapping acute symptoms of mood episodes and symptoms belonging to a current comorbid pathology. Besides that, the use of psychiatrists and semi-structured diagnosis interviews may also have contributed to finding a lower frequency of psychiatric comorbidities in our study than in previous reports. A study also evaluating only euthymic patients (YMRS $<7$ and HAM-D-17 $<9$) reported a similar rate of psychiatric comorbidity (31%) to the one found in our study (Vieta et al. 2001).

An important issue observed in this study, which may have influenced the rate of comorbidities, is that the majority of the sample was formed by bipolar I (90.3%). The literature is controversial regarding the higher frequency of axis I comorbidity between bipolar I and II patients (Coryell et al. 1985; Cassano et al. 1992; Benazzi 1997; McElroy et al. 2001; Mantere et al. 2006). In reports of specific disorders, substance abuse is more common among bipolar I patients than among bipolar II patients. The opposite is true for anxiety and eating disorders (Goodwin and Jamison 2007). A recent study evaluating anxiety disorders comorbidity in euthymic bipolar patients also found no differences in the rates of current and lifetime anxiety between bipolar I and bipolar II patients (Albert et al. 2008). Although a large body of evidence derived from rigorously conducted studies supports the presence of higher comorbidity in bipolar patients, the difference between the comorbidity frequency in bipolar I and II patients is open to debate.

One interesting socio-demographic issue about our sample is the fact that almost 80% were females. According to literature, consultation rates and help-seeking patterns for women are consistently higher than those for men, especially in the case of emotional problems and depressive symptoms (Mackenzie et al. 2006; Judd et al. 2008). Empirical evidence shows that low treatment rates for men cannot be explained by better health, but must be attributed to a discrepancy between perception of need and help-seeking behavior (Möller-Leimkühler 2002). The results found in our study are compatible with those in other researches, which describe higher frequency of anxiety comorbidity in women with bipolar disorder than in equally afflicted men (Graaf et al. 2002; Gaudiano and Miller 2005; McIntyre et al. 2006). Moreover, it is important to highlight that gender is not the only variable that interferes with the frequency of comorbidity.

Another peculiar aspect of this present study is the high frequency of Afro-descendents (72.6%), which is similar to that observed in the general population of Salvador (78% of Afro-descendents), where the study was conducted (Instituto Brasileiro de Geografia e Estatística 2006). Reports revealed potential disparities in treatment, presence of psychosis, history of involuntary commitment and presence of comorbidity with substance use disorder among minorities (Afro-descendents and Hispanics) compared with Caucasian patients (Kennedy et al. 2004; Kilbourne et al. 2005). These findings could reflect either genuine ethnic differences in the presentation of BD or be explained by BD patients from ethnic/racial minority groups receiving less intensive specialized mental health treatment than

Table II. Socio-demographic and clinical differences between euthymic bipolar patients from a specialized center in Brazil with and without psychiatric comorbidities.

| | All BP ($N=62$) *N* (%)/Mean (SD) | BP with comorbidity ($N=17$) *N* (%)/Mean (SD) | BP without comorbidity ($N=45$) *N*(%)/Mean (SD) | *t*-Test or Chi-square *P* |
|---|---|---|---|---|
| Gender (males) | 13 (21%) | 6 (46.1%) | 7 (53.9%) | 0.15 |
| Actual age (years) | 42 (12.73) | 45.8 (11.21) | 39.9 (13.02) | 0.03 |
| Ethnicity (Afro-descendants) | 45 (72.6%) | 14 (31.1%) | 31 (68.9%) | 0.35 |
| Educational level (years) | 10.9 | 11.3 (2.44) | 10.7 (3.97) | 0.72 |
| Marital status | | | | 0.04 |
| Without Partner | 48 (77.4%) | 10 (20.8%) | 38 (79.2%) | |
| With Partner | 14 (22.6%) | 7 (50%) | 7 (50%) | |
| Currently working | | | | 1.00 |
| No | 46 (74.2%) | 13 (28.3%) | 33 (71.7%) | |
| Yes | 16 (25.8%) | 4 (25%) | 12 (75%) | |
| *Type of first episode | | | | 0.03 |
| Mania | 27 (43.5%) | 4 (14.8%) | 23 (85.2%) | |
| Depression | 33 (53.2%) | 13 (39.4%) | 20 (60.6%) | |
| Hospitalization (yes) | 51 (82.3%) | 12 (23.5%) | 39 (76.5%) | 0.15 |
| Rapid cycling (yes) | 3 (4.8%) | 0 (0%) | 3 (100%) | 0.55 |
| Electroconvulsotherapy (yes) | 6 (9.7%) | 1 (16.7%) | 5 (83.3%) | 1.00 |
| Suicide Attempt (yes) | 16 (25,8%) | 8 (50%) | 8 (50%) | 0.02 |
| Use of lithium during life (yes) | 54 (87.1%) | 14 (25.9%) | 40 (74.1%) | 0.67 |
| Family history of suicide (yes) | 15 (24.2%) | 6 (40%) | 9 (60%) | 0.31 |
| Family history of suicide attempt (yes) | 10 (16.1%) | 1 (10%) | 9 (90%) | 0.26 |
| Family history of BD (yes) | 10 (16.1%) | 2 (20%) | 8 (80%) | 0.71 |
| Suicidal ideation during life (yes) | 16 (25.8%) | 7 (43.7%) | 9 (56.3%) | 0.19 |
| Suicide plan during life (yes) | 12 (19.3%) | 5 (41.7%) | 7 (58.3%) | 0.30 |
| Presence of psychosis (yes) | 37 (59.7%) | 10 (27%) | 27 (73%) | 0.93 |
| Type of bipolar disorder | | | | 0.66 |
| Type I | 56 (90.3%) | 15 (26.8%) | 41 (73.2%) | |
| Type II+NOS | 6 (9.7%) | 2 (33.3%) | 4 (66.7%) | |
| Age at first episode (years) | 25.7 (12.01) | 27.2 (12.42) | 24.8 (11.66) | 0.54 |
| Time of illness evolution (years) | 16 (10.94) | 18.7 (11.20) | 15.1 (11.00) | 0.19 |
| Number of hospitalizations | 5.2 (5.34) | 5.5 (4.35) | 5.3 (5.74) | 0.41 |
| Number of episodes pole+ | 5.4 (5.09) | 6.6 (6.37) | 4.9 (4.50) | 0.42 |
| Number of episodes pole - | 4.8 (5.70) | 5.5 (6.60) | 4.7 (5.73) | 0.11 |

SD, standard deviation; BD, bipolar disorder; NOS, no other specification.
*Numbers and percentages do not sum up to total amounts because two of the subjects could not remember which type was their first episode, therefore only 60 subjects answered this question.

Caucasian patients (Gonzáles et al. 2007). However, little is known about ethnic differences in the clinical, psychosocial and treatment characteristics in bipolar patients; and the few studies having a majority of Afro-descendents did not evaluate co-morbidities (Fekadu et al. 2006; Kebede et al. 2006; Negash et al. 2005).

Regarding specific disorders, we found that anxiety disorders were the most frequent comorbid disorders (33.7%), with 22.6% of our sample having at least one anxiety disorder at some point in life. In literature, the lifetime prevalence of any anxiety disorder in bipolar patients is widely varied, and ranges from 18% in clinical studies to 93% in community-based epidemiological studies (McIntyre et al. 2006). To our knowledge, only two other studies evaluated anxiety disorders in euthymic bipolar patients, but one described only the current frequency of comorbid anxiety disorders (7%), while the other reported a current and lifetime frequency of bipolar patients with anxiety disorder (32.4 and 41%, respectively) (Vieta et al. 2001; Albert et al. 2008). In our study, we evaluated both the current and lifetime frequency of comorbid anxiety disorders (33.7%) and the current and lifetime frequency of bipolar patients with anxiety disorders (22.6%). This variation could be partly explained by some methodological differences in the diagnostic criteria used and the definition of euthymia, as being a period of time without acute symptoms and YMRS and HAM-D scores.

The question about whether substance misuse precedes, induces or follows bipolar disorder is still unresolved. Bipolar disorder preceded by substance misuse may represent a clinically milder subtype of bipolar illness. This subtype would be less primary and might be targeted earlier on by primary prevention with programs focused on substance misuse (Pacchiarotti et al. 2007). This could explain the low frequency of substance abuse and dependence

observed (8%), when compared to most of the articles described in literature (28–58%) (Frye and Salloum 2006). Another reason for these differences, besides the euthymia issue, may be the larger ratio of women in our sample. Women, in general, present a lower prevalence of this disorder, when compared to men (Brady and Randall 1999; Greenfield et al. 2007).

The presence of a steady partner, and a more advanced age were the socio-demographic variables most associated with comorbidity. One might suppose that in this population the presence of a steady partner does not necessarily mean having the social network element as a protective factor. In a community-based study conducted in Ethiopia, being married was associated with improvement of social functioning in recent-onset cases of BD. This association, however, was not observed in long-standing cases (Kebede et al. 2006). In our sample, most cases were not recent-onset, like the trend seen in Ethiopia. The influence of marital status in bipolar patients remains unclear, and the data is rare and controversial (Carpenter et al. 1995; Szádóczky et al. 1998). More advanced age could be chronologically associated with the greater probability of developing other psychiatric disorders at some point in time. The literature, in general, does not describe significant differences related to socio-demographic data between patients with and without psychiatric comorbidity (Pini et al. 1999; McElroy et al. 2001; Vieta et al. 2001).

The association between axis I psychiatric comorbidity and the first BD depressive-type episode and a history of suicide attempts is worth noting. These findings are similar to those found in literature (Pini et al. 1999; Vieta et al. 2001). With specific regard to suicide attempts, a recent systematic review showed that the presence of axis I psychiatric comorbidity was associated with a history of suicide attempts at some point in life (Hawton et al. 2005). Such association reinforces the idea that the presence of psychiatric comorbidity increases the severity of the illness and worsens the prognosis of bipolar patients (Colom et al. 2000).

The main limitation of this study was the restricted sample size. Although only a small portion of the total number of patients was excluded ($n = 9$; 14.5%), the small sample prevented us from carrying out a logistic regression aimed at removing confusing factors and decreasing the power of both generalization of the findings and increasing the risk of type II errors. Other important limitations were the retrospective data collection, which increased the risk of a memory bias.

In summary, our study showed that, in literature, the diagnosis of comorbidities in bipolar patients may be overestimated due to methodological issues, such as assessing these individuals in an acute phase of BD. Despite the lower frequency of comorbidities found in our sample, it is important to highlight that more than one-fourth of bipolar patients in our sample had at least one psychiatric comorbidity, the presence of which could negatively influence the prognosis of BD. Longitudinal studies with larger sample sizes are warranted to further investigate the impact of each psychiatric comorbidity in the course of BD and its role as a risk factor for suicide attempts.

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## Statement of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

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**ORIGINAL INVESTIGATON**

# Turning order into chaos through repetition and addition of elementary acts in obsessive-compulsive disorder (OCD)

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**Abstract**

A concept and methodology derived from an animal model provided the framework for a study of rituals in obsessive compulsive disorder (OCD) patients and yielded objective and observable criteria applicable for compulsive rituals across patients. The employed ethological approach should be able to reveal and identify a common structure underlying OCD rituals, pointing to shared psychopathology. Eleven OCD rituals performed by patients in their own home were videotaped and compared with the behaviour of healthy individuals instructed to perform the same rituals. The videotaped rituals were deconstructed into *visits* to specific locations or objects (ritual space), and to the *acts* performed at each location/object (ritual basic components). Quantitative analyses revealed that compulsiveness emanates from the expansion of repeats for some acts and visits, and from the addition of superfluous act types. Best discrimination between OCD and control rituals (90.9% success) was provided by the parameter "maximum of act repeats in a ritual" ($R^2 = 0.77$). It is suggested that the identified properties of compulsive behaviour are consistent with a recent hypothesis that ritualized behaviour shifts the individual's attention from a normal focus on structured actions to a pathological attraction onto the processing of basic acts, a shift that invariably overtaxes memory. Characteristics and mechanisms of compulsive rituals may prove useful in objective assessment of psychiatric disorders, behavioural therapy, and OCD nosology.

**Key words:** *Rituals, compulsive behaviour, CBT, spatiotemporal structure, OCD diagnosis*

## Introduction

Obsessive-compulsive disorder (OCD) is a severe, chronic psychiatric problem (Rapoport 1989a, 1990; Rasmussen et al. 1991) with a prevalence rate of 1–3% (Karno et al. 1988; Weissman et al. 1994). *Obsessions* are recurring, persistent thoughts, impulses, or images that inappropriately intrude into awareness and cause marked distress or anxiety. *Compulsions* are the need to repeat physical behaviours such as checking or mental behaviours such as counting things, and occur in response to an obsession with strictly applied rules (DMS-IV; APA 2000). Altogether, OCD is a disabling disorder with strong severe impact on life quality (El-Sayegh et al. 2003). Diagnosis, assessment, and treatment of OCD are based on various criteria and rating scales (Jenike et al. 1990; APA 2000). However, these rating scales are based on the patients' introspection or their self-reporting, thus providing little information on the form (structure) of compulsive behaviour. Considering the heterogeneity of OCD rituals (Lochner et al. 2003), it is difficult to use rating scales to analyze and quantify OCD rituals, except perhaps to obtain basic information on stress, duration of preoccupation, or relative prevalence of subtypes. Here we aimed to isolate the structural units of OCD rituals in order to enable a quantitative analysis across disparate rituals and thus allow a search for any common underlying mechanisms (Reed 1985; Insel 1988). The isolation of structural units may have bearing on OCD research by: (i) providing observable and measurable, relatively objective, characteristics of OCD rituals; and (ii) distinguishing between components that are unique to OCD rituals and components shared with normal performance of the same activities.

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## Materials and methods

### *Study population*

*OCD patients.* Five male and two female adult patients, meeting DSM–IV (APA 2000) and SCID criteria for OCD and who had compulsions with obvious motor rituals, were videotaped at their homes where they routinely perform rituals. Y-BOCS scores were 15–32, ages were 21–44, and durations of OCD were 10–31 years. After a complete description of the study to the subjects, written informed consent was obtained. The study was approved by the Helsinki Committee of Geha Mental Health Center.

*Control individuals.* A matched healthy individual of similar age and gender was asked to perform the same task that formed the OCD ritual. For example, if a patient described his/her ritual as "I lock my car", the respective control was requested to "lock your car".

### *Procedure*

The psychiatrist (H.H.) and experimenter arrived at the patient's home, described the research and asked again for the patient's approval to participate and be videotaped. It was stipulated that the rituals that the patient wished to display should be the frequently performed recent ones. After an explanation of the videotaping equipment and procedure, videotaping commenced and lasted for 1–2 h, with only the experimenter following the patient with a handheld camera. Patients reported a medium or higher degree of closeness of the videotaped ritual to their off-camera compulsion.

### *Data acquisition and analysis*

A ritual was defined as the set of movements performed to accomplish a task as specified by the patient. For instance, for a patient who described a motor performance as "car locking", this was taken by the experimenter to be its function and the ritual was labeled as "car locking". The ritual included all the movements displayed at the locations and/or objects within the functional action. The beginning and end of a ritual were determined by the patient's activity – for example, when the patient arrived at a specific location, performed a set of acts there, and departed from that location, the start and end of the ritual were respectively arrival and departure from the location. However, when a ritual was less clearly demarcated by spatial location, the patient's behaviour was used to identify the start and end of the ritual – for example, in a "nose-blowing" ritual, start was the point when the patient picked up a tissue paper and end was set when the patient cleaned his/her shirt from pieces of the tissue paper and switched to operating the TV with a remote control. In such a case, it was considered by the experimenter that the remote control is not part of the "nose-blowing" ritual. However, had the patient touched the remote control before cleaning the shirt, then touching the remote control would have been considered as part of the "nose-blowing" ritual. It should be noted that the ascribed function was used as a framework for analysis and not for deciphering the patients' motivation/understanding behind their performance.

Rituals were scored during playback of the video records. Briefly, we listed the *acts* or movements that comprised each ritual, and the *objects or locations* at which these acts were performed. This list served in scoring of behaviour with the *Observer* (Noldus Information Technology, The Netherlands), a software for ethological descriptions. For each ritual, the following 16 parameters were extracted from the videorecordings: duration and incidence of visits and acts performed at each object/location (mean, maximum and minimum values for each variable; = 12 parameters); the overall number of acts and visits in a ritual, the overall ritual duration, the number of act types, and the number of different objects/locations.

### *Statistics*

Because of the large variability in duration of different rituals (for example, a ritual of "arranging shirts in closet" is longer than a "lighting a cigarette" ritual), paired *t*-tests (two-tailed) were employed to compare OCD rituals and their matched controls for each of the 16 parameters. To examine which of these measures discriminates best between patients and controls, a binary logistic regression was employed where the dependent variable was group (patients versus controls) and the covariates were the 16 pertinent measures. The covariates as potential predictors were evaluated using the stepwise forward conditional method to assess the model, with probabilities for stepwise entry and removal set at 0.05 and 0.10, respectively. Calculations were performed using SPSS 14.0 for Windows. Alpha level was set to 0.05.

## Results

Table I depicts a ritual of an OCD patient and of a matched control individual. Columns stand for the objects at which the ritual was displayed. The sequence of acts in the ritual is given by reading from left to right and from top to bottom. As shown,

Table I. Exemplary OCD and control rituals. A ritual of lighting a cigarette by an OCD patient (top) and a respective control (bottom). Objects/locations at which acts were performed are depicted in the top row. The sequence of acts is depicted from left to right and top to bottom for each object/location.

| Cigarette packet | Cigarette | Match box | Match | Chess tool | Object on shelf | Ashtray |
|---|---|---|---|---|---|---|
| OCD patient | | | | | | |
| Put on table | | Put on table | | | | Put on table |
| Pick up | | Pick up | | | | |
| Hold | | Hold | | | | |
| Touch | | Touch | | | | |
| Hold | | Hold | | | | |
| | Pick one | | | | | |
| Put on table | | | | | | |
| | Hold | Put on table | | | | |
| | | Pick up | Take one | | | |
| | Put in mouth | | | | | |
| | | | Touch | | | |
| | | Hold | | | | |
| Turn | | | | | | |
| Touch | | Touch | | | | |
| | | Hold | | Touch | | |
| | | | Light | | | |
| | Hold in mouth | | | | | |
| | | Put on table | | | | |
| Pick up | | Pick up | | | | |
| | | | Throw | | | |
| | | | | | | Remove |
| Put on table | | Put on table | | | | |
| Flip sides | | Flip sides | | | | |
| Flip sides | | Flip sides | | | | Touch |
| | | | | {Touch} ×7 | | |
| | | | | | {Touch} ×4 | |
| | Hold | | | | | Put in the cigarette |
| Control | | | | | | |
| | Pick one | | | | | |
| | Put in mouth | | | | | |
| Put on table | | Pick up | Take one | | | |
| | | | Light | | | |
| | | | Throw | | | |
| | | Put on table | | | | |

the patient started by putting three items on the table: a cigarette packet, a match box, and an ashtray. The patient then picked up the cigarette packet and match box, holding them in one hand and touching them with the other hand. He then held both objects while looking at them, picked out a cigarette, placed the packet and matches back on the table, and so on. Overall, the patient displayed 52 acts over 64.3 s compared with only eight acts over 36.7 s in the control individual that was asked to light a cigarette. Table I thus exemplifies the structural units: *acts* that are performed during *visits* to objects (or locations), and together form the entire *ritual*.

Overall duration of rituals was longer in OCD patients than in their respective controls, but there were no significant differences between patients and controls in the mean duration of a visit to an object/location or in the mean duration of an act displayed in a ritual (Figure 1A). Implicit in these findings is the likelihood that the longer duration of OCD rituals was a result of repeating visits and/or acts. Indeed, the total number of visits to objects/locations and the total number of acts were both significantly higher in OCD compared with control rituals (Figure 1B,C). Similarly, the mean number of times that an object/location was visited, and the average frequency of acts performed during a visit were both significantly higher in OCD (Figure 1B,C). Finally, while in both OCD and control rituals the minimum number of visits in a ritual or of acts in a visit to an object/location was 1 (data not shown), the maximum numbers of such visits and acts were both significantly higher in OCD compared with control rituals (Figure 1B,C). Clearly, the longer duration of OCD rituals does not reflect a slowing down of movements but rather an increase in the repetition of acts and visits.

Figure 2 provides a more detailed portrait of the increase in repeats of visits and acts. It reveals that the incidence of repeated visits was more common in

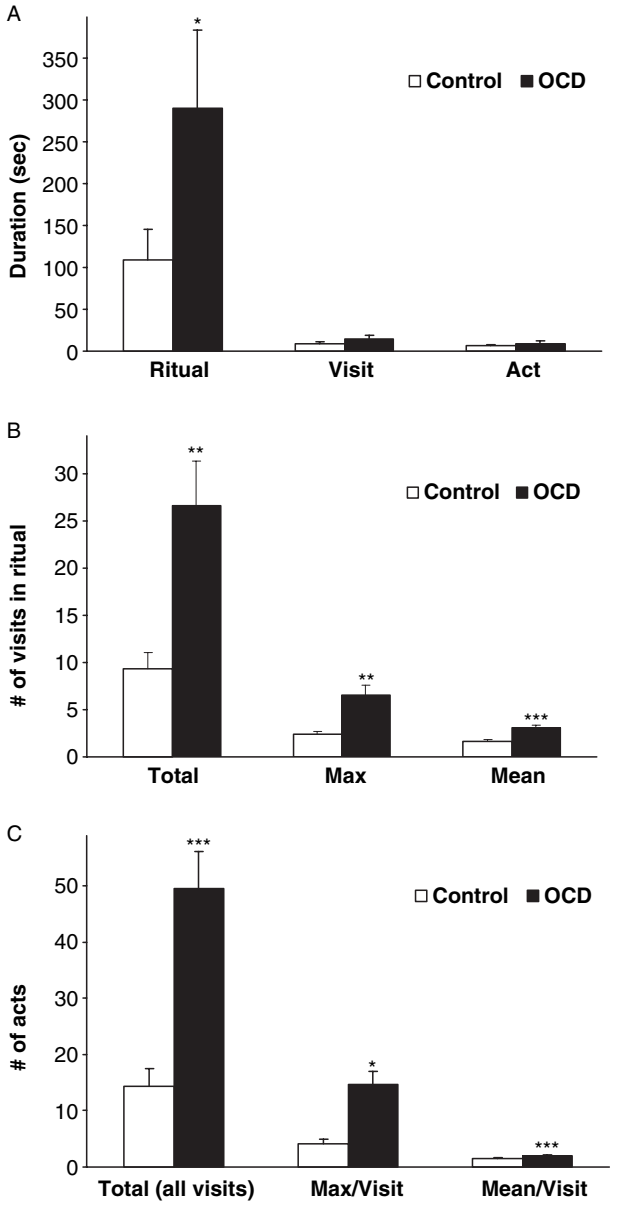


Figure 1. Parameters of three structural units that comprise rituals evaluated in 11 OCD and 11 control rituals. Values are means + SEM per ritual with each ritual contributing one value to the calculations. (A) Durations (s) of rituals, visits, and acts. Duration of OCD rituals was significantly longer than the duration of the corresponding rituals performed by controls ($t_{10} = 2.32$; $P < 0.05$). There were no significant differences in visit duration ($t_{10} = 0.57$; ns) and in act duration ($t_{10} = 0.22$; ns) of OCD rituals compared with their respective control rituals. (B) Total, maximum, and average number of visits in a ritual were all significantly higher in OCD compared with control rituals ($t_{10} = 3.53$, $P < 0.01$; $t_{10} = 3.08$, $P < 0.005$; and $t_{10} = 5.70$, $P < 0.0005$, respectively). (C) Total (left), maximum (center), and average (right) number of acts in a ritual were significantly higher in OCD compared with control rituals ($t_{10} = 5.01$, $P < 0.001$; $t_{10} = 2.74$, $P < 0.05$; and $t_{10} = 3.97$, $P < 0.005$, respectively).

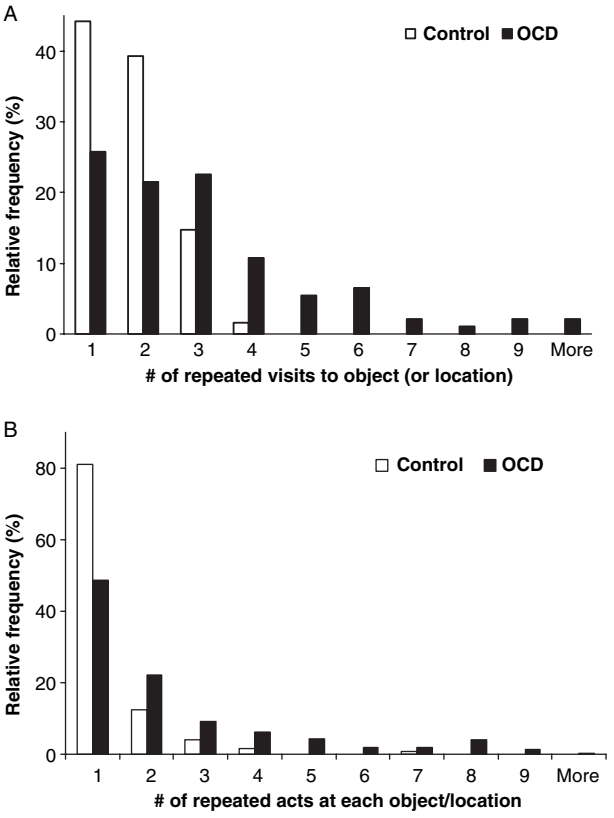


Figure 2. Relative frequency distribution of the number of visit repeats in rituals (a) and of act repeats at each object/location (b) in healthy individuals and OCD patients. (A) The relative frequency distribution of 293 OCD visits and 99 control visits (at all objects/locations and in all rituals). The proportions of objects/locations that were visited only once *versus* objects that were visited repeatedly (44.3 and 55.7% for controls, and 25.8 and 74.2% for OCD rituals, respectively) revealed a significant difference ($\chi^2 = 7.07$, df = 1; $P < 0.01$). (B) The relative frequency distribution of act repeats in the 545 OCD acts and 159 control acts over visits to the various objects/locations in all rituals. As for visits, the number of act repeats was also divided into two categories: one act repeat *versus* two or more act repeats. The proportions of 81.1 and 18.9% in controls compared with 48.7 and 51.3% in OCD patients were significantly different ($\chi^2 = 34.8$, df = 1; $P < 0.001$).

OCD rituals; moreover, it was not unusual for a visit to an object/location to be repeated even six or more times, an expansion rarely observed in controls. The repetition of acts showed a similarly great difference between patients and controls (Figure 2). Strikingly, whereas control individuals performed an act only once in more than 80% of the cases, more than 50% of the acts performed by OCD patients were repeated twice or more during a visit to an object/location.

A logistic regression was used to examine which variables discriminate best between OCD and control rituals. The "*maximum of act repeats in a ritual*" (Figure 1) was the only variable needed to provide an excellent fit to the model (Nagelkerke $R^2 = 0.773$, Wald statistic = 7.632, $P = 0.006$), with a correct classification of 90.9% ($\chi^2 = 19.1$, $P < 0.001$), misclassifying one of 11 control and one

Table II. Content of OCD and control rituals: Extraction of the variety of act types and objects/locations.

| | Overlapping | Unique to OCD | Unique to Control |
|---|---|---|---|
| Acts | | | |
| | Put on table | Touch | |
| | Pick up | Hold in mouth | |
| | Take one | Remove | |
| | Pick one | Flip sides | |
| | Put in mouth | Put in the cigarette | |
| | Light | Hold | |
| | Throw | | |
| Total | 7 | 6 | 0 |
| Objects/Locations | | | |
| | Cigarette packet | Ashtray | |
| | Cigarette | Chess tool | |
| | Match box | Object on shelf | |
| | Match | | |
| Total | 4 | 3 | 0 |

The content of the rituals shown in Table I are depicted here as overlapping acts or visits, or as those that were performed only in the OCD rituals or only in the control ritual. For example, the OCD ritual of lighting a cigarette (Table I) comprised seven objects, compared with only four in the control ritual. The control objects overlapped with those of the OCD ritual, which included additional three objects that were not visited in the control ritual. Two of these objects/locations, the chess tool and the object on the shelf, were entirely irrelevant to cigarette lighting. Similarly, there are seven overlapping act types in both OCD and control rituals of cigarette lighting, and additional six act types in OCD but not in the control ritual. Overall, the OCD ritual comprised almost twice the number of different objects/locations and act types compared with the control ritual.

of 11 OCD rituals. A similarly good fit to the model (Nagelkerke $R^2 = 0.837$, Wald statistic $= 4.352$, $P = 0.037$) and an identical classification rate was obtained if "*maximum number of visits in a ritual*" (Figure 1) was substituted for "*maximum of act repeats in a ritual*" (correct classification of 90.9%, $\chi^2 = 21.7$, $P < 0.001$).

In addition to the increased repeats of acts and visits, OCD rituals comprised an elevation in the number of distinct objects/locations visited and in the variety of act-types performed. The extraction of the variety of visits and acts is illustrated in Table II for the ritual shown in Table I. As shown, objects/locales can be subdivided into three distinct sets. One set is common to patients and controls, consisting of all objects/locales visited by both groups ("Overlapping" column in Table II). The other two sets are unique either to patients or to controls: one of them consists of objects/locales visited by patients but not by controls, and the other set consists of objects/locales visited by controls but not by the patients. These sets appear to represent superfluous visited objects/locations, unnecessary for ritual performance.

Figure 3 depicts the results of a similar analysis for the variety of acts performed in all 11 rituals. The common set is depicted in Figure 3 (gray bar) for all

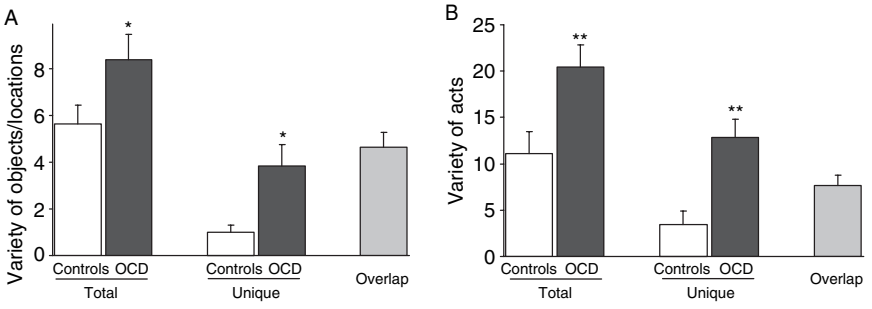


Figure 3. Variety of different objects/locations (a) and act types (b) in rituals. Values are means+SEM, calculated for 11 OCD and 11 control rituals. (A) Total number of different objects/locations in a ritual is depicted on the left for control and OCD rituals, and was significantly higher in OCD rituals than in their respective controls ($t_{10} = 2.95$; $P < 0.05$). The number of unique objects/locations visited by only controls or by only OCD patients is depicted in the central bars, and the number of overlapping objects/locations that were visited in both OCD and control rituals are depicted at the right (gray bar). (B) Total number of different acts in a ritual is depicted on the left for control (clear bars) and OCD (dark bars) rituals, and is twice as large in OCD than control rituals ($t_{10} = 3.7$; $P < 0.005$). The number of acts unique to controls or to only OCD patients is depicted in the central bars, and the number of overlapping acts that were performed in both OCD and control rituals are depicted at the right (gray bar).

rituals as the "overlapping set" and appears to represent a minimum (or necessary) set of objects/locales for the performance of the rituals. As shown, OCD patients had on average a significantly greater repertoire of acts than controls; moreover, they had significantly more superfluous acts compared to controls. Altogether, even when repetitions were excluded, there was a substantial difference between the composition of OCD and control rituals, and the number of unnecessary objects/locales was significantly higher in OCD rituals compared with control rituals. The greater variety in act types and objects/locations was another characteristic of OCD rituals, which together with repetitions accounted for the phenotype of compulsions.

Superfluous act types found in OCD but not in control rituals were typically "touch", "stare", "hold", "check", etc. (see OCD video-clip at Eilam, 2007). For example, in Table I, "touch", was the act with the highest incidence (18 times), and was performed 11 times at objects/locations unconnected to cigarette lighting. The act with the second highest incidence was "hold" and, like "touch", it may be considered as a type of checking. Together, these two acts comprised 44% of the acts in that OCD ritual, but were absent in the control ritual.

## Discussion

### *The structural units of compulsive behaviour in OCD patients: Rituals, visits to objects/locations, and acts at these objects/locations*

In the present study we scrutinized the spatiotemporal structure of OCD rituals. We began with defining a ritual as a set of acts that are linked to the purpose or function that a patient attributes to his/her activity. For analysis, rituals underwent segmentation into a sequence of visits to objects or locations, a segmentation that was based on two notions: first, this analytic approach has been utilized in studying rituals in an animal model of OCD (Szechtman et al. 1998; Eilam et al. 2005); and second, this ritual parsing follows the notion that objects/locations have a pivotal role in segmentation of events (Zacks et al. 2001). Visit content consisted of basic acts (or movements) performed at a specific object/location. Thus, *acts* are the elemental components and *visits* are the higher level of organization – a larger segment comprised of several acts. The sequence of visits comprises the *ritual* and is the germane behavioural unit of compulsive behaviour. A benefit of this concept of structural units lies in overcoming the problem of comparing different rituals (Mataix-Cols et al. 2005). Specifically, how can one compare and quantify different rituals such as hand washing, door locking, or arranging shirts? As shown in this study, the segmentation into visits and acts provides a common means for such quantification. In the following we argue that parsing of rituals into visits and acts has a bearing on three perspectives of OCD. First, it provides a means to identify and quantify changes in rituals, highlighting the involved mechanisms; second, identifying such mechanisms enables conceptualization of the malfunctioning in OCD; and, third, the structural units and the conceptual change that characterize OCD rituals may be utilized in improving diagnostic and therapeutic tools.

### *Why do OCD rituals look "repetitive and normal"?*

Comparing rituals in OCD patients and in healthy controls revealed three characteristics: (i) OCD rituals comprised more repetitions of acts and visits; (ii) OCD rituals comprised surplus acts and visits, some irrelevant to the patient-ascribed function of the ritual; (iii) the duration of visits and acts in OCD did not differ from that of controls, and the prolonged duration of the OCD ritual was due to repeated performance of some components. Because of the latter characteristic, compulsive rituals have been intuitively perceived as repetitive. OCD rituals have also been considered normal because, except for repetitions, it is usually hard to discern in what sense OCD rituals may be abnormal (Rapoport 1989a,b, 1990). We argue that the apparent "normality" in OCD rituals stems from: (i) OCD rituals share a large overlap (more than 50%) of act types and visits with control behaviour; (ii) the duration of visits and acts do not differ in OCD and control rituals. Taking together these two properties, it is sometimes hard to discern what is strange in OCD rituals until one notices the repetitions and the surplus act types that underlie the compulsive ritual (see OCD video-clip at Eilam, 2007).

In addition to repetitions, OCD rituals had twice the number of act types of their respective controls (Table II). Moreover, surplus act types were typically "check", "touch", "clean", etc. These act types were repeated excessively and were performed during frequent visits to a few objects/locations at a rate that was not matched in controls, and perhaps account for labeling OCD rituals as, for example, "checking ritual" (Figure 2). In all, the present study provides a seminal notion on the structural units of compulsive rituals, and by quantitative and qualitative inspection of these units it is possible to discern in what sense compulsive rituals look repetitive but normal.

*Which perceptual and conceptual processes may explain compulsive rituals?*

In a recent account, Boyer and Lienard (2006) suggested that two systems may account for the occurrence of many ritualized behaviours, including OCD rituals. One system is of "inferred threats to fitness"; the second is of "action-parsing". The data collected here strongly support the hypothesis for the involvement of an action-parsing system, where rituals develop by virtue of a shifted focus in action-parsing from mid-ranged actions to finer movements (Boyer and Lienard 2006). Specifically, the authors suggested that behaviour may be described at three levels: (i) simple gestures; (ii) behavioural episodes; and (iii) scripts. For example, in a script of "getting dressed for dinner", there are mid-range episodes such as "put on a shirt", "put on shoes", etc. Each of these episodes consists of simpler (basic) gestures; for example, "put on shoes" comprises "take" the shoes, "insert" the feet into the shoes, and "tie" the laces. Boyer and Lienard (2006) review evidence that most people spontaneously focus on the mid-level of episodes. Indeed, "getting dressed for dinner" is generally described by putting on shoes, shirt, etc., and not by the lower level of gestures. In light of this concept, Boyer and Lienard (2006) hypothesized that an excessive focus on the lower level of basic gestures is what characterizes rituals (including OCD rituals). Indeed, this is precisely what was revealed in the present study. Parsing categories become obvious when behaviour was described in the context of the acts that are performed at a specific location or object (Eilam et al. 2006). In OCD rituals, the focus on certain acts is implicit in the inflated rate of act types and act repetition, reflecting a descent to gesture level.

Zacks et al. (2001) consider an event as a segment of time at a given location that is conceived by an observer to have a beginning and an end. Accordingly, data-parsing is a way of bringing order into a continuous flow of events by establishing hierarchical structural units. Spontaneous focusing at the mid-range units (Zacks et al. 2001) is a form of "chunking" of elementary (basic) gestures (acts), and chunking is a way of simplifying perception and conception. In contrast, a shift to the elementary level involves increased complexity. Hence, Boyer and Lienard (2006) suggested that compulsive behaviour entails a momentary overloading or swamping of working memory, especially when act sequences are represented at the lower parsing level. The authors suggested that such swamping of memory accounts for the temporary relief of anxiety afforded by performance of compulsive rituals. Although the present study does provide strong support for the existence of a focus on lower levels of data-parsing, it does not help to illuminate whether the functional significance of compulsive rituals is indeed to provide relief from anxiety by overtaxing working memory.

*Practical implications of the present analysis of rituals*

Comparing the various parameters measured in OCD rituals revealed that of all the parameters, the "*maximum of act repeats in a ritual*" was the only variable needed to discriminate between OCD and control rituals. The distinct and non-overlapping range of this parameter ($4.1 \pm 0.9$ in control vs. $14.7 \pm 2.4$ in OCD rituals) may therefore provide a simple diagnostic tool: counting more than 10 repetitions of a specific act seems to reliably identify an OCD ritual. The maximum number of visits to an object/location may be another distinctive parameter ($2.4 \pm 0.3$ in control vs. $6.6 \pm 1.0$ in OCD rituals). Thus, the present study delineates observable categories that unequivocally distinguish between rituals of OCD patients and healthy individuals. However, the specificity and implementation of these parameters as OCD predictors needs further elaboration and verification.

The present study demonstrated a coupling of compulsive rituals and specific objects/locations (Eilam et al. 2006). This spatial component of OCD may be of value in behavioural therapy (BT). For example, a patient with a coffee-making ritual had a way of throwing a spoon filled with coffee from a height of 30 cm into the empty cup, claiming that this prevented dirt. That patient may be asked to gradually lower the spoon until pouring the coffee directly into the cup without the spoon-throwing activity. Other therapeutic implementations of the coupling between behaviour and a specific environment could be that of moving to another environment or removing the object at which rituals converge (Penzel 2000, 2003). Thus, attending to the spatial component in compulsive rituals may add means in BT of OCD, in utilizing the spatial-environmental component in exposure and response prevention. Moreover, exposing OCD patients to their own video records may have value in BT. We base this suggestion on our striking experience, when patients who could describe their rituals precisely down to the very small detail were surprised when watching their ritual on video. It is as if there is a gap between the patients' egodystonic awareness of what they do, and observing how that behaviour looks on video. Consequently, exposure of OCD patients to video recordings of their own rituals may increase the efficacy of BT.

## Epilogue

The present analyses of compulsive behaviour provide novel and heuristic segmentation of OCD rituals into structural units, units that may also help in nosology of OC spectrum disorders and OCD (Jenike 1990; Hollander 1993; McElroy et al. 1994; Hollander et al. 2005). Another diagnostic application of these parameters may lie in resolving the debate over whether complex tics in Tourette's syndrome are compulsions or tics. The present account on OCD rituals has identified objective tools for assessment of observable compulsions, and these may be useful in other fields of OCD research. For example, the fine structural units of compulsions (acts) may be performed during parallel brain imaging. Similarly, watching personal video recording of rituals may promote the efficacy of cognitive behavioural therapy. Altogether, the present results provide measurable knowledge that may prove useful in OCD research. Finally, Zacks and Tversky (2001) suggested that the human mind is gifted in bringing order to chaos by perceiving activity as consisting of discrete events that have some orderly relations. The present results illustrate, however, that the OCD mind may also generate chaos out of order (Kubota et al. 2002) by the parsing of activity at the more basic level of discrete acts (gestures) (Boyer and Lienard 2006) and of affinity between acts and specific objects/locations.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**ORIGINAL INVESTIGATION**

# Co-morbidity of bipolar disorder in children and adolescents with attention deficit/hyperactivity disorder (ADHD) in an outpatient Turkish sample

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**Abstract**

This study aimed to assess the prevalence of bipolar disorder (BPD) in children and adolescents with attention deficit hyperactivity disorder (ADHD), and to compare the clinical characteristics of a group with ADHD with a group with co-morbidity of ADHD and BPD. The study includes 121 individuals, aged 6–16 years, with a diagnosis of ADHD. Co-morbidity of BPD was evaluated using the Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime version (K-SADS-PL) and the Parent-Young Mania Rating Scale (P-YMRS). The Child Behavior Checklist (CBCL) was used to assess psychopathology in two groups. Ten children (8.3%) in the ADHD sample received the additional diagnosis of BPD. The ADHD +BPD group had significantly higher scores than the ADHD group on withdrawn, anxiety/depression, social problems, thought problems, attention problems, aggression, externalization, total score items of CBCL, and on the P-YMRS. It could be concluded that BPD is not a rare co-morbid condition in children with diagnosis of ADHD and subjects with this co-morbidity show more severe psychopathology than subjects with pure ADHD. Differential diagnosis of BPD disorder in subjects with ADHD seems crucial in establishing an effective treatment program, and therefore improving mental health outcomes.

**Key words:** *Attention deficit/hyperactivity disorder, bipolar disorder, comorbidity*

## Introduction

Attention deficit/hyperactivity disorder (ADHD) consists of a persistent pattern of inattention, hyperactivity and impulsivity (APA 2000). It is well established that ADHD is frequently comorbid with other psychiatric disorders such as oppositional defiant disorder, conduct disorder, anxiety disorders, depression, tic disorders, substance abuse and bipolar disorders (AACAP 2007). Perhaps the most diagnostically challenging and controversial of co-occurring disorders in ADHD is bipolar disorder (BPD). When they occur in combination, these conditions often complicate the assessment process, clinical diagnosis and treatment. Literature reviews indicate that this topic has received increased clinical and scientific attention in recent years. Many studies have been conducted to assess the rate of this co-morbidity, phenomenology, clinical characteristics and differential diagnosis with BPD.

Despite the increasing amount of research in paediatric bipolar disorder, there is still confusion and controversy surrounding clinical diagnosis of this condition. This confusion is partly due to the lack of awareness and clinical bias, in certain setting, against the diagnosis of mania in children (Geller et al. 1998; Biederman 2003). Symptom overlap between BPD and other childhood-onset psychiatric disorders is another important factor contributing to diagnostic difficulties (Diler et al. 2007). Differential diagnosis with ADHD is an especially crucial issue. Since several symptoms are shared by ADHD and BPD, the debate regarding the validity of paediatric bipolar disorder has shifted from whether paediatric bipolar disorder exists to which symptoms should be considered cardinal symptoms of bipolar disorder in children (Mick et al. 2005). Irritability, impulsivity, hyperactivity, and distractibility are very frequent in both disorders, therefore not specific for mania (Mick et al. 2005). Elation, grandiosity, racing

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thoughts, decreased need for sleep and hypersexuality are common in bipolar disorder and can discriminate BPD from ADHD (Geller et al. 2002; Pavuluri et al. 2005).

Additionally, several instruments such as the Child Behavior Checklist (CBCL) (Achenbach 1991), Young Mania Rating Scale (Young et al. 1978), and the Child Mania Rating Scale (Pavuluri et al. 2006) are considered effective instruments for helping clinicians differentiate mania in an ADHD population.

Results of studies evaluating the effectiveness of the CBCL in differential diagnosis of paediatric bipolar disorder are controversial. A few studies proposed a CBCL paediatric bipolar profile (BPD) based on the significant elevation in the anxiety/depression, attention problems and aggression subscales (Mick et al. 2003; Farone et al. 2005). However, the predictive validity of CBCL-BPD in identifying BPD has been recently questioned (Volk and Todd 2007; Meyer et al. 2008). Some authors have attempted to differentiate ADHD from mania using the Young Mania Rating Scale and the Parent-Young Mania Rating Scale (Fristad et al. 1995; Diler et al. 2007). However, other investigators stated that this instrument is designed to quantify rather than diagnose an episode of mania (Carlson and Kelly 1998).

Regarding the rate of this co morbidity, several studies have investigated the rate of BPD in ADHD and vice versa. Six population-based studies evaluated the co-occurrence of ADHD and BPD in youths, three of which showed elevated rates of BPD in participants who have ADHD; however, three failed to show an association between the two disorders (Galanter and Leibenluft 2008). Clinical studies have found rates of BPD ranging from 8.2 to 23% in youths who have ADHD (Wozniak et al. 1995; Butler et al. 1995; Biederman et al. 1996; Diler et al. 2007). Regarding the rate of ADHD in clinic referred subjects with BPD, rates vary widely across samples, from 4 to 98% (Kowatch et al. 2005; Birmaher et al. 2006; Jaideep et al. 2006). Setting, participants age, age of onset of BPD, referral source and ascertainment bias affect these rates.

The relatively high degree of co-occurrence between these disorders raises the question of probable common aetiological factors. Several studies proposed the role of dopamine transporter genes in ADHD (Cook et al. 1995; Faraone et al. 2005) and BPD (Greenwood et al. 2006). Further, the involvement of the brain-derived neutrophic factor (BDNF) gene in the pathogenesis of ADHD (Kent et al. 2005) and BPD (Geller et al. 2004) was reported. Family studies strongly suggest that paediatric onset bipolar disorder has a strong familial component. Some investigators documented that bipolar disorder and ADHD co segregated in relatives support the hypothesis that ADHD and co morbid bipolar disorder may represent a unique developmental subtype of bipolar disorder (Faraone et al. 1997; Biederman et al. 2004).

Despite increasing research on BPD, paediatric bipolar disorder is still unrecognized in most countries other than the USA. BPD research from outside of the USA is limited and according to some studies, there is significant ethnic differences in the prevalence of BPD (Youngstrom et al. 2005) and clinical presentation of BPD (Kennedy et al. 2004). These findings underlie the need for further assessment of bipolar disorder in children with different ethnic origin.

This study aimed to: (a) assess the prevalence of BPD in children and adolescents with diagnosis of ADHD using a semi-structured interview; (b) compare the clinical characteristics of children with ADHD with a group with ADHD and comorbid BPD.

## Materials and methods

### *Subjects*

The sample consisted of 121 individuals aged 6–16 years (23 girls, 98 boys), with diagnosis of ADHD who had been referred to the ADHD unit at the Child Psychiatry Department of Istanbul School of Medicine, Istanbul University (IUITF), Turkey, between October 2006 and September 2007. All were referred from general out-patient child psychiatry clinic. All consecutive referrals who met the criteria mentioned below were included in the study.

Inclusion criteria were: (1) consensus for the diagnosis of ADHD by at least two child psychiatrists; (2) parent's informed consent and patient's assent to be included in the study.

Exclusion criteria were presence of chronic medical illness, any sensory-motor disability, neurological disorder, diagnosis of pervasive developmental disorder and other developmental disorders.

### *Procedures*

The study protocol was reviewed and approved by the Institutional Review Board at Istanbul School of Medicine and all parents gave written consent for their child's participation in the study. Diagnosis of ADHD was made by the consensus of two child psychiatrists (NMM and GL) according to DSM-IV-TR criteria.

All individuals were referred from the general outpatient clinic to the ADHD unit. Diagnosis of ADHD was reconfirmed using DSM-IV-TR criteria. All parents completed the Child Behavior Checklist

(CBCL) 4–18 and the Parent-Young Mania Rating Scale (P-YMRS) prior to the structured clinical interview. Comorbidity of Bipolar disorder was detected using the Turkish version of Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime version (K-SADS-PL) (Gökler et al. 2004). The K-SADS-PL was administered to the mother and the child separately. In addition to K-SADS, DSM-IV-TR mania criteria was applied. Final diagnosis of BPD was made based on Leibenluft's "the narrow phenotype" definition (Leibenluft et al. 2003). Therefore, only subjects who met the full criteria in DSM-IV-TR for hypomania or mania, including the duration criterion and hallmark symptoms of elevated mood or grandiosity were diagnosed with BPD. The onset of the first episode, the number of episodes, and the offset of the last episode associated with the bipolar disorder were assessed.

*Assessment*

1. The Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version Present and Life Time version: The K-SADS PL is a semi structured diagnostic interview designed to assess current and past episodes of psychopathology in children and adolescents according to DSM-III-R and DSM-IV criteria and **is** worded such that it discerns BPD criteria over and above chronic symptoms. Probes and objective criteria are provided to rate individual symptoms. It can be effectively administered by clinicians or trained interviewers, and has established psychometric properties. The Turkish version of K-SADS-PL was used in this study. The translator demonstrated the reliability and validity of this version (Gökler et al. 2004).
2. Child Behavior Check List: The CBCL is one of the best-studied, empirically derived checklists that measure psychopathology (Achenbach 1991). The CBCL includes 118 problem behaviour items rated from zero (not at all typical of the child) to two (often typical of the child). It has 11 scales: delinquency, aggression, withdrawal, somatic complaints, anxiety/depression, and social problems, thought problems, attention problems, internalization, externalization, and total score. Scores on the scales are reported as t scores with a mean of 50 and a standard deviation of 10. A cut-off point of 70 has been traditionally recommended as a clinically meaningful cut-off point (Achenbach 1991).
3. Parent-Young Mania Rating Scale: The P-YMRS is an 11-item rating form adapted from the Young Mania Rating Scale (YMRS) (Gracious et al. 2002). Parents rate their child's manic symptoms on five explicitly defined grades of severity, with item scores ranging from 0 to 4 (and three items ranging from 0 to 8). The P-YMRS yields a total score that can range from zero to 56, with higher scores representing greater psychopathology. Ratings were based on the reported presence of symptoms over the previous week (Gracious et al. 2002).

*Statistics*

Statistical analysis was done using SPSS version 11.0. Two groups (ADHD and ADHD + BPD) were compared in terms of sociodemographic data and clinical characteristics based on CBCL sub scores and P-YMRS. Sociodemographic data was compared using the Pearson chi-square test. CBCL subscores and P-YMRS scores were analyzed using the Mann–Whitney *U*-test and the Pearson chi-square test.

## Results

A total of 121 children and adolescents, with the diagnosis of ADHD were evaluated. The mean age of the total group was $10.59 \pm 2.19$ years. It included 98 boys (81%) and 23 girls (19%).

Ten children (8.3%) received the additional diagnosis of BPD (ADHD + BPD). There was no statistically significant difference between the two groups in terms of mean age and gender (Table I).

The age of onset of bipolar disorder in ADHD + BPD group ranged between 7 and 12 years (mean age: $8.9 \pm 2.2$ years). Two patients (20%) in the ADHD + BPD group had BPD type II and eight patients (80%) had BPD type I, of whom two were rapid cycling and one of them was rapid cycling with psychotic features (Table II).

The ADHD + BPD group had significantly higher scores than the ADHD group in the CBCL subscales including; withdrawn ($P = 0.005$), anxiety/depression ($P = 0.023$), social problems ($P = 0.004$), thought problems ($P = 0.043$), attention problems ($P = 0.011$), aggression ($P = 0.00$), externalization ($P = 0.002$) and total score ($P = 0.006$) items (Figure 1).

Further assessment evaluating the rate of CBCL-proxy showed that 55 subjects had CBCL-PBD profile. However, only 10 of this group were diagnosed with bipolar disorders. Therefore, the

Table I. Demographics of two groups (Pearson Chi-square test).

| | | Age | | | |
|---|---|---|---|---|---|
| | *N* | Mean (SD) | Minimum–Maximum | *z* | *P* |
| ADHD | 111 | 10.62 (2.166) | 7–16 | −0.638 | 0.524* |
| ADHD+BPD | 10 | 10.20 (2.530) | 7–14 | | |
| | Boys | Girls | Total | df | *P* |
| ADHD | 90 (91.8%) | 21 (91.3%) | 111 (100.0%) | 1 | 0.933* |
| ADHD+BPD | 8 (8.2%) | 2 (8.7%) | 10 (100.0%) | | |

ADHD, attention-deficit hyperactivity disorder; BPD, bipolar disorder; *$P>0.05$.

positive predictive validity of CBCL proxy in this study was 18%.

The ADHD+BPD group had a significantly higher P-YMRS mean score than the ADHD group ($31.60\pm4.42$ versus $11.25\pm5.97$ and $P=0.00$) (Table III).

## Discussion

The goal of this study was to assess the prevalence of BPD in children and adolescents with the diagnosis of ADHD and to compare the clinical characteristics of children with ADHD with a group with co morbid ADHD and BPD. The prevalence of BPD was 8.3% in this population and the ADHD+BPD group had significantly higher scores on the CBCL items (withdrawn, anxiety/depression, social problems, thought problems, attention problems, aggression, externalization and total score), and P-YMRS than the ADHD group.

The first point that needs to be discussed is the prevalence of BPD in our ADHD group. The majority of studies report a higher prevalence than our findings. Biederman et al. (1996) assessed 140 children with ADHD at baseline and then again 4 years later, and they found that BPD was diagnosed in 11% of ADHD children at baseline and in an additional 12% at 4-year follow-up (Biederman et al. 1996), and they reported that their findings are consistent with a 22% rate among hospitalized ADHD children reported by Butler et al. (1995). In these studies, diagnosis of mania was made according to DSM-III-R criteria and K-SADS-E (epidemiological version) was used. A recent study from Turkey with similar assessment methods reported the similar rate (8.2%) in prepubertal, clinical referred children with ADHD. The low rate, in our study and Diler's study seems to be related with applying Leibenlufts' "the narrow phenotype" criteria.

Comparing the clinical features of the two groups using CBCL and P-YMRS showed that the groups can be distinguished by using both tools. Most of the previous studies comparing CBCL scores between individuals with ADHD and BPD accompanying ADHD showed that the later group displays higher scores in several subscales of CBCL (Geller et al. 1998; Hazell et al. 1999; Diler et al. 2007). Compatible with some of these studies, we found that the ADHD+BPD group had significantly higher rates than the group with ADHD on the withdrawn, anxiety/depression, social problems, thought problems, attention problems, aggression, and externalization subscales of CBCL. Mick et al. (2003) and Faraone et al. (2005) studies reported a relatively homogenous profile of CBCL associated

Table II. Characteristics of ADHD+BPD group.

| | Age | Gender | Age of first episode of BPD | Number of episodes | Type of BPD |
|---|---|---|---|---|---|
| Case 1 | 7 | Male | 7 | 1 manic episode | BPD 1 |
| Case 2 | 7 | Male | 7 | 1 manic episode+1 depressive episode | BPD 1 |
| Case 3 | 14 | Male | 12 | 2 manic+1 depressive episodes | BPD 1 |
| Case 4 | 10 | Female | 7 | 5 manic episodes | BPD 1 rapid cycling |
| Case 5 | 9 | Male | 7 | 5 manic episodes | BPD 1 rapid cycling |
| Case 6 | 12 | Male | 12 | 2 depressive +1 hypomanic episodes | BPD II |
| Case 7 | 9 | Male | 7 | 3 manic episodes | BPD 1 |
| Case 8 | 9 | Female | 9 | 4 manic episodes | BPD 1 rapid cycling with psychotic features |
| Case 9 | 11 | Female | 9 | 2 manic episodes | BPD 1 |
| Case 10 | 14 | Male | 12 | 2 depressive +1 hypomanic episodes | BPD II |

ADHD, attention-deficit hyperactivity disorder; BPD, bipolar disorder; BPD 1, bipolar disorder 1; BPD II, bipolar disorder II.

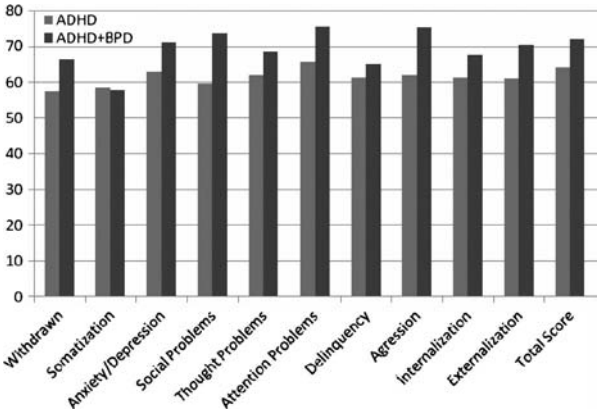


Figure 1. Comparison of the mean CBCL scores between two groups (Mann–Whitney *U*-test). CBCL, Child Behavior Checklist; ADHD, attention-deficit hyperactivity disorder; BPD, bipolar disorder.

with paediatric bipolar disorder with clinically significant elevations in the anxiety/depression, attention problems and aggression subscales. However, evaluating the predictive validity of CBCL profile of BP in our study revealed a poor positive predictive validity (18%) for CBCL- BPD profile. In addition to this a recent longitudinal study examined diagnostic and functional trajectories of individuals with the CBCL-BPD profile from early childhood through young adulthood. The results of this study showed that children with the CBCL-BPD profile are at risk for ongoing, severe psychiatric symptomatology, including behaviour and emotional comorbidities in general, and bipolar disorder, anxiety, ADHD and cluster B personality disorders in particular. The authors concluded that the value of this profile might be in predicting ongoing comorbidity and impairment, rather than any one specific DSM-IV diagnosis (Meyer et al. 2008). Although the present study showed the poor predictive validity of CBCL-BPD profile in identifying youths with BPD, it seemed that subjects with co-morbidity of ADHD and BPD had more clinical impairment and more behavioural problems as indicated by higher scores in the CBCL subscales.

Consistent with previous studies, our study reconfirms that P-YMRS is a powerful instrument in differentiating ADHD and BPD (Youngstrom et al. 2004; Diler et al. 2007). While the mean total scores of the ADHD group was $11.25 \pm 5.975$, the mean total scores of ADHD+BPD were $31.60 \pm 4.427$ ($P = 0.00$). Therefore, it could be concluded that along with findings based on detailed clinical examination, P-YMRS could help clinicians to assess BPD symptoms and differentiate this group from ADHD.

Regarding the age of onset of BPD, in the present group it was $8.9 \pm 2.2$ years old. The previous studies reported a different range of onset for BPD. Biederman et al. (2004) reported that the average age at onset of BPD in ADHD children was $6.3 \pm 4.7$ years, and stated that in 55%, onset was before the age of 6 years in another study. The reported average age of onset of manic episodes in another study from Turkey was $9.24 \pm 0.68$ years. The age of onset of BPD in Spain was reported as $13.2 \pm 3.5$ (Soutollo et al. 2005) and in Brazil $9.6 \pm 3.5$ (Tramontina et al. 2003). It is not clear if the difference in the age of onset is related with ethnic difference, or it could be related to sample characteristics. Further studies need to assess this point.

In conclusion, the present study revealed a significant rate of comorbidity of BPD in children with ADHD and it has been detected that children with comorbidity of BPD can be distinguished from children with ADHD alone based on clinical examination and supporting tools such as P-YMRS. Further, we found that CBCL-BP profile does not have a high positive predictive value in identification of this group.

Table III. Comparison of the mean P-YMRS scores between two groups (Mann–Whitney *U*-test).

| | Group | *N* | Mean (SD) | *z* | *P* |
|---|---|---|---|---|---|
| P-YMRS | ADHD | 111 | 11.25 (5.975) | | |
| | ADHD+BPD | 10 | 31.60 (4.427) | −5.074 | 0.00* |

ADHD, attention-deficit hyperactivity disorder; BPD, bipolar disorder; P-YMRS, Parent-Young Mania Rating Scale; *$P < 0.05$.

The present study suffers from some limitations such as small sample size and including only clinic-referred children. However, since there is a shortage of studies on this topic from non-western countries, it may contribute to the knowledge of BPD presentation in different cultures. In addition, it may help raise clinician awareness about the presence of BPD in subjects with ADHD.

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None.

## Statement of Interest

None.

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**ORIGINAL INVESTIGATION**

# Pathophysiology of NSS in ADHD

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**Abstract**
Attention deficit/hyperactivity disorder (ADHD) is the behavioural disorder most commonly diagnosed in childhood. In addition to the main symptoms of inattention, impulsiveness and hyperactivity, neurological soft signs (NSS) are often associated with ADHD. NSS are discrete motor and sensory disorders that cannot be linked to specific cerebral lesions. We review all the scientific contributions on NSS in ADHD. The conclusions support the presence of an alteration in the neural networks for motor control inhibition, at the base of the pathophysiology of NSS in children with ADHD, as well as a possible central role of dopamine in these neural circuits.

**Key words:** *ADHD, NSS, control inhibition, neural networks, dopamine*

## Introduction

Neurological soft signs (NSS) have been described as non-normative performance on a neurological examination of motor and sensory functioning in the absence of a focal lesion (Shafer et al. 1983). Some examples of NSS include difficulty in the fluid execution of rapid alternating movements such as pronation and supination of the hand (dysdiadochokinesis), motor slowness, dysgraphesthesia (Schonfeld et al. 1989), and difficulty in sequencing complex motor tasks. The origin of NSS is unknown. Nichols and Chen (1981) found that only a small number of many possible perinatal complications discriminated children diagnosed as having soft signs from normal controls. NSS have been found to be associated with IQ deficits, hyperactivity and learning disorders (Nichols and Chen 1981). These neurological abnormalities have longitudinal stability and are positively correlated with poor functional outcome in adulthood (Pine et al. 1996). Several investigators found positive correlations between neurological soft signs and increased risk of psychiatric disorders, such as depression and ADHD (Rasmussen and Gillberg 2000), and a strong association between NSS and ADHD (Denckla and Rudel 1978; Gillberg 1998). Shaffer et al. (1985) reported that adolescents with early soft signs had significantly lower IQs and were more likely to have a psychiatric disorder characterized by anxiety, withdrawal and affective disorders (Shaffer et al. 1985) as well as schizophrenia in adulthood (Leask et al. 2002). During the past 32 years, a number of standardized neurological test instruments have been used in research and clinical practice to identify and quantify NSS. One of the first was the Physical and Neurological Examination for Soft Signs (PANESS) (Guy 1976). Then, Touwen and Prechtl developed the Examination of the Child with Minor Neurological Dysfunction (Touwen and Prechtl 1970) as a quantitative examination for children with possible minor neurological dysfunction (MND), often referred to as "soft signs" (Touwen and Sporrel 1979). In clinical practice, The Revised Neurological Examination for Subtle Signs (NESS, Denckla 1985) is sensitive to soft developmental changes and to revealing soft motor deficits in central nervous system development. Denckla proposed a clear distinction between "soft signs" that, although soft, are abnormal at any age and those that would be normal if found in a younger child. In fact, motor ability and neuroanatomical structures show substantial growth, elaboration and myelination during early childhood (Denckla 1985). Although it is common to observe soft signs in typically developing younger children, persistence of soft signs into later childhood and adolescence suggests motor dysfunction and could

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be a marker for atypical neurological development (Larson et al. 2007). NSS are variable and their presence alone should not be considered either diagnostic or the unique basis for explaining complex behavioural and neurological diseases (Dijxhoorn et al. 1987).

## NSS and ADHD

Attention deficit/hyperactivity disorder (ADHD) is the behavioural disorder most commonly diagnosed in childhood. It usually manifests before a child reaches 7 years of age and consists of a persistent pattern of inattentiveness, impulsiveness and/or hyperactivity. Besides the "core" symptoms, the motor ability of ADHD children is often significantly poorer than it should be based on their age and level of intellectual functioning. Gillberg and Rasmussen (2000) examined the longer-term outcome of 55 subjects, aged 22 years, affected by ADHD, particularly when combined with developmental coordination disorder (DCD), previously referred to as attention deficit disorder (ADD) or minimal brain dysfunction (MBD), at initial workup at 7 years of age. In this context, MBD requires the presence of both attentional deficit and signs of either fine-motor, gross-motor or visual perception/conceptualization dysfunction. None of the subjects had received stimulant treatment. They were compared with 46 age-matched subjects not affected by such diagnoses. In the ADHD/DCD group 58% had a poor outcome characterized by remaining symptoms of ADHD, antisocial personality disorder, alcohol abuse, criminal offending, reading disorders, and low educational level compared with 13% in the control group. The authors determined that increased NSS were very useful as a screening tool for psychopathology, and diagnosis of ADHD (Rasmussen and Gillberg 2000). Dickstein et al. (2005) studied NSS in 17 children with ADHD and in 20 normal controls (NC) with no significant group differences in hand or foot lateral preference. They found that subjects with ADHD were slower than NC on repetitive motor tasks (Dickstein et al. 2005), consistent with some previous studies reporting that children with ADHD had impaired repetitive motor responses (Rubia et al. 1999b; Epstein et al. 2003). Uslu et al. (2007) underlined that certain factors investigated by the Neurological Examination for Subtle Signs (NESS), such as speed of movement, dysrhythmia and overflow with timed movements, provide important information that could increase our understanding of the neurobiological bases of ADHD and the clinical implications of neurological soft signs. They studied a group of 30 children with ADHD using the NESS and found an increase in overflow movements in children with ADHD. This could indicate a deficit in cortical inhibitory functions, which is a cardinal neurophysiological feature of ADHD (Uslu et al. 2007). This was also underlined in a study by Mostofsky et al. (2003) in which 42 children with ADHD showed significantly more overflow movements than 30 NC, predicting performance on measures of motor response inhibition (Mostofsky et al. 2003). At the same time, dysrhythmia and slowed speed of movement are associated with functional deficits in the cerebellum and basal ganglia (Kandel 2000). Mostofsky et al. (2003) also underlined a significant effect of sex on the association between ADHD and Total Overflow movements; in fact, in boys the presence of ADHD is associated with significantly more overflow movements than in girls, and the number of movements is higher in children with the full syndrome of impulsive, hyperactive and inattentive symptoms (Mostofsky et al. 2003).

### *NSS and clinical subtypes of ADHD*

Meyer and Sagvolden (2006) studied 528 South African children (264 with symptoms of ADHD and 264 normal controls) of both genders, divided in two age groups, 6–9 and 10–13 years, who were assessed using the following tests: the Grooved Pegboard, which measures manual dexterity, complex coordination and movement speed; the Maze Coordination Task, which measures complex coordination, goal-directed fine movements, accuracy and stability of movement; and the Finger Tapping Test, which is a sample measure of finger movement and speed (Meyer and Sagvolden 2006). All tasks were performed with both hands. Problems in motor control were found primarily in children between 6 and 9 years of age. In fact, motor speed and accuracy on both repetitive and sequential tasks increased with age in healthy children (Denckla 1973), confirming that in order for an examination to be useful it must be standardized for different ages. This study also shows that in children with ADHD motor control problems are independent from cultural differences (in fact, they are detected in Europe and Africa as well as in other countries) and are not related to hand preference. Compared with children who had no mental disorders, all ADHD clinical subtypes (C, combined; I, predominantly inattentive; HI, predominantly hyperactive/impulsive) performed worse on the Grooved Pegboard and Motor Coordination Task than on the Finger Tapping Test. This result replicates the findings of a European study (Seidman et al. 1997). The impairment was most severe in the ADHD-C subtypes and less severe in the ADHD-I and HI subtypes in both

genders, with slight differences in performance between hands. Pitcher et al. (2003) found that type and degree of movement difficulty differed between subtypes and that males with ADHD-I and ADHD-C had significantly poorer fine motor ability ($P<0.001$) than control children (Pitcher et al. 2003). In a preceding study they showed that boys in the ADHD-I subtypes had great difficulty with timing and force output and showed greater variability in motor outcomes (Pitcher et al. 2002). Thus, it is likely that poor fine motor skills make greater demands on sustained attention. Furthermore, there is a strong association between inattention and movement difficulties, as greater inattention is predictive of greater difficulty in motor coordination (Pitcher et al. 2003). These findings indicate the need for increased recognition of the clinical and research implications of the relationship between ADHD and motor dysfunction (Pitcher et al. 2002).

## The "network inhibition hypothesis" at the base of the pathophysiology of NSS In ADHD

The neuroanatomical basis of NSS remains poorly understood, and it has yet to be established whether the disorder is due to specific or to diffuse brain abnormalities (Dazzan and Murray 2002).

The excessive overflow movements in children with ADHD appear to reflect immaturity of the neural networks involved in inhibitory control (Mostofsky et al. 2003). Houk and Wise (1995) described the interconnections and the role of the basal ganglia, the cerebellum and the cerebral cortex in planning and controlling action (Houk and Wise 1995). Using blood oxygen level-dependent functional magnetic resonance imaging, Cao et al. (2006) showed decreased regional homogeneity in the frontal-striatal-cerebellar circuits, consistent with the hypothesis of abnormal frontal-striatal-cerebellar networks in boys with ADHD (Cao et al. 2006).

### *Role of cerebral cortex in inhibitory control*

The neural mechanisms underlying habituated motor responding and motor response inhibition in children with ADHD, were studied by comparing fMRI activation during a Go/No go task in 25 children with ADHD and 25 typically developing (TD) children, aged 8–13 years. Increased intrasubject variability (ISV), measured in response time, is reported in children with ADHD across various tasks. For TD children, increased pre-supplementary motor area (pre-SMA) activation during No/Go events was associated with less ISV, while the reverse was true in ADHD children for whom increased pre-SMA activation was associated with more ISV. In contrast, ADHD patients with less ISV showed greater prefrontal activation. These data suggest a functional anomaly of the pre-SMA in ADHD and the recruitment of prefrontal circuits as a compensatory mechanism by which some children with ADHD are able to achieve more consistent performance despite abnormalities in pre-SMA activation (Suskauer et al. 2008a). The pre-SMA area is connected to the anterior prefrontal areas (Dum and Strick 1991) and striatal projections from the pre-SMA largely extend to the caudate nucleus and the middle and rostral putamen (Lehericy et al. 2004). Anatomic imaging studies of children with ADHD reported localized anomalies in pre-SMA area, including reduced volume (Mostofsky et al. 2002) and thickness when compared with control children (Shaw et al. 2006). This area plays an important role in motor planning and switching from automatic to voluntary controlled actions (Hoshi and Tanji 2004). A possible explanation is that abnormality in the pre-SMA circuits is central to impaired response inhibition in ADHD, regardless of task demand (Suskauer et al. 2008b).

### *Role of basal ganglia in inhibitory control*

Deficits in repetitive motor tasks, documented in children with ADHD (Dickstein et al. 2005), provide further evidence of dysfunctional dopaminergic circuits in cortical and basal ganglia structures that result in the inability to regulate motor excitation and inhibition in the pathophysiology of ADHD (Casey et al. 1997; Durston et al. 2003). Schulz et al. (2005) examined inhibitory control in adolescents with ADHD during childhood using fMRI with the Stimulus and Response Conflict Tasks. They found positive correlations between prefrontal and basal ganglia activation and ADHD symptom intensity (ratings). This evidence suggests that difficulty with inhibitory control may represent a core deficit in ADHD and raises the possibility that the increased frontostriatal activation normalizes with the concomitantly remission of symptomatology (Schulz et al. 2005).

### *Role of cerebellum in inhibitory control*

Another region in the brain showing deviance associated with ADHD is the cerebellum. This is true when it is measured algorithmically as a single unit and when its different components are considered (Castellanos et al. 2002), and even more when the posterior cerebellar vermis is measured (Mostofsky et al. 1998; Castellanos et al. 2001). In

fact, in the cerebellum of males with ADHD the size of the posterior vermis is significantly decreased; further, within the posterior vermis the inferior posterior lobe (lobules VIII–X) is involved in this reduction, whereas the superior posterior lobe (lobules VI/VII) is not (Mostofsky et al. 1998). The dopamine membrane transporter (DAT) is a specific marker of DA axons (Ciliax et al. 1995) and the cerebellar vermis contains selective dopamine-transporter-like immunoreactive axons. Further, within the vermis labelled axons were present only in portions of a subset of lobules. In lobules II, III and IV, DAT-IR axons were found primarily in the depths of the intracentral and preculminate fissures and to a lesser degree in the more external folia of these lobules. In lobules VIIIA and VIIIB, DAT-IR axons were present in both the external and the internal folia, but the density of immunoreactive axons was greater in the internal folia (Melchitzky and Lewis 2000). Another fMRI study demonstrated that the reduced volume of the inferior posterior cerebellar hemispheres is correlated with a poor clinical outcome in patients with ADHD (Mackie et al. 2007). Thus, both the cerebellar vermis and the cerebellar posterior lobe have an important function in motor control (Ito 1984).

In an fMRI study, Sakai et al. (2000) showed some of the interactions between the pre-SMA area and the posterior lobe of the cerebellum. The authors found that the pre-SMA area was selectively active in response selection, whereas the cerebellar posterior lobe was selectively active in timing adjustment, and that the primary motor cortex received connections from both the pre-SMA and the cerebellum (Sakai et al. 2000). Both the basal ganglia and the cerebellum have recurrent connections with the prefrontal cortex, which is the site of high-level information processing because of its activity related to working memory, action planning and decision-making. The activity of the cortical neurons could be the result of the recurrent dynamics of the cortico-basal ganglia and the cortico-cerebellar networks to provide common representations of the cerebellum and the basal ganglia working together (Doya 2000) (see Figure 1).

In ADHD, inborn developmental abnormality of the brain-impaired function of neurocircuitries is important for attention and for the motor system. Exploration of the cerebellum's influence on cortico-striatal-thalamo-cortical (CSTC) circuits (Alexander et al. 1986), which determine the choice, the initiation and the performance of complex motor and cognitive responses (Graybiel 1998), seems very promising for clarifying the pathophysiology of ADHD.

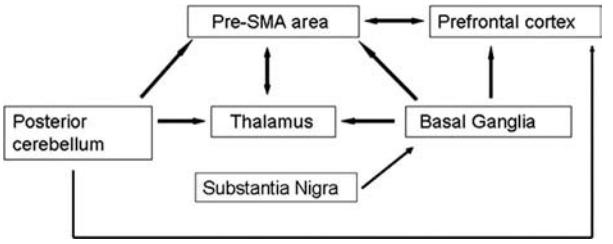


Figure 1. Principal neural networks involved in inhibitory control. This model graphically represents the neural mechanisms for the processing of response selection, and timing adjustment in motor control. The prefrontal cortex is specialized for unsupervised learning, and has been regarded as the site of high-level information processing, because of its activity related to working memory, action planning, and decision making. The information processing is guided by the input signal itself, but may also be regulated by the ascending neuromodulatory inputs from: the pre-supplementary motor area (Pre-SMA), active in response selection, the cerebellar posterior lobe, active in timing adjustment, and from the basal ganglia. The basal ganglia are specialized for reinforcement learning, which is guided by the reward signal encoded in the dopaminergic input from the substantia nigra. The thalamus receives the posterior cerebellum and basal ganglia inputs, and projects to the Pre-SMA area.

### *Animal models and the "network inhibition hypothesis" of NSS in ADHD*

Animal models are helpful in medical research since they have simpler nervous systems, more easily interpretable behaviours, more homogeneous genetics, a more easily controlled environment, and a greater variety of interventions available (Sagvolden et al. 2008). In the literature, there are no investigations on neurological soft signs in animal models of ADHD, but some interesting findings are useful to clarify the pathophysiology of NSS in ADHD. There is a variety of commonly used animal models for ADHD: e.g., the spontaneously hypertensive rats (SHR), the dopamine transporter knockout and knockdown (DAT KO and DAT KD) mice, and the Coloboma mice. Irrespective of the genetic determinants of each of these models, these animal models show possible impairments of response inhibition as being related directly to abnormal catecholamine function in the prefrontal cortex and/or basal ganglia. (Groman et al. 2008). SHR are a selectively bred line originating from normotensive Wistar–Kyoto (WKY) rats and display many characteristics that resemble ADHD symptoms, such as hyperactivity and inattention (Sagvolden 2000). Dopamine release is decreased in SHR prefrontal cortex and norepinephrine concentrations are elevated (Russell et al. 2000a). The noradrenergic system appears to be hyperactive as a result of impaired alpha-2A adrenoceptor regulation (Russell et al. 2005). Russell et al. (2000) investigated possible long-term effects of methylphenidate treatment on dopaminergic function in striatal slices of

SHR compared to their WKY control rats, suggesting that presynaptic mechanisms controlling dopamine release had been altered in SHR rats (Russell et al. 2000b). Effective ADHD treatments, including methylphenidate, amphetamine and atomoxetine, reduce impulsive behaviour, probably by enhancing response inhibition, in rats (Navarra et al. 2008). Some studies evaluate both cognitive and motor function of DA-depleted rats after intracerebral neonatal microinjections of 6-hydroxydopamine (6-OHDA). This experimental model, in developing rats, is strikingly similar to the clinical syndrome of MBD (Shaywitz et al. 1976; Archer et al. 1988). Animal models provide us with important findings in order to understand the anatomic bases of motor learning and motor control. Recent anatomical studies proposed a cerebellar and basal ganglia interaction, based on the identification of a di-synaptic pathway originating from cerebellum and projecting to the striatum *via* the thalamus (Ichinohe et al. 2000). Rossi et al. (2008) studied striatal long-term depression (LTD), a crucial form of synaptic plasticity involved in motor learning after cerebellar lesions in rats. Authors showed that the cerebellum controls striatal synaptic transmission in general, and synaptic plasticity in particular, supporting the notion that the two structures operate in conjunction during motor learning (Rossi et al. 2008).

## NSS and pharmacological treatments

### *Effect of methylphenidate on NSS in patients with ADHD*

Lerer and Lerer (1976) published the first study about the effect of methylphenidate (MPH) on NSS in patients with ADHD. These authors found that out of 40 children with three or more NSS, 29 showed marked improvement or complete resolution of NSS following a 60-day treatment with MPH. They also underlined that administration of the placebo did not appreciably change the neurological status of 20 hyperactive children and that behavioural improvement, which was studied by means of Conners' Abbreviated Teacher Rating Scale, did not always correspond to resolution of the abnormal neurological signs (Lerer and Lerer 1976). Rubia et al. (2003) demonstrated the effectiveness of persistent administration of methylphenidate on deficits in motor timing in ADHD children and extended the use of methylphenidate from the domain of attentional and inhibitory functions to the domain of executive motor timing (Rubia et al. 2003).

Motor system excitability can be investigated in vivo by means of single and paired pulse transcranial magnetic stimulation (TMS). Moll et al. (2000) studied motor system excitability in 18 drug-naive ADHD children, aged 8–12 years, compared with 18 age-matched healthy children using the TMS. They provided evidence of inhibitory deficits within the motor cortex of ADHD children and of an enhancement of inhibitory mechanisms in this brain region after the oral intake of 10 mg of methylphenidate (Moll et al. 2000). Deficits in repetitive motor tasks provide further evidence that dysfunctional dopaminergic circuits in cortical and basal ganglia structures cause the inability to regulate motor excitation and inhibition in the pathophysiology of ADHD (Casey et al. 1997; Durston et al. 2003).

## Conclusion

Multiple abnormalities of the motor system have been identified in some children with ADHD including persistence of overflow movements (Denckla and Rudel 1978), impaired timing of motor responses (Rubia et al. 1999a) and deficits in fine motor abilities (Pitcher et al. 2003). The presence of excessive overflow movements in children with ADHD appears to reflect immaturity of the neural networks involved in inhibitory control (Mostofsky et al. 2003). This review analyzes all the scientific contributions on NSS in ADHD (see Table I) and supports the evidence of a "network inhibition hypothesis" at the base of the pathophysiology of NSS in ADHD, where the interconnections between

Table I. Recent studies included in this review.

| Reference | ADHD | Controls | Mean age | Evaluation scale |
|---|---|---|---|---|
| Pitcher et al. 2003 | 104 | 39 | 10 years | Movement Assessment Battery for Children (MABC) and the Purdue Pegboard Test |
| Mostofsky et al. 2003 | 42 | 30 | 9.8 years | The Physical and Neurological Examination for Soft Signs, Conflicting motor response task, Controlateral response task |
| Dickstein et al. 2005 | 17 | 20 | 10.6 years | The Revised Physical and Neurological Examination for Soft Signs |
| Meyer and Sagvolden 2006 | 264 | 264 | 6–13 years | The Grooved Pegboard, the Maze Coordination Task and the Finger Tapping Test |
| Uslu et al. 2007 | 30 | 74 | 9.20 years | Neurological Examination for Soft Signs (NESS) |

basal ganglia, cerebellum and cerebral cortex have a central role in the inhibition of voluntary movements. The finding of selectively containing dopamine transporter-like immunoreactive axons in the cerebellar vermis (Melchitzky and Lewis 2000) suggests that dopamine has a central role; this is also supported by the effect of methylphenidate on NSS, documented in the articles cited above. The importance of the dopamine function in the genesis of NSS derives from evidence that deficits in repetitive motor tasks are due to dysfunctional dopaminergic circuits in cortical and basal ganglia structures. Dysfunction of the nigro-striatal dopamine branch causes several NSS associated with ADHD, including impaired force and timing regulation of muscle groups, and symptoms include poor motor control (Kadesjo and Gillberg 1999). The finding of slowed speed of movement in repetitive motor tasks is connected to functional deficits in the cerebellum and basal ganglia. NSS are spontaneously present in drug-naive children with ADHD. This evidence supports the possibility of a dopamine dysfunction prior to the administration of drugs also because of marked improvement or complete resolution of NSS in children with ADHD after treatment with MPH (Lerer and Lerer 1976).

More studies are needed to assess the sensory and motor soft signs associated with ADHD and to integrate clinical evidence with neuroimaging findings and neuropsychological dysfunction. Direct investigation of the cortical processes leading to motor overflow may provide a more complete understanding of the pathological relevance of motor overflow and NSS in general (Hoy et al. 2008). The comparability of future studies can be improved by using the same structured rating scale for NSS; moreover, a useful examination must be standardized for different ages. In an attempt to elucidate the role of NSS in children with ADHD, it is crucial that repeated neurological assessment be included in the medical examination of drug-naïve and treated children with ADHD.

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## Statement of Interest

The authors disclose any commercial or other associations that might pose a conflict of interest in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# Irreversibility of cardiac autonomic dysfunction in female adolescents diagnosed with anorexia nervosa after short- and long-term weight gain

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**Abstract**

Anorexia nervosa (AN) patients may present with cardiac autonomic system dysfunction. Power spectral analysis of heart rate variability (HRV) is a reliable noninvasive examination for the quantitative assessment of the central sympathovagal interaction that modulates cardiovascular autonomic function. In the present study, HRV parameters were assessed in female adolescent AN inpatients in the malnourished phase at admission, at discharge when achieving weight restoration, and 24–36 months after discharge, when considered remitted. Nineteen normal-weight female controls were similarly assessed. Spectral analysis of HRV was done with the fast Fourier transform algorithm. At admission and discharge, patients underwent routine laboratory examinations and responded to questionnaires assessing eating-related preoccupations, behaviors, and personality attributes, depression and anxiety. Compared with the controls, AN patients had significantly lower heart-rate and HRV, lower total power and low frequency components, elevated high frequency components, and decreased low to high frequency power ratio as assessed with the power spectral analysis at all three evaluation points. These disturbances were not correlated with the baseline laboratory and psychometric measures. Our preliminary findings suggest that female adolescent AN inpatients may have a cardiovascular autonomic dysfunction in the form of vagal abnormality present not only in malnourished patients, but also persisting following short-term and long-term weight restoration.

**Key words:** *Anorexia nervosa, heart rate, heart rate variability, autonomic nervous system*

## Introduction

Anorexia nervosa (AN) is an eating disorder (ED) characterized by voluntary restriction of food and loss of weight of at least 85% of desired weight (1), that often leads to severe malnutrition and an exceedingly high mortality risk compared with most other psychiatric illnesses (Sullivan 1995; Casiero and Frishman 2006). Although the mechanisms associated with increased mortality in AN are still poorly understood, it is likely related to disturbances in cardiovascular function resulting from a host of malnutrition-related physical complications, including electrolyte imbalances, hypotension, bradycardia or arrhythmias (Neumarker 1997; Neumarker et al. 1997). The median length of time between diagnosis and death in AN patients is around 10 years (Millar et al. 2005). Whereas some studies have found that a substantial percentage of deaths linked to AN occur at or above the age of 65 years (Reas et al. 2005), the long-term survival of AN in other studies does not differ from that expected in the studied populations (Korndorfer et al. 2003).

Many disturbances found in AN patients can be attributable to autonomic nervous system (ANS) dysregulation, likely affecting thermoregulation, vascular motility, heart rate and rhythm, and blood pressure (Kollai et al. 1994; Galetta et al. 2003). Indeed, almost 80% of AN patients have cardiovascular abnormalities, mainly bradycardia, hypotension, arrhythmias and repolarization disorders. Additionally, AN patients may present with reduced left ventricular mass (Galetta et al. 2003; Romano et al. 2003) and alterations in sympathovagal

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balance (Kollai et al. 1994; Kreipe et al. 1994; Petretta et al. 1997; Rechlin et al. 1998; Casu et al. 2002; Galetta et al. 2003; Cong et al. 2004; Melanson et al. 2004; Platisa et al. 2005; Platisa et al. 2006), the latter being of parrticular relevance in increasing the risk of cardiovascular mortality and sudden death in AN (Neumarker 1997).

Heart rate variability (HRV) and related measures are a reliable noninvasive technique enabling quantitative assessment of cardiovascular autonomic regulatory responses to autonomic regulatory mechanisms (Task Force 1996). Heart rate (HR) is not constant, but oscillates around a mean value. These oscillations are due to modulations of autonomic nervous system (ANS) activity which control HR. Changes in sinus rate over time, or more precisely, the standard deviation of intervals between successive R waves (SDRR) of the cardiac cycle, are termed heart rate variability (HRV). Representation of the relative share (also called "power") of oscillations of different time ranges into frequency distributions (using Fourier transform) have shown that HRV signals are concentrated into at least three distinct frequency bands: high-frequency (HF), low-frequency (LF), and very-low frequency (VLF) (Task Force 1996). The HF band has respiration as the primary rhythmic stimulus ("sinus arrhythmia") and is mediated by changing levels of parasympathetic tone. The LF band is affected by the oscillatory rhythm of the baroreceptor system and is mediated by sympathetic influences. The VLF component has not yet been given a precise physiological meaning and is subject to considerable debate, having been attributed variously to thermoregulatory processes, peripheral vasomotor activity, and the rennin–angiotensin system. It is considered to be a predominately sympathetic indicator (Akselrod et al. 1981).

The ANS links the central nervous system (CNS) and the cardiovascular system. Efferent links in the neural control of heart rhythm consist of sympathetic and parasympathetic fibres innervating the sinus node. Because sympathetic and parasympathetic firing alters spontaneous sinus node depolarization, cardiac rate and rhythm convey information about autonomic influences to the heart (Elghozi et al. 2001). Taken together, power spectrum analysis of HRV, which is reliable and noninvasive, can utilize the assessment of peripheral ANS system to provide a general indication of central cardiovascular autonomic regulatory responses (Task Force 1996).

The aim of the present study was to evaluate the ANS parameters in a group of female adolescent AN patients at three time points: in the acute malnourished underweight phase, when achieving weight restoration, and following long-term weight gain. The ANS parameters were compared to normal-weight healthy control women, matched for age and gender. In line with the high mortality risk found in AN patients overtime, we hypothesized that malnourished AN patients will show disturbances in HR, HRV and spectral indexes compared to healthy controls, that will persist also at short- and long-term weight restoration.

## Methods

The study underwent the standard procedure for approval by the Helsinki Ethics Committees of the Ben-Gurion University of the Negev, and the Chaim Sheba Medical Center, Tel Hashomer. Participants, or their parents or other legal guardians in the case of minors under the age of 18 years, gave their written voluntary consent after having received detailed information about the study.

### *Participants*

*Experimental group.* Twenty-four females meeting DSM-IV (American Psychiatric Association 1994) criteria for AN (all diagnosed with restricting-type AN (AN-R)) were recruited from the adolescent ED inpatient department at the Chaim Sheba Medical Center, Tel-Hashomer, Israel. Patients were excluded from the study if diagnosed with lifetime or current schizophrenic spectrum disorder, bipolar disorder, substance use disorder, organic brain disorder, cardiovascular disorder, and a medical disorder potentially affecting food consumption and weight (diabetes mellitus or thyroid disorders), or if taking any medication.

*Control group.* This group included 19 healthy female volunteers matched to the experimental group for age and time of day of electrocardiogram recordings. Control women had no lifetime or current medical and psychiatric disorders and no stigmata indicative of an ED. Their weight was within 85–115% of ideal body weight (IBW) since puberty according to the Metropolitan Life Insurance (1959) criteria (there are no established Israeli IBW criteria), and they had regular menstrual cycles since menarche.

Patients were assessed at three time points: (1) within 7 days of admission, (i.e. during the acute malnourished phase of the illness; $n=24$); (2) at discharge, when achieving their desired weight and maintaining it for at least two consecutive weeks ($n=12$). 3) At long-term follow-up (24–36 months after discharge; $n=6$). Controls were assessed once. No differences were found in baseline demographic, clinical, laboratory, psychological, and cardiovascular parameters between AN patients who participated, or

did not participate in the second and third evaluations. Duration of hospitalization was $4.23 \pm 4.7$ months.

*Instruments.* AN was diagnosed independently by two certified child and adolescent psychiatrists with the Eating Disorder Family History Interview (EDFHI) (Strober 1987), according to the DSM-IV (American Psychiatric Association 1994) criteria. The EDFHI is a structured clinical interview designed to gather detailed information on weight and eating history that has been extensively used in studies of ED patients (Pollice et al. 1997), including in Israeli samples (Stein et al. 2005). Duration of AN-R before admission was $2.5 \pm 2.8$ years. Other psychiatric morbidity was similarly assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition (SCID-I/P, Version 2.0) (First et al. 1995) according to the DSM-IV criteria (American Psychiatric Association 1994).

A structured questionnaire specified the participants' demographic data, All participants were also administered the following self-rating scales:

*The Eating Attitudes Test-26* (EAT-26) (Garfinkel and Newman 2001; Garner et al. 1982) is the most widely used scale to assess eating-related preoccupations and behaviors. It consists of 26 items that gather into three factors and a total score: dieting, refraining from fatty food and physical appearance; bulimic symptomatology and preoccupation with food; and personal control over eating. Higher scores indicate greater disturbance. A total score of 20 or above is indicative of eating-related pathology. The EAT-26 has been previously validated in Israeli ED populations (Koslovsky et al. 1992).

*The Eating Disorders Inventory-2 (EDI-2)* (Garner 1991) is considered the gold standard for the assessment of core ED traits and ED-related personality attributes (Thiel and Paul 2006). It includes 91 items which gather into eleven subscales that measure drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation, and social insecurity. Higher scores indicate greater pathology. The EDI-2 has been previously shown to differentiate Israeli ED patients from controls (Niv et al. 1998; Stein et al. 2005).

*The Beck Depression Inventory (BDI)* (Beck et al. 1961) is a 21-item inventory measuring symptoms of depression. Higher scores indicate more depressive symptoms. The BDI has been previously used in eating disorders patients (Pollice et al. 1997), including in Israeli samples (Stein et al. 2005)

*The State Anxiety Inventory (STAI)* (Spielberger et al. 1973) assesses the anxiety at the time of the evaluation (State–Anxiety, 20 items) and the general tendency to display anxiety (Trait–Anxiety, 20 items). Higher scores indicate more anxiety symptoms. The STAI has been previously used in eating disorders patients (Pollice et al. 1997), including in Israeli Samples (Yackobovitch-Gavan et al. 2009).

*Procedure.* AN-R inpatients were interviewed within 7 days of admission with the EDFHI and the SCID, after being considered medically stable. The self-rating scales were also administered at that time by trained research assistants. A complete medical and neurological evaluation was performed at admission and thereafter monthly, until discharge. Routine laboratory examinations, including glucose, sodium, potassium, calcium, phosphorous, urea, creatinine, complete blood count, iron, folic acid, vitamin B-12, free thyroxin index, triiodothyronine, thyroid stimulating hormone, cortisol and urinalysis were collected between 08:00 and 09:00 h, after an overnight fast within the first week of hospitalization, and thereafter monthly, until discharge. Only those inpatients whose medical condition was stable throughout the study period were analyzed. Weight and height were obtained at admission and thereafter monthly, until discharge. For the sake of reducing internal variability, all measurements were taken during the morning hours by a single investigator, using standardized procedures (Tanner 1994). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (Bray 1992).

It is the standard policy of this department to discontinue psychotropic treatment of AN patients within the first week of hospitalization, and to wait for about 4 weeks before stating serotonin specific reuptake inhibitors (SSRI) treatment if required, and if the BMI is 17 $kg/m^2$ or greater. In the case of severe agitation, patients can be temporarily treated with clonazepam or prothiazine, or occasionally with low doses of risperidone or olanzapine, as required.

In the current study, no patients received any psychotropic medication at the first evaluation on admission, whereas eight of the 12 patients assessed at discharge were treated with SSRIs. In these cases, the drug regimen was stable for at least 4 weeks before the respective evaluation. The six patients assessed at long-term follow up did not receive any psychotropic treatment.

### *Electrographic (ECG) recordings*

All ECG recordings were performed between 10:00 and 12:00 h to avoid circadian bias. ECG recordings were acquired by connecting the participants in a seated position at complete rest to a Holter monitor (Oxford 4-24). To minimize extraneous stress, the room used was quiet and the temperature maintained at 25°C (to prevent activation of thermo-

regulatory mechanisms which affect power spectra). A detailed explanation of the procedure was given. Participants were instructed to breathe normally.

Segments of lead II ECG were amplified, digitalized (500 Hz, width pass 0.05–35 Hz), and stored using a personal computer connected to an analogue/digital converter (National Instrument Inc.)-based software system (Biopac System, Inc). After classification of QRS couples configuration, the frequency histogram of RR intervals was displayed and ECG strips of the intervals in both tails of the RR distribution were manually confirmed until no QRS complex was mislabelled as either an artefact or an ectopic beat. Premature ventricular beats, electrical "noise", or aberrant beats along with the preceding and following intervals were rejected. Ventricular premature complexes and other artefacts were rejected applying thresholds at $\pm 15\%$ of reference RR duration. Only normal-to-normal intervals R–R were analyzed.

### *Analysis of HRV*

HRV was analyzed in both time and frequency domain. In the time domain, we calculated the mean value of HR, and the standard deviation of the successive difference in RR intervals (HRV). In the frequency domain, power spectrum density (PSD) of HRV signals was calculated using fast Fourier transform. The spectral components (peaks) were assessed in term of frequency (in Hz) and in power, which was calculated for each individual component (the area under the portion of the power spectrum density curve related to each component): VLF band (0.01–0.04), LF bands (0.04–0.15 Hz) and HF bands (0.15–0.4 Hz), and total power (TP, 0.01–0.4 Hz), represented by the total area under the curve. Because total power varied greatly among individual participants, it was determined in both absolute units and as normalized values. The representation of LF and HF in normalized units emphasizes the controlled and balanced behaviour of the two branches of the ANS. Normalized units were calculated for the LF and the HF components as: $\text{Power}_{\text{NU}} = (100 \times \text{Absolute power})/((\text{Total power} - \text{VLF}) \times \text{SD of successive difference in R–R intervals})$.

### *Data analysis*

To compare group effects, the variables were analyzed by analysis of variance (one-way ANOVA). Because of the skewness of the data, logarithmic transformation was performed on the absolute units of the spectral components of HRV before the statistical analysis.

The groups were compared with Scheffe's post-hoc comparison test. Because of the skewness of the data, logarithmic transformation was performed on the absolute units of the spectral components of HRV before the statistical analysis. Chi-square tests were used for categorical data.

We used linear regression analyses to assess the relationship between disease duration and HR and HRV indices.

## Results

The assessment of the anthropometric data revealed no difference in the age of the malnourished AN patients ($15.9 \pm 0.45$ years) and the controls ($15.6 \pm 0.45$ years). One AN patient was diagnosed additionally with major depressive disorder, two with an obsessive compulsive disorder (OCD), and another two with social phobia; another seven patients were diagnosed with a combination of a depressive disorder (major depression or dysthymia), and an anxiety disorder (OCD or a different anxiety disorder). No differences have been found in any of the physiological and psychological parameters introduced between AN patients with or without a comorbid diagnosis at baseline.

Comparison of the BMI of the 19 controls ($21.0 \pm 0.3$ kg/m$^2$) with that of the AN patients as assessed on admission ($15.5 \pm 0.3$ kg/m$^2$, $n = 24$), discharge ($19.5 \pm 0.4$ kg/m$^2$, $n = 12$), and long-term follow-up ($21.5 \pm 1.1$ kg/m$^2$, $n = 6$), revealed significant between-group differences [$F(3,57) = 46.9$, $P < 0.0001$]. Specifically, the BMI of the AN patients on admission was exceedingly low in comparison to the controls. It increased significantly during hospitalization, reaching almost normal ranges at discharge (Bray 1992). The six patients assessed with the EDFHI at follow-up were all considered remitted (Strober et al. 1997; Yackobovitch-Gavan et al. 2009), as their BMI was within normal range (Bray 1992), they had regular menstrual periods, and none had evidence of restricting, bingeing and/or purging behaviours for at least 12 consecutive months.

The results of the laboratory tests of the malnourished and weight restored AN were within normal range on both admission and discharge, with no differences in any of these variables in the 12 patients assessed on both evaluation points (results not shown).

Analysis of the psychometric testing revealed that the AN patients scored higher on all EDI-2 subscales (except for the bulimia subscale) compared to Israeli norms (Niv et al. 1998; results not shown). Their mean EAT-26 score both on admission ($38.9 \pm 5.1$) and discharge ($46.5 \pm 7.6$) was considerably higher

than the cut-off point of 20 required to define eating-related pathology (Garner et al. 1982), being significantly higher also in comparison to Israeli standards (Koslovsky et al. 1992). The mean BDI score on admission (23.2 ± 3.7) signified moderate-severe depressive symptoms, whereas the mean score at discharge (31.1 ± 6.5) represented severe depression (BDI scores moderate severe depression = 20 − 29, severe depression ≥ 30, see Rotherham-Borus and Ttrautman, 1988). The mean scores for the STAI State-Anxiety and Trait-Anxiety both on admission (51.9 ± 5.3 and 46.4 ± 4.5, respectively) and discharge (63.4 ± 4.6 and 45.5 ± 11.05, respectively) represented moderate to severe anxiety (Spielberger et al. 1970), being significantly elevated in comparison to the mean scores of 32.5 ± 9.0 for STAI state-anxiety and 37.4 ± 9.2 for STAI trait-anxiety in the general Israeli population (Teichman and Melineck 1979). No significant differences between admission and discharge were found in the scores of the psychometric scales of the twelve patients assessed on both evaluation points (results nit shown).

The electrophysiological results are presented in Table I. The malnourished AN patients exhibited significantly lower mean HR and HRV compared with the control group [$F(3,57) = 10.1$, $P < 0.0002$ and $F(3,57) = 25.2$, $P < 0.0001$, respectively]. Moreover, the AN group continued to exhibit lower mean HR and HRV in comparison to the control women both at discharge ($P < 0.015$ and $P < 0.0001$, respectively), and at the long-term follow-up assessment ($P < 0.05$ and $P < 0.0001$, respectively).

In the case of the spectral indexes used for analysis of ANS regulation, malnourished AN patients had significantly lower total power ($F(3,57) = 7.3$, $P < 0.00035$), lower LF components power (in normalized units) ($F(3,57) = 9.8$, $P < 0.00001$) and higher HF components power ($F(3,57) = 9.8$, $P < 0.00001$), compared to the control group (Table I). The ratio of low to high frequency power was significantly decreased in the malnourished AN patients compared to the controls ($F(3,57) = 8.1$, $P < 0.0002$). Moreover, these differences between AN patients and controls were still present at the discharge and follow-up assessments (Table I).

Bradycardia (i.e. HR < 50 bpm) was observed in four malnourished AN patients (16.7%) and in two AN patients at discharge (16.67%). By contrast, none of the control participants had evidence of bradycardia.

All electrophysiological findings retained their significance following Bonferroni correction. HR, HRV, and the spectral indexes were not correlated with the duration of AN before assessment and with the baseline laboratory and psychometric data. No differences were shown in any of the ECG parameters between the AN patients receiving, or not receiving SSRIs on discharge, and no between-group differences were found in the respiratory rate (range 13–17 cyclic/min).

Table I. Power spectral analysis of heart rate variability (HRV) in anorexia nervosa (AN) patients and normal controls

| | Controls I N = 19 | Starvation phase II N = 24 | Weight restoration III N = 12 | Follow-up IV N = 6 | One-way ANOVA |
|---|---|---|---|---|---|
| Time domain: | | | | | |
| Heart rate[1] (bpm) | 81.6 ± 1.8 | 65.7 ± 2.3 | 69.8 ± 3.4 | 69.3 ± 3.7 | I ≠ II, III, IV |
| Heart rate variability[2] (ms)* | 0.27 ± 0.03 | 0.09 ± 0.009 | 0.09 ± 0.02 | 0.08 ± 0.01 | I ≠ II, III, IV |
| Frequency domain: | | | | | |
| Total power[3]: | 539.6 ± 32.3 | 409.6 ± 19.5 | 373.8 ± 31.9 | 380.5 ± 36.7 | I ≠ II, III, IV |
| Absolute (log) power values of the frequency bands: ($ms^2$): | | | | | |
| VLF | 4.4 ± 0.07 | 4.3 ± 0.08 | 4.4 ± 0.08 | 4.1 ± 0.1 | |
| LF | 5.3 ± 0.07 | 4.7 ± 0.07 | 4.7 ± 0.1 | 4.7 ± 0.2 | |
| HF | 5.4 ± 0.08 | 5.3 ± 0.07 | 5.0 ± 0.1 | 5.2 ± 0.2 | |
| Power (normalized units): | | | | | |
| LF%[4] | 48.0 ± 0.5 | 37.0 ± 1.7 | 38.9 ± 2.6 | 37.9 ± 1.6 | I ≠ II, III, IV |
| HF%[5] | 52.0 ± 0.5 | 63.0 ± 1.7 | 61.0 ± 2.6 | 62.1 ± 1.6 | I ≠ II, III, IV |
| LF/HF[6] | 0.93 ± 0.02 | 0.62 ± 0.05 | 0.68 ± 0.1 | 0.62 ± 0.04 | I ≠ II, III, IV |

Results are expressed in mean ± SEM; bpm, beats per minute; ms, milliseconds; $ms^2$ log values of frequency bands: VLF, very low frequency; LF, low frequency; HF, high frequency; *SD of successive difference in R–R intervals.

[1] $F(3,57) = 10.1$, $P < 0.0002$.

[2] $F(3, 57) = 25.2$, $P < 0.0001$.

[3] $F(3, 57) = 7.3$, $P < 0.00035$.

[4] $F(3, 57) = 9.8$, $P < 0.0001$.

[5] $F(3, 57) = 9.8$, $P < 0.0001$.

[6] $F(3, 57) = 8.1$, $P < 0.0002$.

## Discussion

The present naturalistic prospective preliminary study shows that malnourished female adolescent AN inpatients assessed at rest have significantly lower mean HR and HRV, lower total power and LF components, higher HF components, and decreased low to high frequency power ratio compared to a matched control group. These disturbances in the various spectral components may indicate a shift of sympathovagal balance toward vagal tone predominance and a reduced sympathetic tone. These results are confusing, since vagal tone activation might underlie susceptibility toward bradycardia, usually accompanied by an elevation in total power, whereas the reverse occurs during sympathetic activation (Task Force 1996). Our results are also not characteristic of any peripheral ANS disturbance. Thus, we may speculate that the phenomenon detected in our study reflects a dysfunction of central nervous system regulation in AN patients, rather than a peripheral abnormality. Moreover, HRV measures fluctuations in autonomic inputs to the heart rather than the mean level of autonomic inputs (Task Force 1996). Thus, both autonomic withdrawal and a high level of sympathetic input to the point of saturation can lead to diminished HRV. Altogether, we may speculate that the physiological periodic fluctuations of the ANS activity are abnormal in these AN patients. Alternatively, our findings to be related to reduced responsiveness of sinus nodal.

Most importantly, these cardiac and spectral disturbances indicative of cardiovascular vagal abnormality still persist in the 12 AN patients examined at weight restoration, and in the six patients examined 24–36 months after discharge. At that time, these six patients exhibit normal BMI and are considered remitted from AN according to standardized accepted criteria. Both at discharge and at long-term follow-up, cardiac and spectral parameters are still significantly different from the controls, although at these two evaluation points there is no difference in BMI between patients and controls.

Several studies assessing cardiac autonomic control in malnourished underweight AN patients have found marked decrement of sympathetic tone (LF) (Nudel et al. 1984; Kreipe et al. 1994; Rechlin et al. 1998), whereas other studies, similar to ours, show an increase in vagal tone activity (Kollai et al. 1994; Petretta et al. 1997; Casu et al. 2002; Galetta et al. 2003; Cong et al. 2004; Vigo et al. 2008), with a concomitant decrease in sympathetic activity. Such an imbalance can be detrimental, potentially contributing to the higher cardiovascular mortality risk seen in AN patients (Petretta et al. 1997). In line with these results, Melanson et al. (2004) have found reduced HRV in AN patients who are ill for a prolonged period of time. By contrast, Platisa et al. (2005) have found that when standing, AN patients show higher HR, and reduced total HRV and HF spectral power as compared to controls.

Taken together, the studies showing reduced HRV in AN support the notion that malnourished AN patients have abnormal vagal tone activity. Platisa et al. (2006) evaluated linear and non-linear measures of HRV, and found differences in both measures in acute and chronic AN patients over a 24-h assessment period. Specifically, acute AN was characterized by decreased HR and increased HRV, whereas chronic AN patients showed increased HR, reduced HRV and higher differences between awakening and sleeping conditions. Also, Lesinskiene et al. (2008), in a meta-analysis of heart rate and QT interval alteration in anorexia nervosa, showed that bradycardia and relationship between HR and BMI decrease as the disease continues. In contrast to these findings, in our AN sample, the HR and HRV parameters were similar in the acute and chronic phases, and there was no significant correlation between length of disease and HRV parameters.

We have also shown that despite short-term weight restoration and long-term remission characterized with maintenance of normal BMI, female adolescents hospitalized because of AN continue to exhibit cardiovascular vagal hyperactivity. By contrast, other studies have shown reversibility of cardiac abnormalities in AN patients following weight restoration. Thus, Rechlin et al. (1998) have found that the HRV parameters of weight-restored female AN patients are not different from those of control women. Yoshida et al. (2006) have reported that after short-term refeeding (32.4 days), the mean daytime HR of female AN patients rises significantly (from 54.9 to 69.4 bpm) despite no increase in BMI; additionally, the changes in sympathetic activity have been negatively correlated with the changes in HRV. In another study (Ulger et al. 2006), underweight female AN adolescent patients have shown low HR and low R wave amplitudes in V6 lead compared to control girls. Following weight restoration, HR has increased to normal levels, as assessed at one year follow-up. By contrast, although R wave amplitudes in V6 lead have increased after refeeding, they have not reached the levels found in the control group, suggesting that some functional cardiac abnormalities may persist in weight restored AN patients.

The inconsistencies in the findings of the studies assessing HR and HRV in AN may also reflect differences in the patients' gender, age, nutritional status, psychiatric comorbidity, and mineral and electrolyte levels. Whereas mineral and electrolyte alterations are known to affect the function of the

ANS and to contribute to disturbances in cardiac autonomic function, it is important to note that no such alterations have been found in our sample.

The alterations in HR, HRV, and spectral power components found in our AN individuals not only in the malnourished condition, but also at discharge and long-term follow-up, suggest that these alterations are not the mere result of acute malnourishment, but rather represent the consequence of prior illness. Alternatively, the presence of vagal hyperactivity after long-term remission from AN, with the assumed absence of confounding nutritional influences, may suggest that these autonomic changes may putatively reflect a premorbid trait of AN, that might have existed even before the appearance of acute weight reduction (Kaye et al. 2004). If this the case, then the voluntary food restriction and weight reduction found in AN patients puts this already vulnerable group at a particularly high risk to develop cardiac morbidity. Alternatively, the increased ANS involvement in future to be AN patients may have a role in increasing anxiety (Freedman et al. 2007), this when bearing in mind that elevated anxiety is considered a pivotal predisposing factor for the development of AN (Strober 2004)

We additionally found in the ECG recordings of some AN patients several segmentations in which the R–R interval fluctuations are abnormal as compared to matched healthy controls (Figure 1). This highly unusual finding is not reflected by the spectrum analysis method and may indicate a form of control-dysregulation.

Apart from possible contributions to our understanding of altered ANS functioning in AN patients, HRV may yield important insights concerning cardiovascular morbidity in these conditions. Experimental and clinical studies demonstrate that cardiovascular autonomic regulation plays an important role in cardiac morbidity and mortality (Kleiger et al. 1987; Verrier 1987; Singer et al. 1988; Tsuji et al. 1994; Carney et al. 1995). Reduced HRV is itself associated with sudden cardiac death, at least in non-psychiatric patients (Singer et al. 1988). Moreover, anxiety symptoms are associated with a 4- to 6-fold increase in sudden cardiac death and cardiac arrhythmias (Coryell et al. 1986; Haines et al. 1987), presumably because of some abnormality in the autonomic control of the heart (Yeragani et al. 2007a,b,c, 2008; Bar et al. 2008).

In light of the evidence that mortality is increased in AN, and since changes in HRV are also predictive risk factors for cardiovascular morbidity and mortality, follow-up prospective longitudinal studies in larger AN samples are required to determine the course and effect of autonomic dysregulation in these patients.

We found no improvement in core ED traits (EAT-26 and EDI-2), EDI-2 related personality attributes, depression and anxiety following weight restoration. Other studies have also found similar findings (Bastiani et al. 1995; Pollice et al. 1997; Stein et al. 2005). One can argue that the lack of improvement following short-term weight restoration may be associated with the presence of considerable psychological distress in adolescent AN inpatients who are still not adjusted to undesired changes in weight and physical appearance. Alternatively, the inclination towards dysphoria and behavioural over-control at weight restoration – demonstrated in our AN sample in the high scores of depression, anxiety, and EDI-2-Perfectionism – may be explained by a potential increase in brain serotonin concentrations in weight restored AN patients (Kaye et al. 2005).

## Limitations

We have not followed our comparison group to check whether alterations in cardiac parameters would be found also in normal individuals over time, reflecting a possible time-period effect. We have not been able to control for the effects of psychiatric comorbidity, as similar to previous studies (Halmi et al. 1991), most of our AN inpatients are diagnosed with at least one comorbid DSM-IV Axis I disorder. Moreover, as we have studied only severely ill AN inpatients, our findings cannot be generalized to less severe populations. Fourthly, SSRI treatment is known to affect the function of the ANS and may contribute to

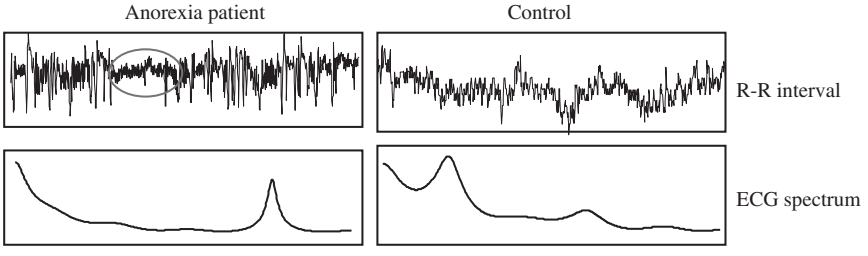


Figure 1. Spontaneous fluctuations in interbeat interval (R-R interval) of a 600-s time period during supine rest. The power spectra (ECG spectrum) show the heart rate variations in all frequency bands. Y-Axis spectra: PSD (ms2r103Hz) Spectral power ranged from 0.0 to 5x104.

disturbances in cardiac autonomic function. However, all malnourished patients and the six remitted patients assessed at long-term follow-up have not received psychotropic medications. Furthermore, the cardiac findings are almost identical in the eight patients receiving SSRIs and the four patients not receiving SSRIs on discharge. These findings suggest that the HRV disturbances in our AN patients are likely the result of the eating disorder itself, rather than a consequence of the influence of psychotropic treatment.

A major limitation of the present study is the considerable dropout rate, both in the weight restoration stage, and specifically at the follow-up assessment. Nevertheless, no differences have been found in any of the baseline physiological and psychometric assessments between AN patients assessed, or not assessed, at stage 2 and 3. Dropout at weight restoration has been associated with the release of several patients from the department before achieving their desired weight, the development of physiological disturbances that required the discontinuation of the study, and technical problems of the spectral analyzer. Our department is a tertiary care centre that hospitalizes patients from all over Israel, thus reducing the likelihood of continuation of treatment in our service following discharge. This may have potentially accounted for our difficulties in locating some former patients at follow-up, whereas others were serving in the Israeli army, thus being unable to participate. Altogether, we suggest that the limitations noted in our study allow our findings to be considered at this time only as preliminary, requiring a much larger cohort to draw more definite conclusions about cardiac ANS abnormalities in AN.

## Conclusions

The present preliminary study found that female adolescent inpatients diagnosed with AN have cardiovascular autonomic control dysfunction that may be irreversible after short- and long-term weight gain. This ANS dysfunction may increase the risk of mortality in AN, including that of sudden death.

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None.

## Statement of interest

The authors have not declared any conflicts of interest.

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**ORIGINAL INVESTIGATION**

# Planning in borderline personality disorder: Evidence for distinct subpopulations

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**Abstract**

*Objective.* Borderline personality disorder is a severe mental disorder, whereas previous studies suggest executive functions may be impaired. The aim of this study was to evaluate executive planning in a sample of 85 individuals. *Methods.* Planning was assessed by means of the Tower of London (Drexel University version) task. Latent class cluster analysis models were adjusted to the data. *Results.* We identified two different subpopulations of borderline personality disorder patients, one of them with significantly reduced performance. *Conclusion.* Neuropsychological mechanisms may be involved in borderline personality disorder, at least in a subgroup of patients.

**Key words:** *Borderline personality disorder, neuropsychological tests, impulsivity*

## Introduction

Borderline personality disorder (BPD) is a psychiatric condition characterized by affective disturbance, impulsivity, disturbed cognition, and intense unstable relationships (Herpertz et al. 2007). BPD affects 1–2% of the population and it is associated with high levels of suffering and impairment and a significant increase in the suicide risk (Hyman 2002), as well as a greater frequency of utilization of various forms of treatment compared to other mental disorders (Bender et al. 2001). Major depressive disorder, anxiety disorders and substance abuse are frequently comorbid with BPD and in this context have a poorer prognosis (Skodol et al. 2002).

Executive functions (EF) are the cognitive skills responsible for the development of complex, goal-oriented behaviours, including set-shifting and set maintenance, interference control, inhibition, integration across space and time, planning and working memory (Pennington and Ozonoff 1996). Neuropsychological correlates of BPD are currently not known, since studies that have been carried out for the past two decades have shown inconsistent results. While some of them conclude that there is a link between executive dysfunction and BPD (Burgess 1991; van Reekum et al. 1996; Bazanis et al. 2002; Coolidge et al. 2004; Dinn et al. 2004; Stevens et al. 2004; Beblo et al. 2006), other identified no differences between patients and control individuals (Driessen et al. 2000; Sprock et al. 2000; Kunert et al. 2003). A detailed review of studies of cognition in BPD can be found in (Fertuck et al. 2006). A meta-analysis concluded that BPD patients perform more poorly than controls in every aspect of EF (Ruocco 2005). This author identified a smaller, albeit statistically significant, effect size for cognitive flexibility, whereas the domain of planning showed a much larger effect relative to healthy controls. We studied planning among BPD patients and normal individuals. Using

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latent class cluster analysis (LCCA) we found the presence of cryptic substructure among BPD patients based on their EF performance.

## Methods

### *Subjects, clinical and neuropsychological assessment*

Individuals were ascertained from outpatient consultants to the departments of Psychiatry, Odontology or Surgery of the Clinical Hospital of the University of Chile. The sample consisted of 85 individuals. Sixty percent the sample (51 subjects) met criteria for BPD, while 34 subjects did not. Diagnostic suspicion of BPD was established through the abbreviated IPDE interview. The BPD module of the complete version of IPDE was administered to those individuals that scored high on this part of the abbreviated IPDE. Using the abbreviated version of the interview, at least another personality disorder was suspected in 88.9% of the BPD patients (at least one cluster A disorder in 60%, at least one cluster B disorder other than BPD in 57.8%, and at least one cluster C disorder in 66.7%). Control individuals did not meet criteria for any personality disorder. Both for BPD patients and control subjects, presence of Axis I pathology, including current or past psychotic disorders or bipolar disorder, or neurological or medical illnesses interfering with the neuropsychological evaluation were exclusion criteria. BPD patients were free of medication for at least 2 weeks prior to evaluation.

Measures of impulsivity were also performed, using the Overt Aggression Scale modified for outpatients (OAS-M), and Barratt Impulsiveness Scale version 10 (BIS-10). OAS-M (Yudofsky et al. 1986; Coccaro et al. 1991) is an instrument for rating aggressiveness (OAS-A), irritability (OAS-I) and suicidality (OAS-S). These features are scored independently and the three scores are added to obtain a total (OAS-T). This measure is state-dependent. On the other hand, BIS-10 (Patton et al. 1995) is a trait-dependent instrument. It is a self-report, Likert-type questionnaire that estimates impulsiveness that manifests as motor (BIS-M), attentional (BIS-A), and non-planning (BIS-NP) impulsiveness. It has a significant, positive correlation with BPD diagnosis (Fossati et al. 2007).

Executive planning was assessed by means of the Tower of London, Drexel University version (TOL-DX) task. The test is composed by a board of three different sized bars and three beads of different colors. The examinee must construct a given configuration while following the specific constraints of the task. The following scores are recorded: *total move score* (TMS: number of movements that the subject needed to accomplish the pattern that exceed the minimum of movements required); *latency time* (LT: time that goes from the reception of the instruction to the initiation of the action); *execution time* (ET: time that it takes to the subject to solve the exercises, starting from the first movement that he performs); and *problem solving time* (PST: the sum of the latency and execution times). Ten problems are presented in an increasing degree of difficulty. Problems 1 through 5 can be solved in a minimum of two to five moves, while the rest of the problems require six or seven moves to be solved. The resolution of the more difficult problems (6–10) could increase the sensitivity of the measure (Culbertson and Zillmer 1998) and were therefore considered for the following analysis.

All the individuals gave their written consent to participate in this study, which was approved by the Ethics Committee of the Clinical Hospital of the University of Chile and was performed in accordance with the Declaration of Helsinki.

### *Statistical analysis*

Mean comparisons were carried out through parametric (Student's $t$) and non-parametric (Mann–Whitney $U$) tests when corresponding, using SPSS 17.0 software. Correlations were investigated using Pearson's coefficient.

Latent Class Cluster Analysis (LCCA) models containing one through 10 classes were fitted to the data using Latent Gold 4.0 software (Statistical Innovations, Belmont, MA). To avoid ending up with local solutions (a well-known problem in LCA), we used multiple sets of starting values as automatically implemented in Latent GOLD. The Bayesian Information Criterion (BIC) was used to select the best fit model. Because we were dealing with sparse contingency tables, we estimated $P$ values associated with $L^2$ statistics by (500 replicates) rather than relying on asymptotic $P$ values. To obtain a bootstrap estimate of the $P$ value corresponding to the difference in log-likelihood value between two nested models, such as two models with different numbers of latent classes or different number of discrete factors follows a procedure where the $-2$LL-difference statistic is defined as $-2 \times$(LLH0 – LLH1), where H0 refers to the more restricted hypothesized model (say a K-class model) and H1 to the more general model (say a model with K+1 classes). Replication samples were generated from the probability distribution defined by the ML estimates under H0. The estimated bootstrap p value is defined as the proportion of bootstrap samples with a larger $-2$LL-difference value than the original sample (Vermunt and Magidson 2005).

This approach was overall comparable with the selection of the best fitting model when using parsimony criteria such as the BIC.

The analysis included as indicators the TMS, LT, ET, and PST scores in the TOL-DX as well BIS and OAS-M scores. As covariates for the model, we used gender, age, and BPD diagnosis. We did not consider the presence of interactions between variables and the basic assumption of local independence of the standard latent class model was supported. Next, we relaxed the local independence assumption by allowing for interactions between variables, as well as for direct effects of covariates on variables (Vermunt 1997). Latent GOLD calculates bivariate variable–variable and variable–covariate residuals that can be used to detect which pairs of observed variables are more strongly related. Therefore, bivariate residuals greater than 3.84 were included iteratively for each model to identify significant correlations between the associated variable–variable and variable–covariate pairs inside each class. An alpha level of 0.01 was chosen to consider a variable or covariate as significant.

The data generated by LCC was used to assign each individual to its corresponding cluster, and means of the scores of each cluster on clinical and neuropsychological measures were compared by parametric (ANOVA followed by Tukey test) or non-parametric (Kruskal–Wallis *H* followed by Mann–Whitney *U*) tests.

## Results

The sample was composed of 85 individuals, 71.8% female and 28.2% male. Their mean age was 31.3 years. Sixty percent of the sample was affected by BPD. Table I shows the scores of controls and BPD patients on clinical measures (BIS and OAS total and subscales). There are significant differences ($P<0.01$) on each one of them.

Table I. Controls' and borderline personality disorder patients' scores (mean ± SD) on clinical measures.

| | Control | BPD |
|---|---|---|
| BIS total** | 57.44 ± 10.96 | 76.35 ± 12.34 |
| BIS-M** | 14.44 ± 3.61 | 24.75 ± 5.14 |
| BIS-A** | 22.21 ± 4.57 | 26.8 ± 4.88 |
| BIS-NP** | 20.79 ± 4.84 | 24.8 ± 5.68 |
| OAS-total** | 1.56 ± 2.21 | 38.27 ± 21.33 |
| OAS-A** | 1.18 ± 1.87 | 28.94 ± 19.76 |
| OAS-I** | 0.35 ± 0.77 | 6.53 ± 2.05 |
| OAS-S** | 0.03 ± 0.17 | 2.98 ± 3.41 |

BPD, borderline personality disorder; BIS, Barratt Impulsiveness Scale, including total, motor (BIS-M), attentional (BIS-A), and non-planning (BIS-NP) impulsiveness; OAS, Overt Aggression Scale, modified for outpatients, including total, aggressiveness (OAS-A), irritability (OAS-I) and suicidality (OAS-S) scores. **Significant differences ($P<0.01$) between both groups.

Table II shows the scores of controls and BPD patients on the TOL-DX. No significant differences ($P>0.05$) were identified between both groups. Significant correlations at the 0.05 level were found between OAS scores (except OAS-S) and ET, and between OAS-I and PST. No correlation was found between BIS scores and TOL-DX parameters (data not shown).

### *LCC analysis*

A three-class model was found to best fit the data. Of the proposed indicators, LT, ET, PST, and BIS total and subtotals and OAS score proved to have a significant effect on cluster membership ($P<0.01$). TMS score did not have an effect ($P=0.94$). Among the covariates, diagnosis was shown to be significant in terms of cluster membership ($P<0.01$). Age and gender did not have an effect ($P=0.027$ and $P=0.62$, respectively). Figure 1 depicts the three-class model.

All of the control individuals belonged to cluster 2. Meanwhile, 79.1% of BPD patients belonged to cluster 1, and 20.9% to cluster 3. Cluster 1 and cluster 3 were made up exclusively of BPD patients, and these two clusters behave in a similar fashion regarding the OAS and BIS scores. However, as to the TOL-DX performance, cluster 1 is indistinguishable from cluster 2 (which is made up of non BPD individuals), while cluster 3 separates from the other two clusters. This latter group presents higher times of latency, execution, and total problem solving. Tables III–V present the comparison between the scores of controls and of the two BPD patients subgroups on clinical and neuropsychological measures.

## Discussion

In the present study we identified two subpopulations among the BPD patients distinguishable on the basis of their performance on a task that measures EF. BPD patients tended to score higher on clinical

Table II. Controls' and borderline personality disorder patients' scores (mean ± SD) on problems 6–10 of the Tower of London-DX task.

| | Control | BPD |
|---|---|---|
| TMS | 17.06 ± 1.65 | 17.27 ± 9.79 |
| LT | 15.65 ± 10.35 | 22.45 ± 20.94 |
| ET | 84.41 ± 35.14 | 106.02 ± 60.75 |
| PST | 103.82 ± 36.56 | 118.61 ± 59.5 |

BPD, borderline personality disorder; TMS, total move score; LT, latency time; ET, execution time; PST, problem solving time.

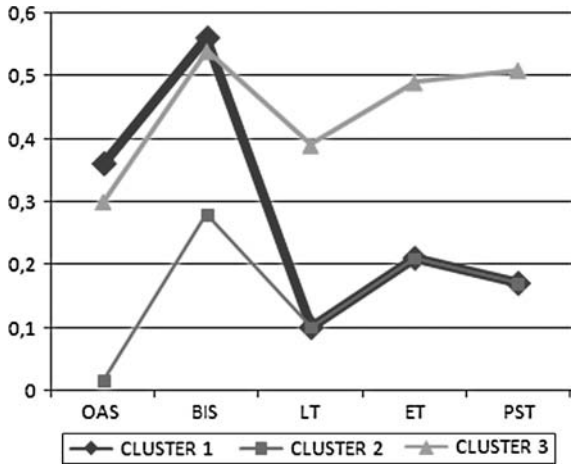


Figure 1. Three-class model for impulsivity measures and executive planning. OAS, Overt Aggression Scale, modified for outpatients; BIS, Barratt Impulsiveness Scale; LT, latency time; ET, execution time; PST, problem solving time.

self-report measures (BIS, OAS), but one subgroup performed poorer on all time indices of the TOL-DX, whereas a second subgroup performed comparably to controls. This suggests that neuropsychological mechanisms are involved in BPD, at least in a subgroup of patients.

The literature regarding the neuropsychological performance of BPD patients comprises discordant results whose comparison is not straightforward, however meta-analytical techniques point to the existence of differences with control individuals, specially in the domain of planning (Ruocco 2005). When tower tasks have been employed, the pattern of impairment observed is comparable to that found by our group (Beblo et al. 2006), with differences in the time of resolution of the task but not in the number of moves. Discordant conclusions (Kunert et al. 2003) could be explained by existence of heterogeneity within the population of patients that has been demonstrated in the present study. The work of (Ruocco 2005) also suggests that this discrepancy is likely due to low limited statistical power of individual studies.

Table III. Controls (cluster 2) and borderline personality disorder patients (clusters 1 and 3) scores (mean±SD) on Barratt Impulsiveness Scale.

| Cluster | 1 | 2 | 3 |
|---|---|---|---|
| BIS total | 76.65±13.32 | 57.44±10.96** | 75.27±8.77 |
| BIS-M | 25.13±5.17 | 14.44±3.61** | 23.36±5.03 |
| BIS-A | 26.9±5.28 | 22.21±4.57** | 26.45±3.14 |
| BIS-NP | 24.63±5.79 | 20.79±4.84** | 25.45±5.5 |

Significant differences (*$P<0.05$ and **$P<0.01$, respectively) with the other two clusters. BIS, Barratt Impulsiveness Scale, including total, motor (BIS-M), attentional (BIS-A), and non-planning (BIS-NP) impulsiveness.

Table IV. Controls (cluster 2) and borderline personality disorder patients (clusters 1 and 3) scores (mean±SD) on Overt Aggresion Scale modified for outpatients.

| Cluster | 1 | 2 | 3 |
|---|---|---|---|
| OAS total | 39.88±22.02 | 1.56±2.2** | 32.45±18.3 |
| OAS-A | 30.45±20.52 | 1.18±1.87** | 23.45±16.34 |
| OAS-I | 6.65±2.07 | 0.35±0.77** | 6.09±2.02 |
| OAS-S | 3±3.24 | 0.03±0.17** | 2.91±4.13 |

**Significant differences ($P<0.01$) with the other two clusters. OAS, Overt Aggression Scale, modified for outpatients, including total, aggressiveness (OAS-A), irritability (OAS-I) and suicidality (OAS-S) scores.

Clinical differences between BPD patients subgroups are not evident with the measures used in this study. Thus, BIS total and subscales scores, as well as OAS total and subscales scores are not significantly different between these subpopulations. Further studies are needed to find out whether these neuropsychological patterns correlate with clinical severity, response to treatment, among others.

The findings of the present study indicate that executive dysfunction is not an essential feature of BPD. In fact, despite the absence of dysfunction in the TOL-DX task, cluster 1 individuals exhibit high levels of impulsivity and aggression. A possible explanation for this is that these clinical features are not mediated by neuropsychological mechanisms, but the possibility of the existence of executive dysfunction that has not been detected by the present study must also be acknowledged. TOL-DX is mainly although not exclusively a measure of working memory, planning and strategy use (Unterrainer and Owen 2006), but other domains of EF, such as the inhibition of habitual or prepotent responses were not evaluated. Furthermore, whereas abstract and decontextualized problems like the one we presented here demand the activation of dorsolateral prefrontal cortex, situations with affective involvement require the activation of orbitofrontal cortical regions (Castellanos et al. 2006), a region that may be compromised in BPD (Berlin et al. 2005) and that was not evaluated here. Administration of other EF tasks, including those where an affective component is involved, could be necessary

Table V. Controls (cluster 2) and borderline personality disorder patients (clusters 1 and 3) scores (mean±SD) on Tower of London, Drexel University version.

| Cluster | 1 | 2 | 3 |
|---|---|---|---|
| LT | 14.7±5.94 | 15.65±10.35 | 50.64±30.67** |
| ET | 86.18±24.39 | 84.41±52.14 | 178.18±93.96** |
| PST | 86.18±24.39 | 103.82±36.56 | 228.82±86.97** |

**Significant differences ($P<0.01$) with the other two clusters. LT, latency time; ET, execution time; PST, problem solving time.

for the purposes of this line of research. Another limitation of the present study is that included only outpatients. We must also address the fact that our sample shows a high frequency of comorbidity with other personality disorders, thus making difficult to ensure that our findings are specific of BPD. This phenomenon has been acknowledged both in research as well as in clinical practice, where practically all patients meet criteria for more than one personality disorder. This is viewed as a limitation of the categorical classification system (Livesley 2008). An answer to that limitation could be an extension of the current model with the integration of a dimensional approach (Livesley 2007). Until that new framework is well defined, the categorical diagnosis is helpful for communication and reproduction of our findings.

Considering that both daily life activities as well as medical and psychological treatment compliance are goal-oriented complex behaviors, executive dysfunction is a major source of impairment in psychiatric disorders (Royall et al. 2002). Therefore, although differences between healthy and affected individuals are neither generalized nor large enough as to be useful as markers of the disorder, recognizing its presence should be a part of an integral diagnosis that can properly estimate the functional status and that could have prognostic relevance. It could be hypothesized that the amount of benefit obtained from psychotherapy, a process that relies on memory and language, could be diminished in those patients with cognitive dysfunctions. These dysfunctions should be also considered when deciding about pharmacological treatment, given that drugs can influence EF either positively or negatively (Arnsten et al. 1995, 1999; Mehta and Riedel 2006). Furthermore, non-pharmacological, cognitively oriented interventions that have proven useful in other disorders (Wasserstein and Lynn 2001) could have a role in BPD.

The present study contributes to the neuropsychological characterization of BPD and poses questions regarding the significance of executive dysfunctions in this disorder, proposing that these could have diagnostic and prognostic relevance.

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## Statement of interest

No conflict of interest declared.

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**ORIGINAL INVESTIGATION**

# Paternal age and common mental disorders

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**Abstract**

*Introduction.* There is evidence in the literature that there are associations between advancing paternal age and psychosis or more specifically schizophrenia, but not enough to support a strong link between advancing paternal age and common mental disorders. *Objective.* This study aims to explain the association between paternal age at birth and common mental disorders in progeny during their adulthood. *Methodology.* This is a sub-study from a larger survey which was planned to study the epidemiology of mental disorders in Malaysia. Respondents who could remember the age of parents at birth were included in the study. The diagnosis of common mental disorders (CMD) was made using the CIS-R (Clinical Interview Schedule-Revised) instrument in the PROQSY (Programmed Questionnaire System) format. Association between paternal age at birth and CMD was studied using logistic regression, after controlling for age, gender, ethnicity and presence of family history of mental disorders. *Results.* Respondents with paternal age at birth of 19 and below and 50 above and had higher rates of 10 and 25% for common mental disorders ($\chi^2 = 7.007$, $P = 0.072$) with odds ratios of 2.89 (95% CI of OR = 1.1–7.6) and 4.28 (1.4–12.7). *Discussion*. Progenies of fathers under 20 and over 50 had higher risk for mental disorders. Factors such as immaturity in sperm of teenage fathers, mutation in germ line of older fathers, environmental and psychosocial factors could have contributed to increased prevalence of common mental disorders in the progeny.

**Key words:** *Paternal age, common mental disorders*

## Introduction

Advanced parental age-associated mutation in the gametes is related to higher rates of congenital disorders in the progenies (Tarin et al. 1998; Thacker 2004), as in Down's syndrome being 1 in 300 if maternal age at birth is 35 and 1 in 100 if the maternal age is 40 and above (Thacker 2004). Association between advanced paternal age of 50 and above with increased risk of schizophrenia has been noted in studies using cohort and case–control study designs (Brown et al. 2002; Dalman and Allebeck 2002). A three-country study (Denmark, Sweden and Australia) examining the effect of parental age at birth as a risk factor for psychosis showed that in Denmark and Sweden the risk of psychosis was higher among the offspring of older fathers and very young mothers (20 and below) (El-Saadi et al. 2004). To our knowledge there is no available literature on the association between parental ages at birth and risk of common mental disorders per se in the progenies. This study which is a part of a larger epidemiological survey, the Malaysian Mental Health Survey, MMHS, (2004–2006) was designed to examine the prevalence, of risk factors and quality of life associated with psychiatric disorders, while this study aims to explain the association between paternal age and presence of common mental disorders in adult progenies.

## Methodology

### *Sampling*

The required sample size was estimated to be 4300 respondents at 90% confidence level by taking into

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account the disorder with the least prevalence rate in the population, panic disorder (Singleton et al. 2003). Multistage cluster sampling method was used to sample respondents. First stage involved selection of states (clusters) by geographical distribution with one state from the west coast (Penang), one from the east coast (Kelantan), one from the middle of the peninsular Malaysia (Negeri Sembilan) and one state from the East Malaysia (Sarawak). Federal Territory was included to represent the capital of the country. The second stage of sampling involved selection of enumeration blocks (EB) by ethnic distribution, from which households were later selected systematically. Enumerators, upon visit to the listed households, made a list of the eligible samples in the household and chose two respondents, preferably a male and a female, with the aid of random selection tables provided. All Malaysian citizens aged 16 and above were eligible to participate in the survey with exception of those unable to answer questions due to physical impediments, the elderly (aged 60 and above) who had low scores (0–4) for Elderly Cognitive Assessments Questionnaire (ECAQ) (Saroja et al. 1995), those who were not members of the household (e.g., relatives who were visiting) and those who were not at home after three consecutive attempts to contact them. The selected respondents were interviewed upon verbal consent using the instruments in the absence of other family members.

Enumerators for this study, who were college students, medical students, research assistants and contract staff from the Statistical Departments were trained and mock interviews were carried out for three days before the actual commencement of the fieldwork. Only those who showed satisfactory agreement with the trainers were kept in the survey. The enumerators were assisted by trainers on the field on the first day of data collection and watched interviews being carried out by trainers upon consent by the respondents.

### *Instruments*

Instruments used in this study were the Clinical Interview Schedule Revised (CIS-R) (Lewis et al. 1992) in Programmed Questionnaire System (PROQSY) format (Lewis 1994) to diagnose common mental disorders which include mixed anxiety and depressive disorder, depressive episode, generalized anxiety disorder, phobic disorders, panic disorder and obsessive compulsive disorder. The instrument has 14 subsections, each assessing an individual symptom. A total score of 12 and above after summation of scores for individual sections indicate presence of clinically diagnosable mental disorders. The PROQSY generates an automatic ICD-10 diagnosis for each respondent based on the answers given, thus providing an outcome equivalent to as being seen by a psychiatrist. Psychosis screening questionnaire (PSQ) was used to screen for psychosis (Bebbington and Nayani 1995), Alcohol Use Disorders Identification Test (AUDIT) questionnaire for alcohol use disorders (Babor et al. 1992), Items from Diagnostic Interview Schedule (DIS) (Robins et al. 1982) for drug use and Short-forms 12 (SF-12) (Ware et al. 1996) for quality of life assessment. Subjects who were screened positive for psychosis using PSQ or were in the highest and lowest 5% scores for CIS-R or AUDIT were identified and interviewed by psychiatrists using Schedule for Clinical Assessment in Neuropsychiatry, SCAN (Wing et al., 1990) within a month from data collection. Age of parents at birth of the respondents was determined by the respondents being asked to estimate the parental age as in these categories, 20–29, 30–39, 40–49, 50–59 years old and older. Family history of mental illness was assessed only in the first-degree family members, i.e. parents, siblings and children. Presence of family history of mental disorders was assessed using an item from WHO-Mental Health Risk Factor analysis instrument. The item was as follows: "Has any of your parent, sibling or children ever had mental disorders?"

### *Ethical approval*

Ethical approval was obtained from the National University of Malaysia Ethical Committee.

### *Analysis*

Analysis was made using the Statistical Packages for Social Sciences (SPSS) software version 12. Descriptive statistics including chi-square tests were used to study association between parental age at birth and common mental disorders. Logistic regression models were developed to study parental age at birth and the presence of mental illness after adjusting for age, gender, ethnicity and presence of family history of mental disorders.

## Results

Three thousand six hundred and sixty-six adults from five states in Malaysia participated in the Malaysian Mental Health Survey (MMHS). A total of 2174 respondents or 59% from the MMHS population could estimate the age of at least one parent and were included in this study. Ninety-one percent of those respondents ($n = 1972$) knew the

age of both of their parents whereas 183 subjects only knew the age of mother and 19 subjects only that of their fathers.

Prevalence rate for common mental disorders was 6% ($n=130$) with 95% confidence interval of 5.53–6.47. The most frequently obtained diagnosis was mild mixed anxiety and depressive disorder ($n=59$), followed by mixed anxiety and depressive disorder ($n=34$), generalised anxiety disorders ($n=15$), phobic disorders ($n=14$) and mild depressive episode ($n=8$).

Forty-one percent of the MMHS subjects did not know the age of parents at birth and thus could not be included in this analysis. The included and excluded respondents were compared for prevalence of mental disorders and demographic characteristics. The two groups do not have significantly different rates for common mental disorders (6 and 5%) and no significant difference in demographics (Table I).

Fifty-four percent ($n=1181$) of the respondents were born to mothers aged 20–29 years old followed by mothers aged 30–39 years old ($n=599$, 28%). Forty-five percent of respondents ($n=929$) were born to fathers 20–29 years old, followed by 35% born to fathers 30–39 years old. More mothers were aged below 20 ($n=294$, 13%) in comparison to fathers ($n=78$, 3%) and there were more fathers aged 40 and above ($n=248$, 12%) in comparison to mothers ($n=81$, 4%).

Profiling of parental age and presence of common mental disorders among respondents are shown in Table II. No association was observed between maternal age at birth and presence of common mental disorders among respondents ($\chi^2=7.007$, $P=0.072$). Respondents with paternal age at birth of 19 and below and 50 above and had higher rates for common mental disorders of 10 and 25% while respondents whose fathers were aged between 20 and 49 years had rates between 5–6% ($\chi^2=7.007$, $P=0.072$) plotted as a J-shaped curve (Figure 1).

Table I. Knew parental age versus those who did not.

| Factor | Knew parental age | Did not know parental age | Test statistics ($\chi^2$, $P$) |
|---|---|---|---|
| *Presence of CMD* | | | |
| No | 2043 (94) | 1413 (95) | 0.88 |
| Yes | 131 (6) | 79 (5) | 0.3495 |
| *Gender* | | | |
| Female | 1334 (62) | 909 (61) | 0.19 |
| Male | 830 (38) | 583 (39) | 0.6603 |
| *Ethnicity* | | | |
| Malay | 1196 (55) | 773 (52) | 18.58 |
| Chinese | 564 (26) | 424 (28) | 0.000 |
| Indian | 248 (11) | 134 (9) | |
| Others | 166 (8) | 161 (10) | |
| *Age* | | | |
| ≤19 | 216 (10) | 79 (5) | 339.69 |
| 20–29 | 468 (21) | 226 (15) | 0.000 |
| 30–39 | 497 (23) | 311 (21) | |
| 40–49 | 443 (20) | 334 (22) | |
| 50–59 | 326 (15) | 286 (19) | |
| ≥ 60 | 185 (9) | 256 (17) | |
| Missing | 39 (2) | | |

Respondents born to fathers aged 19 years and below were at increased risk of common mental disorders by 2.89 times and for paternal age of 50 and above the risk increased by 4.28 times. Presence of a family member with mental illness was associated with a three-fold risk for having mental disorders themselves. Gender had no impact on the rate of common mental disorders in the model. Age groups of 19 and below and 20– 29 had significantly higher odds for common mental disorders of 3.80 and 2.45 in comparison to the reference groups of 30–39 years old. Malays and Indians were five times more likely to have common mental disorders than the Chinese (Table III).

## Discussion

This is a sub-study from a Malaysian Mental Health Survey, MMHS. A total of 2174 respondents who form 59% of the MMHS population were included in this analysis. The included numbers of respondents suffice for estimation at 80% confidence level. Rate of mental disorders did not differ significantly across those who knew age of parents at birth and those who did not. Demographic comparison showed similar distribution of gender across the two groups. There was excess of people aged 50 and above in the excluded group suggesting that the older population could have more difficulties in remembering parental age. The older group in this study population had younger parents in comparison to the younger counterparts which reflect the younger age of marriages in the earlier generation. Maternal age at birth did not show any association with the presence of common mental disorders. Logistic regression model that explains the association between paternal age and presence of common mental disorders was developed upon controlling for age, gender, ethnicity and presence of mental disorders among first-degree family members. Being born to teenage fathers or fathers aged 50 and above was found to be significantly associated with increased rate of common mental disorders. This suggests a non-monotonic relationship between paternal age and common mental disorders as highlighted by the three country study for psychosis (El-Saadi et al. 2004). A study conducted in Israel showed similar non-monotonic pattern for paternal age and schizophrenia (Malaspina et al. 2001). Another interesting comparison is a study showing

Table II. Parental age at birth and presence of common mental disorders among respondents.

| Parental age at birth (years) | | Presence of common mental disorders | | Total | Test statistic |
|---|---|---|---|---|---|
| | | No *n* (%) | Yes *n* (%) | | ($\chi^2$, *P*) |
| Mother's age | ≤19 | 267 (91) | 27 (9) | 294 | $\chi^2=7.01$ $P=0.0717$ |
| | 20–29 | 1118 (95) | 63 (5) | 1181 | |
| | 30–39 | 567 (95) | 32 (5) | 599 | |
| | ≥ 40 | 75 (93) | 6 (7) | 81 | |
| | Don't know* | 16 (85) | 3 (15) | 19 | |
| Father's age | ≤19 | 70 (90) | 8 (10) | 78 | $\chi^2=28.180$ $P=0.000$ |
| | 20–29 | 878 (94) | 51 (6) | 929 | |
| | 30–39 | 695 (94) | 41 (6) | 736 | |
| | 40–49 | 196 (94) | 12 (6) | 208 | |
| | ≥ 50 | 30 (75) | 10 (25) | 40 | |
| | Don't know* | 174 (95) | 9 (5) | 183 | |

* Categories not included in the analysis. Respondents who know only the age of one parent at birth are displayed in "Do not know" category in profiling for the age of the other parent.

learning capacities to be a U-shaped curve in progenies of very young fathers and old fathers (Auroux et al. 1989).

Having older parents has psychological consequences on children (Hare and Moran 1979) and increased parental age could result in earlier loss of parents which has been identified as a risk factor for schizophrenia (Hare and Moran 1979; Dalman and Allebeck 2002). However this has only been observed for older fathers, not for mothers. Consanguinous marriages or marriages between biologically related or blood relatives have been reported to contribute to the inheritance of many genetic and congenital disorders (Kumaramanickavel et al. 2002; Al-Ghazali et al. 2006). This has been described when parents are of similar ancestry as seen in autosomal disorders like haemoglobinopathies, glucose-6-phosphate dehydrogenase, and ocular genetic disorders such as retinitis pigmentosa (Kumaramanickavel et al. 2002; Al-Ghazali et al. 2006). Practice of arranged marriages among relatives like cousins, uncles and nieces still occur in Asia where the man can be much older especially between uncles and nieces. Incest or violence could contribute to marriages among young women and older men, which are under-reported, denied or covered up. The available record for year 2000–2007 shows that the highest numbers of incest cases were

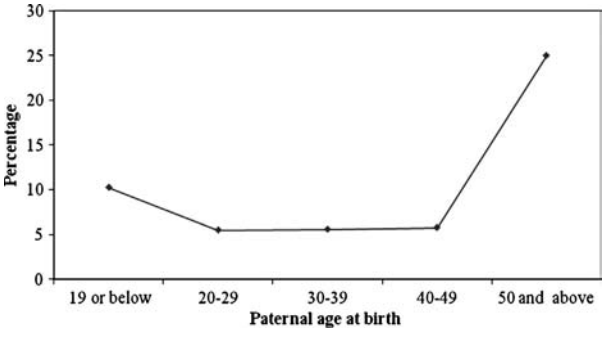


Figure 1. Common mental disorders across paternal age probands. * Respondents who did not know the age of father at birth were not included in the analysis.

Table III. Paternal age at birth and presence of common mental disorders during adulthood among respondents.

| Factors | Significance (*P*) | Adjusted odds ratio | 95.0% Confidence interval | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Gender | | | | |
| Male† | | | | |
| Female | 0.105 | 1.482 | 0.921 | 2.387 |
| Age | 0.001 | | | |
| 19 and below | 0.001 | 3.803 | 1.768 | 8.180 |
| 20–29 | 0.013 | 2.458 | 1.213 | 4.980 |
| 30–39† | | | | |
| 40–49 | 0.122 | 1.797 | 0.855 | 3.777 |
| 50–59 | 0.965 | 1.021 | 0.413 | 2.523 |
| 60 and above | 0.452 | 0.608 | 0.166 | 2.224 |
| *Ethnicity* | 0.001 | | | |
| Malays | 0.001 | 5.039 | 1.992 | 12.746 |
| Chinese† | | | | |
| Indians | 0.002 | 5.236 | 1.837 | 14.927 |
| Others | 0.785 | 0.792 | 0.147 | 4.255 |
| *Mental illness among family members* | 0.007 | | | |
| No† | | | | |
| Yes | 0.001 | 3.131 | 1.596 | 6.143 |
| Do not know | 0.294 | 3.274 | 0.358 | 29.975 |
| Refused | 0.670 | 0.025 | 0.000 | 571246.493 |
| *Father's age at birth (years)* | 0.029 | | | |
| <19 | 0.032 | 2.887 | 1.096 | 7.603 |
| 20–29† | | | | |
| 30–39 | 0.746 | 0.918 | 0.547 | 1.540 |
| 40–49 | 0.676 | 1.162 | 0.575 | 2.350 |
| 50 < | 0.009 | 4.281 | 1.442 | 12.712 |

† Reference category.

involving fathers, followed by uncles and stepfathers. There are no available published articles on this issue due to cultural sensitivity (Ministry if Women, Family and Community Development Malaysia, 2008). Such marriages and problematic family environments would have adverse effect on the children contributing to psychiatric and other common mental health problems either through the mother–foetal connection or a dysfunctional family environment as the child grows. Studies have showed the association between familial adversity, dysfunction and increased psychiatric symptomatology and mental disorders among adolescents (Forehand et al. 1998; Goebert et al. 2000).

The association between advanced paternal age at birth and common mental disorders among progenies could be due to the possibility of a gene–environmental interaction. The rates of de novo mutation in the male germ line has been reported to increase with age (Crow et al. 1999) and association between accumulation of de novo mutation and increasing risk of schizophrenia has been highlighted in other studies (Malaspina et al. 2001; Dalman and Allebeck 2002; Sipos et al. 2004). Accumulation of random mutation could have also contributed to higher rates of common mental disorders in the progenies of older fathers in this study.

As for younger fathers, immature spermatids produced or low activity of DNA repair or antioxidant enzymes could have contributed to elevated risks of de novo genetic disorders (El-Saadi et al. 2004). Lower sperm counts, semen volumes, total number of sperms, lower percentage of motile form of sperm and abnormal placentation as a result of immature spermatids have also been associated with increased risk of adverse birth outcomes among children of younger fathers (Chen et al. 2008). Fathering at young ages has also been associated with social disadvantages (Chen et al. 2008).

There are several limitations to this study. The use of the CIS-R only identifies subjects with common mental disorders and does not detect co-morbidities or psychotic illness. It was not able to detect subjects with schizophrenic spectrum disorders who may later develop schizophrenia. This is a study on the epidemiology of mental disorders that covers a wide geographical area with interview processes taking about 30 min to 1 h. Parental age at birth was assessed as a variable and this study was not designed to look specifically into the relationship between paternal age and mental disorders. Therefore many details and specific information such as exact age of parents when respondents were born, mental health status of parents and for those with family history of mental disorders, the exact family member who suffered and details of the illness were not able to be elicited.

A significant proportion of respondents, 41% could not remember the age of parents when they were born and were excluded from the survey. The obtained sample size of 2174 gives a significant power of 80%, at estimated sample size of 2628. The excluded group consists of higher rates of older respondents and respondents from Chinese and "Others" ethnic group. This slight excess of Chinese and "Others", and those aged 50 and above who did not know the paternal age is due to general poor cooperation and difficulties in remembering details. This does not significantly affect the study outcome. Regression analysis showed a strong and undeniable association between presence of common mental disorders and extreme paternal ages after controlling for demographic and family history of mental disorders.

As respondents were only asked for presence of family members with mental illness inferences cannot be made as to whether definitively the older fathers had mental illnesses. Advanced paternal age at birth and psychosis (El-Saadi et al. 2004) or more specifically schizophrenia (Dalman and Allebeck 2002; Malaspina et al. 2001; Sipos et al. 2008) has been reported, but the dearth of studies which examine paternal age and common mental disorders suggest that research which can specifically examine the relationship between paternal age and common mental disorders should be carried out.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**ORIGINAL INVESTIGATION**

# Dopamine transporter genotype influences *N*-acetyl-aspartate in the left putamen

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**Abstract**

*Introduction.* Dopaminergic activity in the brain is modulated by the dopamine transporter (DAT). Several lines of evidence suggest that a variable number of tandem repeats (VNTR) polymorphism of the *DAT1* gene (SLC6A3) influences its gene expression. The aim of this study was to determine whether the *DAT1 VNTR* polymorphism alters the metabolic ratios NAA/Cho, NAA/Cr, Cho/Cr and Ins/Cr in the left dorsolateral prefrontal cortex, anterior cingulate cortex, and putamen in healthy subjects and psychiatric patients irrespective of clinical diagnosis. *Material and Methods.* Sixty-four individuals (30 patients with bipolar disorder, 18 patients with obsessive-compulsive disorder, and 16 healthy subjects) participated in the study. The 3′-UTR VNTR polymorphism of *DAT1 (SLC6A3)* gene was genotyped in all individuals. $^{1}$H-MRS was performed in the above-mentioned brain regions. *Results.* The individuals with the homozygous *DAT1* 10-repeat genotype presented significantly higher ratios of NAA/Cho and NAA/Cr in the left putamen compared to the group of individuals with the 9/9-repeat or 9/10-repeat genotype. *Conclusion.* The VNTR polymorphism of the *DAT1*-gene modulates NAA/Cho and NAA/Cr in the left putamen independent of psychiatric diagnosis status. These results suggest an association of *DAT1* VNTR polymorphism, dopaminergic activity, and neuronal function in putamen.

**Key words:** *DAT1, SLC6A3, putamen, magnetic resonance spectroscopy, NAA*

## Introduction

The neurotransmitter dopamine plays an important role in regulation of basic brain functions including motor behaviour, motivation and working memory. Pharmacological evidence suggests that dysfunction of the dopaminergic system could be involved in the aetiology of different psychiatric disorders, among others bipolar disorder (BD) and obsessive-compulsive disorder (OCD).

Dopaminergic activity in the brain depends on the synthesis, re-uptake or catabolism of dopamine in pre-synaptic neurons. The dopamine transporter (DAT) mediates uptake of dopamine from the synaptic cleft into neurons and, in this way, regulates dopaminergic neurotransmission in the brain (Giros and Caron 1993).

Reflecting the DAT1-encoding gene (*DAT1*, *SLC6A3*), a 40-bp variable number of tandem repeats (VNTR) polymorphism was identified in exon 15 (Vandenbergh et al. 1992). The tandems are repeated between 3–13 times, with greatest frequency in the 9- and 10-repeat forms in most human populations (Mitchell et al. 2000). As this VNTR resides in the 3′ untranslated region, it does not affect the protein's amino acid sequence. However, VNTRs in untranslated regions of genes have been shown to play an important role in the regulation of transcription efficiency, mRNA stability or mRNA sub-cellular localization (Mignone et al. 2002).

Investigation of the functional role of the *DAT1* VNTR revealed inconclusive results. An in vivo SPECT study in abstinent alcoholics and healthy

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controls revealed a reduced availability of the dopamine transporter in putamen in individuals with the heterozygous 9-/10-repeat genotype compared to individuals with the homozygous 10-repeat genotype (Heinz et al. 2000). A recent study in children with attention deficit hyperactivity disorder also reported a higher DAT availability in children with the homozygous 10-repeat genotype (Cheon et al. 2005). In contrast to these findings, in another study preliminary data on 14 recently detoxified cocaine abusers and 30 healthy controls showed increased striatal DAT availability in patients with a homozygous or heterozygous 9-repeat genotype (Jacobsen et al. 2000). Two further studies could not find any influence of the *DAT1* VNTR genotype on DAT availability in the striatum (Martinez et al. 2001; Krause et al. 2006). Nevertheless, taken together these in vivo SPECT studies suggest that expression of the *DAT1* gene might be modulated by the allele frequency of the VNTR polymorphism.

Proton magnetic resonance spectroscopy ($^1$H-MRS) is an imaging method which allows in vivo determination of several brain metabolites such as *N*-acetyl-aspartate (NAA), choline-containing compounds (Cho), creatine + phosphocreatine (Cr) and inositol + myo-inositol (Ins). NAA is a generally accepted marker for neuronal density and function, even though its exact role in brain metabolism is still unclear. Cho primarily reflects cell membrane phospholipids and cell membrane turnover. Cr represents cell energy metabolism and Ins participates in phospholipid metabolism and signal transduction.

Because an absolute quantification of these compounds is difficult to achieve, most studies focus on specific ratios of the markers of interest.

So far, little is known about the possible effects of genes on these brain metabolites measured by $^1$H-MRS. A recent study reported an effect of the glutamate receptor 3 genotype on NAA measures in the dorsolateral prefrontal cortex (Marenco et al. 2006). These authors were able to show a significant reduction of NAA/Cr levels in the right dorsolateral prefrontal cortex in homozygous A-Allele carriers of a single nucleotide polymorphism in the metabotropic glutamate receptor 3.

The aim of the present study was to determine whether the *DAT1* VNTR polymorphism, irrespective of clinical diagnosis, alters the metabolic ratios NAA/Cho, NAA/Cr, Cho/Cr and Ins/Cr in dopaminergic modulated cortical and subcortical brain regions. In order to address this issue, we acquired $^1$H-MRS data from the left dorsolateral prefrontal cortex, left posterior medial frontal (dorsal anterior cingulate) cortex, and the left putamen in healthy individuals and psychiatric patients suffering from bipolar or obsessive-compulsive disorder.

## Materials and Methods

### *Subjects*

Thirty euthymic patients with bipolar I disorder, 17 patients with obsessive-compulsive disorder and 16 healthy control subjects participated in the study. Written informed consent was obtained from all subjects and the study was approved by the local ethical committee. Subjects were recruited from the outpatient unit of the Department of Psychiatry and Psychotherapy of the Saarland University Hospital. The diagnoses of bipolar I disorder and OCD were confirmed by using the German version of the Structural Clinical Interview for DSM-IV (Wittchen et al. 1997). All bipolar disorder patients were receiving stable medication at the time of the study. Twelve were receiving one or two mood stabilizers (three lithium, five sodium valproate, two carbamazepine and five lamotrigine) and/or antidepressants (five) and antipsychotics (six). Eight patients with OCD received treatment with a SSRI, two with a tricyclic antidepressant drug and six were without any drug treatment.

### *$^1$H-MRS*

Single-volume proton magnetic resonance spectroscopy was performed on a 1.5-Tesla Siemens Magnetom Sonata (Siemens, Erlangen) using a spin-echo sequence with water-suppression and 128 scan averages (TE = 30, TR = 1500). Regions of interest were determined in left dorsolateral prefrontal cortex, left posterior medial frontal (dorsal anterior cingulate) cortex and left putamen according to an exactly predefined and standardized algorithm with multiple rechecking procedures in a T2-gradient echo image (TrueFISP) with 24 slices each in three orthogonal orientations. The positions of the voxels were visually inspected and adjusted based on identifiable anatomical landmarks in reference to standard brain atlases (Talairach and Tournoux 1998; Duvernoy 1999). Voxel-size was $10 \times 35 \times 10$ mm for the middle frontal gyrus, $12 \times 20 \times 15$ mm for the posterior medial frontal (dorsal anterior cingulate) cortex and $10 \times 22 \times 15$ mm for the putamen (Figure 1).

All spectra were post-processed by using the Siemens Medical Solutions spectroscopy software package on a Leonardo workstation. Following acquisition, low frequency filtering for removal of the residual water signal and correction for frequency shifts were performed. Data were zero-filled from 1024 to 2048 points. After apodization of the time-domain signal by a Hanning function with 700 ms width, the data were Fourier transformed to the frequency domain. The spectra were baseline corrected with a sixth-order polynomial and first-order

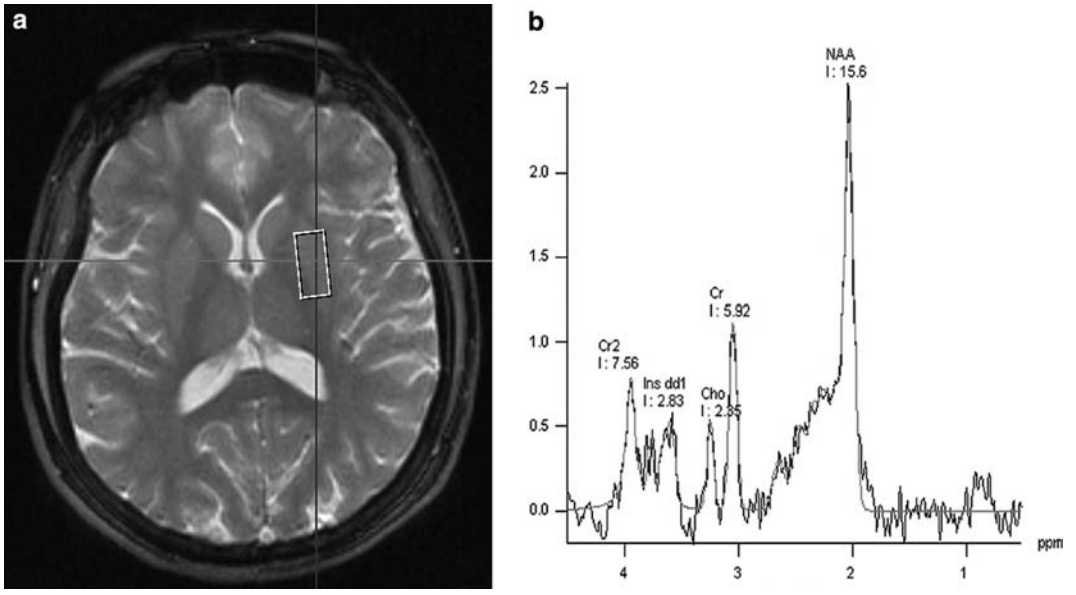


Figure 1. (a) Magnetic resonance imaging demonstrating the localization of the volume of interest in the left putamen. (b) Proton MR spectrum.

phase-corrected. Curve-fitting to the metabolic peaks was applied using Gaussian or Lorentzian line shapes at known frequencies. Relative metabolite concentrations for *N*-acetyl groups (NAA), choline containing compounds (Cho), creatine and phosphocreatine (Cr) and inositol plus myoinositol (Ins) were determined and the metabolic ratios NAA/Cr, NAA/Cho, Cho/Cr and Ins/Cr were calculated.

*Genotyping*

For the genotyping of the 3′-UTR VNTR of *DAT1*, genomic DNA was extracted from EDTA blood by standard procedures. *DAT1* VNTR-specific primers (forward, 5′-AGCTCAGGCTACTGCCACTC-3′; reverse, 5′- AAAAAGCCATTCGCAAACAT – 3′) were used for polymerase chain reaction (PCR). The thermocycler protocol involved an initial denaturation cycle (4 min, 94°C), 35 cycles of denaturation (30 s, 95°C), annealing (30 s, 53.8°C), and extension (30 s, 72°C), followed by a final extension step (7 min, 72°C), and terminating at 4°C. This procedure resulted in fragments of 560 and 600 bp in length. Genomic DNA was amplified by PCR in a final volume of 50 µl containing 100 ng genomic DNA, 10 pmol of each primer (MWG Biotech), 0.2 mM of each dNTP (MBI Fermentas), 7.5 mM $MgCl_2$, and 1 unit of Taq Polymerase (Eurogentec, Seraing, Belgium). For control, 10 µl of PCR product were visualized on 1–2% agarose gels stained with ethidium bromide. Fragments were subsequently analyzed by automated genotyping using an ABI 310 sequencer.

*Statistics*

Statistical analysis was performed using SPSS 14. All tests were two-tailed. Significance level was $P<0.05$. As initial analyses, the influence of the variables gender and handedness on the dependent variables (metabolite ratios) was tested with one-way analysis of variance (ANOVA), and the influence of age and education was evaluated calculating Pearson correlation coefficients.

There is evidence to suggest that the homozygous 10-repeat genotype is associated with higher DAT availability as compared to the 9/9 or 9/10 variants (Heinz et al. 2000, Cheon et al. 2005). Therefore, since the group sizes for the 9/9 genotype were rather small (two controls, three bipolar patients, four OCD patients), we pooled subjects with the 9/9 and 9/10 genotypes in our analysis in order to compare them to subjects with the homozygous 10-repeat genotype.

Multivariate analyses of variance (MANOVA) with *DAT1* VNTR polymorphism and diagnosis as independent factors and with ratios of NAA/Cr, NAA/Cho, Cho/Cr and Ins/Cr as dependent variables were calculated separately for each of the three brain regions: left dorsolateral prefrontal cortex, posterior medial frontal (dorsal anterior cingulate) cortex and putamen. In case of significance post-hoc univariate analyses for each metabolic ratio were calculated.

Additionally, for patients with bipolar disorder the factors treatment with antipsychotics on the one hand, and prior psychotic symptoms on the other, where entered to the MANOVA additionally to the

factor DAT1 intending to analyze the influence of these intervening factors on metabolite ratios.

## Results

### *DAT1 genotype distribution*

Thirty-five individuals (19 BD, eight OCD, eight controls) had the homozygous *DAT1* 10-repeat genotype (10/10) and 29 individuals (11 BD, 10 OCD, eight controls) had the homozygous 9-repeat (9/9) or the heterozygous 9/10-repeat (9/10) genotype (Table I). The second group consisted of nine individuals with the 9/9 genotype (three BD, four OCD, two controls) and 20 individuals with the 9/10 genotype (eight BD, six OCD, six controls).

The two main groups (10/10 genotype versus 9/9 and 9/10 genotype) exhibited no significant differences regarding mean age ($41.7 \pm 12.8$ vs. $35.8 \pm 12.6$, $P = 0.07$, Table I) or gender (14 male, 22 female vs. 15male, 14 female, $P = 0.33$). However, the two groups differed from each other with respect to the duration of education ($12.3 \pm 2.1$ vs. $13.7 \pm 2.7$ years, $p = 0.02$). The distributions of the *DAT1* VNTR alleles are consistent with the results of previous investigation (Heinz et al. 2000; Mitchell et al. 2000).

### *DAT1 genotype and MRS ratios*

Statistical analyses did not reveal significant influences of sex, age, handedness or education on metabolite ratios.

From MANOVA with the independent factors DAT1 and diagnosis, a significant influence of *DAT1* genotype on metabolite ratios in the left putamen was observed in the multivariate analyses with the independent factor genotype ($F = 3.28$, $P = 0.021$, Table II). The post-hoc univariate analyses revealed that the individuals with the homozygous 10/10 genotype presented significantly higher ratios of NAA/Cho (+32%, $F = 11.23$, $P = 0.002$) and NAA/Cr (+17%, $F = 7.20$, $P = 0.010$) in the left putamen compared to the group of individuals with the homozygous 9/9 or heterozygous 9/10 genotype. As there were no significant interactions between DAT1 genotype and diagnosis, this finding was illustrated pooled – over the diagnostic groups (Figure 2). No associations between *DAT1* VNTR polymorphism and the other metabolic ratios in putamen or the metabolic ratios in the left dorsolateral prefrontal cortex or the left posterior medial frontal (dorsal anterior cingulate) cortex were observed. As well, interactions between DAT1 genotype and diagnosis were not significant.

Multivariate analyses with the independent factors *DAT1* genotype/treatment with antipsychotics, and *DAT1* genotype/prior psychotic symptoms in patients with bipolar disorder did not reveal any significant influence of antipsychotic treatment or of prior psychotic symptoms on metabolite ratios.

Table I. Demographical and genotype data of subjects.

| | Genotype | | | |
|---|---|---|---|---|
| | 9/9 | 9/10 | 10/10 | *P* |
| BD (*N*) | 3 | 8 | 19 | |
| OCD (*N*) | 4 | 6 | 8 | |
| Controls (*N*) | 2 | 6 | 8 | |
| | 9 | 20 | 35 | |
| | | 29 | 35 | |
| Age (years; mean ± SD) | $35.8 \pm 12.6$ | | $41.7 \pm 12.8$ | 0.07 |
| Gender (male/female) | 15/14 | | 14/22 | 0.33 |
| Education (years; mean ± SD) | $13.7 \pm 2.7$ | | $12.3 \pm 2.1$ | 0.02 |

BD, bipolar disorder; *N*, number; OCD, obsessive-compulsive disorder; SD, standard deviation.

## Discussion

In this study we found a significant influence of *DAT1* genotype on the metabolic ratios NAA/Cho and NAA/Cr in the left putamen but not in the left dorsolateral prefrontal cortex or the left posterior medial frontal (dorsal anterior cingulate) cortex. In more detail, individuals with the homozygous 10/10 genotype exhibited increased ratios of both NAA/Cho and NAA/Cr in the left putamen compared to the individuals with the homozygous 9/9 or heterozygous 9/10 genotype. These results provide evidence for enhanced NAA levels in the left putamen of subjects homozygous for the 10/10 *DAT1* genotype. As NAA is generally assumed to be a marker for neuronal density and function (Burtscher and Holtas 2001), therefore this finding may indicate that individuals with the 10/10 genotype possess a higher density of neurons or neurons with a higher functionality in the left putamen than individuals with the other genotypes. The *DAT1* genotype appears, therefore, to modulate the neuronal function in the left putamen. This effect could be mediated by a modulation of the activity of the dopamine transporter at pre-synaptic neurons, leading to an altered synaptic dopaminergic activity, which may influence the functionality of post-synaptic neurons and, consequently, NAA levels in the left putamen.

So far, only one study investigated the connection between dopaminergic activity and NAA levels in the putamen. Ellis et al. (1997) reported that drug naïve, but not levodopa treated patients with idiopathic Parkinson's disease showed significantly reduced NAA/Cho ratio in the putamen compared to healthy controls. They hypothesized that the reduced

Table II. $^{1}$H-MRS results in left dorsolateral prefrontal cortex, left posterior medial frontal (dorsal anterior cingulate) cortex and in putamen depending on DAT1 genotype and diagnosis.

| | Controls | | Bipolar disorder | | OCD patients | | Factor DAT1 | | | Interaction DAT1 × Diagnosis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9/9 or 9/10 repeats ($n$ = 8) Mean ± SD | 10/10 repeats ($n$ = 8) Mean ± SD | 9/9 or 9/10 repeats ($n$ = 11) Mean ± SD | 10/10 repeats ($n$ = 19) Mean ± SD | 9/9 or 9/10 repeats ($n$ = 9) Mean ± SD | 10/10 repeats ($n$ = 8) Mean ± SD | df | $F$ | $P$ | df | $F$ | $P$ |
| DLFPC *(multivariate analysis)* | | | | | | | 4, 42 | 0.16 | 0.96 | 8, 86 | 0.44 | 0.90 |
| NAA/Cho | 4.35 ± 1.9 | 3.80 ± 1.1 | 4.26 ± 1.5 | 3.84 ± 1.1 | 3.30 ± 1.1 | 3.90 ± 1.6 | | | | | | |
| NAA/Cr | 2.26 ± 0.4 | 1.94 ± 0.5 | 2.19 ± 0.5 | 2.05 ± 0.4 | 2.05 ± 0.4 | 2.23 ± 0.6 | | | | | | |
| Cho/Cr | 0.56 ± 0.1 | 0.51 ± 0.0 | 0.55 ± 0.2 | 0.57 ± 0.2 | 0.64 ± 0.1 | 0.63 ± 0.2 | | | | | | |
| Ins/Cr | 0.44 ± 0.2 | 0.42 ± 0.1 | 0.34 ± 0.1 | 0.38 ± 0.1 | 0.45 ± 0.1 | 0.44 ± 0.1 | | | | | | |
| PMFC *(multivariate analysis)* | | | | | | | 4, 51 | 1.22 | 0.31 | 8, 104 | 0.60 | 0.77 |
| NAA/Cho | 2.81 ± 0.4 | 2.78 ± 0.5 | 2.65 ± 0.5 | 2.41 ± 0.6 | 2.32 ± 0.4 | 2.60 ± 0.6 | | | | | | |
| NAA/Cr | 1.92 ± 0.4 | 2.09 ± 0.2 | 1.98 ± 0.5 | 1.96 ± 0.5 | 1.86 ± 0.2 | 2.00 ± 0.3 | | | | | | |
| Cho/Cr | 0.68 ± 0.1 | 0.77 ± 0.1 | 0.77 ± 0.2 | 0.83 ± 0.2 | 0.81 ± 0.1 | 0.80 ± 0.2 | | | | | | |
| Ins/Cr | 0.41 ± 0.1 | 0.49 ± 0.1 | 0.44 ± 0.1 | 0.50 ± 0.1 | 0.46 ± 0.1 | 0.48 ± 0.1 | | | | | | |
| Putamen *(multivariate analysis)* | | | | | | | 4, 39 | 3.28 | 0.021 | 8, 80 | 0.91 | 0.51 |
| *Post-hoc univariate analysis* | | | | | | | | | | | | |
| NAA/Cho | 2.43 ± 0.9 | 3.03 ± 0.7 | 2.64 ± 1.3 | 3.45 ± 0.8 | 2.78 ± 0.7 | 3.20 ± 0.8 | 1, 42 | 11.2 | 0.002 | | | |
| NAA/Cr | 1.43 ± 0.6 | 1.92 ± 0.3 | 1.62 ± 0.4 | 1.83 ± 0.3 | 1.87 ± 0.5 | 2.06 ± 0.5 | 1, 42 | 7.20 | 0.010 | | | |
| Cho/Cr | 0.58 ± 0.1 | 0.65 ± 0.1 | 0.68 ± 0.2 | 0.55 ± 0.1 | 0.70 ± 0.2 | 0.65 ± 0.1 | 1, 42 | 1.04 | 0.31 | | | |
| Ins/Cr | 0.16 ± 0.1 | 0.20 ± 0.1 | 0.19 ± 0.1 | 0.20 ± 0.1 | 0.23 ± 0.1 | 0.21 ± 0.1 | 1, 42 | 0.07 | 0.79 | | | |

SD, standard deviation; DLPFC, dorsolateral prefrontal cortex; PMFC, posterior medial frontal cortex; OCD, obsessive-compulsive disorder.

putaminal NAA/Cho ratio might reflect loss of nigrostriatal dopamine terminals. In line with this notion, one could assume that, more generally, a lower number of nigrostriatal dopamine terminals or a lower dopaminergic activity in the striatum may be associated with reduced NAA/Cho ratios. According to this assumption, increased ratios of NAA/Cho and NAA/Cr that were found in the present study in individuals carrying the homozygous *DAT1* VNTR 10/10 repeat genotype might be associated with a higher dopaminergic activity in the left putamen in these individuals. The exact physiological mechanism of this possible association between dopaminergic activity and NAA level remains unresolved and deserves further investigations.

Another unresolved question relates to the fact that the investigated VNTR polymorphism of *DAT1* resides in the 3′ untranslated region of the gene. Therefore, it has no direct influence on the amino acid sequence of the DAT1 protein, and it remains unclear how this polymorphism may exert an effect on the function of the dopamine transporter. Nonetheless, VNTR polymorphisms in untranslated regions have indeed been shown to be able to regulate the translation, degradation and localization of mRNA (Mignone et al. 2002). This may be one way in which the investigated VNTR modulate dopamine transporter availability and dopaminergic activity in the putamen.

It is also important to note that the effects of *DAT1* VNTR genotype on metabolic ratios were seen in all individuals irrespective of diagnostic status. This suggests that the observed genetic effect may relate to a basic mechanism of dopaminergic regulation in the putamen. Compatible with this finding, in a previous study we were unable to detect any changes of metabolic ratios in the putamen in patients with bipolar disorder compared to healthy control subjects (Scherk et al. 2006).

Nevertheless, the postulated association between *DAT1* genotype and NAA level in the putamen may also contribute to the patho-physiological mechanisms involved in bipolar and obsessive-compulsive disorder, as the striatum has been hypothesized to be an integral component of neuronal networks that are affected in these diseases (Saxena et al. 2001; Strakowski et al. 2005).

The results of this study are limited by some methodological aspects. We did not obtain a differentiation between proportions of grey or white

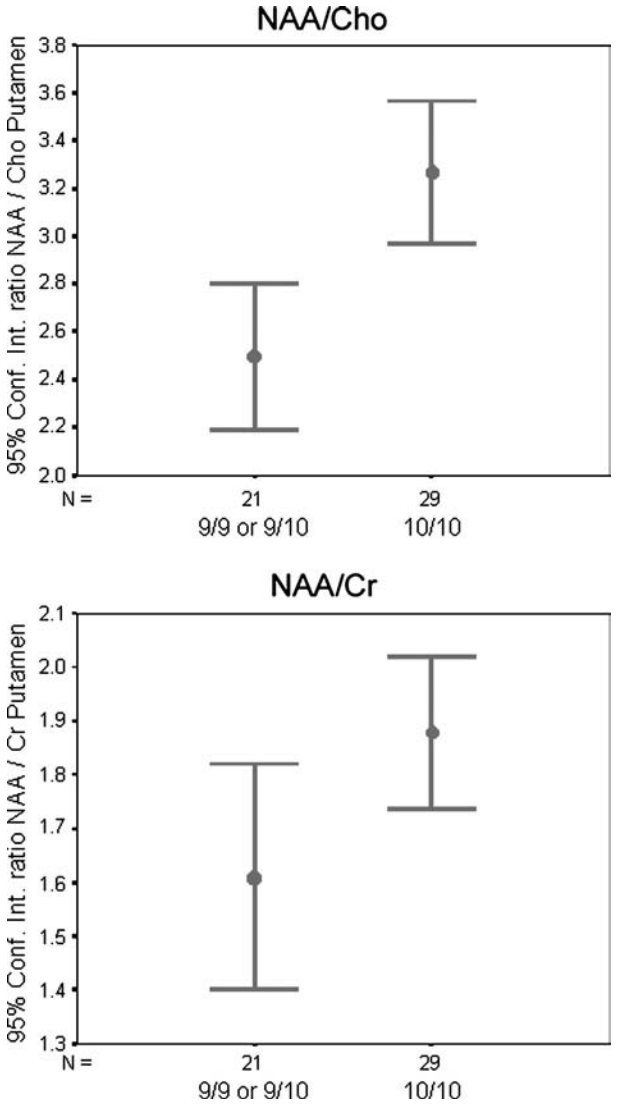


Figure 2. Error bars plot of NAA/Cho and NAA/Cr ratios in left putamen. Individuals with DAT1 VNTR 10–10 genotype exhibited significantly increased ratios of NAA/Cho ($P=0.001$) and NAA/Cr ($P<0.05$) compared to individuals with the 9/10 or 9/9 genotype.

matter in the investigated volumes of interest. Therefore, we can not definitely exclude that differences of grey and white matter proportions between different subjects might have influenced the metabolite concentrations, although the positioning of the volumes of interest were visually inspected and adjusted based on identifiable anatomical landmarks in reference to standard brain atlases (Talairach and Tournoux 1998; Duvernoy 1999).

Moreover, we only estimated relative metabolite concentrations and computed metabolite ratios instead of absolute metabolite concentrations. Although this is a standard method to estimate changes of metabolites, nonetheless this method has some restrictions as some studies have shown that the creatine level is not as stable as previously thought. Therefore, using only the NAA/Cr ratio might lead to false results as creatine could provide an inconsistent denominator. To eliminate this problem we calculated both the NAA/Cr and NAA/Cho ratios as well as the Cho/Cr ratio. The finding of increased values of both NAA/Cr and NAA/Cho ratios, on the one hand, and of an unchanged Cho/Cr ratio on the other hand can be considered as clear evidence for a reduction of NAA even though we were unable to assess the absolute concentration of NAA itself.

We pooled patients with bipolar I or obsessive compulsive disorder with healthy subjects. Therefore, possible effects of diagnosis on metabolites may mimic the reported genotype effect due to asymmetric diagnosis-by-genotype distribution. This is unlikely, because we observed an asymmetry only with respect to the 10/10 genotype in patients with bipolar disorder and the multivariate analysis did not reveal any effect of diagnosis of bipolar disorder in putamen on metabolites.

Through statistical analysis we were able to show that the treatment with antipsychotics did not influence the metabolite ratios in patients with bipolar disorder. Nevertheless a possible influence of drugs on NAA ratios cannot be excluded.

Initially several statistical tests were performed. However, the main MANOVA analyses with factors DAT1 and diagnosis were calculated only for the three regions, and only when multivariate analyses resulted in significant findings, post-hoc univariate tests were added. That way, the probability of getting false-positive findings because of multiple testing was reduced.

One of the strengths of this study is the relatively large sample of both healthy subjects and patients with bipolar disorder or obsessive compulsive disorder.

Taken together, the present findings are consistent with the view that the *DAT1* VNTR polymorphism may modulate dopaminergic activity in the putamen, and that altered NAA levels may be an indicator for this genetic modulation of neuronal function in the putamen. It seems to be worthwhile to investigate the relationship between dopaminergic activity and NAA levels in further studies.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# Dose-related immunohistochemical and ultrastructural changes after oral methylphenidate administration in cerebrum and cerebellum of the rat

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**Abstract**

Methylphenidate is a piperidine derivative and is the drug most often used to treat attention deficit/hyperactivity disorder of children and young adults. Our aim is to investigate dose-dependent dopamine-2 receptor and glial fibrillary acidic protein expression and ultrastructural changes of the rat brain, to demonstrate possible toxicity of the long-term and high dose use of the methylphenidate. In this study, 27 female prepubertal Wistar albino rats, divided into three different dose groups (5, 10 and 20 mg/kg) were treated orally with methylphenidate dissolved in saline solution for 5 days per week during 3 months. At the end of the third month, tissues were removed and sections were collected for immunohistochemical and ultrastructural studies. We believe that methylphenidate causes dose-related activation of the dopaminergic system in several brain regions especially in ventral tegmental area and also causing neuronal degeneration and capillary wall structural changes such as basal membrane thickness and augmentation of the pinostatic vesicle in the endothelial cells. Also, increased dose of Ritalin is inducing astrocytes hypertrophy especially astrogliosis in pia-glial membrane and this is the result of the degenerative changes in prefrontal cortex region due to high dose methylphenidate administration. The dose-related accumulation of the astrocytes in capillary wall might well be a consequence of the need for nutrition of the neuronal tissue, due to transport mechanism deficiency related to neuronal and vascular degeneration. Thus, we believe that the therapeutic dose of methylphenidate must be kept in minimum level to prevent ultrastructural changes.

**Key words:** *ADHD, psycopharmacology, brain development, dopamine D2 receptor occupancy*

## Introduction

Methylphenidate, mostly known as Ritalin, is a piperidine derivative and is the drug most often used to treat attention deficit/hyperactivity disorder (ADHD), one of the most common behavioural disorders of children and young adults (Amini et al. 2004). Methylphenidate hydrochloride (MPH) is a psychostimulant with a pharmacological profile similar to amphetamine and cocaine, and all these drugs are known as indirect dopamine agonists while the dopaminergic system of the brain plays a central role in reward and motivation (Amini et al. 2004; Yang et al. 2006a).

The great concern about the treatment of MPH is that since in children the central nervous system (CNS) continues its maturation and growth well into the second decade of life, the risk therefore exists for adverse interactions between the developing CNS and long-term psychostimulant treatment (Benes 1998; Castner and Goldman-Rakic 1999; Giedd

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et al. 1999). Brandon et al. (2003) reported that the adolescent exposure to MPH induces neuronal changes associated with increased addiction liability in rats. Schteinschnaider et al. (2000) reported a case of 8-year-old boy of cerebral arteritis following methylphenidate use and others suggested that the mechanism that provokes cardiovascular strokes could be a consequence of vasculitis plus initial hypertension, considering the chemical and pharmacological similarity between amphetamines and methylphenidate like reported by others (Delaney and Estes 1980; Rothrock et al. 1988; Trugman 1988).

Neuroplasticity in the developing brain is regulated by c-*fos* and *fosB* expression through modulation of down-stream genes, such as *substance P, dynorphin*, and *cdk5* (Chase et al. 2005). Several researchers suggested that the ADHD is associated with dysfunction of dopaminergic circuits, including prefrontal, subcortical regions (e.g., striatum), limbic regions (e.g., hippocampus) and cerebellar vermis (Castellanos 1997; Mostofsky et al. 1998; Dinn et al. 2001; Anderson et al. 2002; Krain and Castellanos 2006). Dopamine action alters gene regulation in striatal neurons (Yano and Steiner 2005). Psychostimulants such as amphetamine and methylphenidate produce changes in gene regulation in striatal projection neurons (Harlan and Garcia 1998; Torres and Horowitz 1999). MPH binds to the dopamine transporter (DAT) and increases striatal extracellular dopamine (Volkow et al. 2001). Yano and Steiner (2005) demonstrated that acute methylphenidate alters the expression of c-*fos* and substance P preferentially in the sensorimotor striatum. Chase et al. (2005) reported that the MPH exerts its effects at several sites throughout the immature brain which may have implications for the long-term treatment of ADHD and even low doses of MPH increases the expression of two immediate early genes in multiple areas of the brain. Recently, Andreazza et al. (2007) reported that MPH may induce transient DNA damage in the hippocampus, striatum and peripheral blood without any increase in micronuclei frequency, suggesting that such damage might have been repaired by endogenous repairing systems.

The expanded use of methylphenidate stimulates the need to expend researches on the potential toxicity and neuroultrastructural consequences of this drug, particularly during early stages of brain development. The purpose of the present study is to determine dose-dependent changes in D2 receptors, Glial fibrillary acidic protein (GFAP)-positive cells and possible ultrastructural degeneration in the prefrontal cortex, ventral tegmental area and cerebellum of young rat brain after oral methylphenidate administration.

## Materials and methods

### *Animals and treatment*

The experimental protocol was approved by the local Ethical Committee for animal studies and conducted at Gazi University Faculty of Medicine. In the experimental protocol, 27 female Wistar albino rats with a weight of 110 g ($\pm$20), divided into three different dose groups (5, 10 and 20 mg/kg) with their control groups, and the doses of the methylphenidate (MPH) were chosen according to the studies which indicate that, in rats, the metabolism rate of this drug was faster than humans (Brandon et al. 2001; Schenk and Izenwasser 2002; LeBlanc-Duchin and Taukulis 2004). Female rats were selected for this study according to the previous studies reporting that female rats are more anxious than male rats and their nervous systems may be more susceptible to alteration by psychomotor stimulants (Camp and Robinson 1988; Blanchard et al. 1991; Wooters et al. 2006). Prepubertal (25-day-old) rats were treated orally with MPH dissolved in saline solution for 5 days per week during 3 months. We gave MPH orally since this is the route of administration used therapeutically for ADHD. The animals were synchronized to a light–dark cycle (lights on from 08:00 to 20:00 h) beginning at least 2 weeks before the commencement of experiments. These conditions were maintained for 12 weeks during March–May to avoid the possibility of seasonal rhythms affecting the findings.

### *Tissue sampling*

At the end of the third month, all the animals were anaesthetized by ketamine hydrochloride (Ketalar, Parke-Davis, Istanbul, Turkey) 30 mg/kg intramuscularly. For muscle relaxation, 2% xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) 6 mg/kg was used. Then, they were perfused with 1.25% glutaraldehyde and 1% paraformaldehyde solutions.

Following perfusion, the cerebral hemispheres were removed from the skull and were separated from each other by a median section. Cerebrum and cerebellar vermis from each group were fixed in neutral formalin for 72 h and processed for paraffin embedding. Sections of 4–5 μm thickness were processed for polylysin microscope slides.

### *Immunohistochemistry*

For immunohistochemical examination of glial fibrillary acidic protein (GFAP), slides were stored in a microwave oven in 0.1 M citrate buffer, (LabVision, Cat # AP-9003-500, USA). Endogenous peroxidase activity was blocked in 3% hydrogen peroxide

(Lot#AHP40114, Cot#TA-125-HP, LabVision, Fremont, CA, USA). Epitopes were stabilized by application of serum blocking solution (Lot#AUB40204, Cot#TA-125-UB, LabVision, USA). Sections were incubated with and GFAP (Glial Fibrillary Acidic Protein) Ab-4, rabbit polyclonal antibody (Cat # RB-087-A, Lab Vision, USA), diluated in UltraAb Diluent (Lot#AUD40706, Cot#TA-125-UD, LabVision, USA) 60 min at room temperature. After that, the biotinated secondary antibody (Goat antirabbit, Lot#RBN40218, Cot#TR-125-BN, LabVision, USA), was applied. Then, streptavidin peroxidase (Lot#SHR40211, Cot#TS-125-HR, Lab Vision, USA) was applied to the slides. AEC (Lot#AH41013, Cot#TA-125-HA, Lab Vision, USA) was used as chromogen. Afterwards, the slides were counterstained with haematoxylin.

For immunohistochemical examination of Dopamine 2 Receptor (D2DR), Zymed Histostain-Plus Broad Spectrum kit used (Lot#50681666, Cot#85-9043, San Francisco, CA, USA). Endogenous peroxidase activity was blocked in 3% hydrogen peroxide (Lot#AHP40114, Cot#TA-125-HP, LabVision, Fremont, CA, USA). Epitopes were stabilized by application of serum blocking solution (Lot#50681666, Cot#85-9043). Sections were incubated with Dopamine 2 Receptor (D2DR, mouse polyclonal antibody Lot#F2204, Cot#sc-5303, Santa Cruz, CA, USA) diluated in PBS (phosphate buffered saline, pH 7.4, Lot#50381417, Cot#00-3000, Zymed, San Francisco, CA, USA) overnight at $+4^{\circ}$C. After that, the biotinated secondary antibody (Lot#50681666, Cot#85-9043, biotinated secondary antibody) was applied. Then, Streptavidin peroxidase (Lot#506 81666, Cot#85-9043) was applied to the slides. 3-Amino-9-ethylcarbazole (Lot#AH41013, Cot#TA-125-HA, Lab Vision, USA) was used as chromogen.

Afterwards, all slides were counterstained with Mayer's haematoxylin. Slides were examined with Photo-light microscope (DM4000B Image Analyze System and, Leica, Germany) and Leica DFC280 plus camera. The number of immune positive cells is measured manually by using QWin software programme in consecutive areas for serial cutaways taken from each subject (Aktas et al. 2009)

### *Statical analysis*

The normality of the distributions of the variables was controlled by Shapiro–Wilk test. Shapiro–Wilk normality test results are showed that the distributions of the variables were not normal while group variances were not homogeneous according to Levene's test. So, the differences between the independent groups were analyzed by Kruskal–Wallis test and then multiple comparisons between pairs of groups were carried out according to Dunn test. Dependent groups were compared by Friedman test followed by Dunn's multiple comparison. Results have been expressed as number of observations ($n$), mean $\pm$ standard deviation, median and min–max values. $P$ value less than 0.05 was considered significant.

All statistical analyses were performed with the SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL, USA).

### *Electron microscopic study*

All tissues were sacrified and immersion fixation method in 0.1 M phosphate buffer containing 2.5% glutaraldehyde for 2–3 h; then they were post fixed in 1% osmium tetra oxide ($OsO_4$) and dehydrated in a series of graded alcohols (50, 60, 70, 80, 90, 96 and 100% ethanol). After passing through propylene oxide, the specimens were embedded in Araldite CY 212, 2-dodecenyl succinic anhydride, benzyldimethyl amine and dibutylphitalate. Semi-thin sections were cut and stained with toluidine blue and examined with a $BH_2$ Olympus light microscope. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a Carl Zeiss EM 900 transmission electron microscope (TEM).

## Results

### *GFAP*

In the cerebrum, glial fibrillary acidic protein (GFAP) positive (+) cells were statistically meaningfully ($P<0.001$) more numerous than the cerebellum in all groups (Table I).

In the control group, the histological evaluation of the prefrontal cortex displayed GFAP (+) astrocytes especially on molecular layer of the cortex. A thin layer

Table I. Comparison of the GFAP (+) cell number of the cerebrum and the cerebellum for all dose groups.

| | Mean $\pm$ standard deviation<br>Median<br>$n=6$ | |
|---|---|---|
| Groups | Cerebellum | Cerebrum |
| Control | 48.83 $\pm$ 0.75<br>49.00 (48.00–50.00) | 84.50 $\pm$ 1.05$^{***}$<br>84.50 (83.00–86.00) |
| Low dose | 56.33 $\pm$ 5.79<br>54 (50.00–64.00) | 96.50 $\pm$ 3.62$^{***}$<br>96.00 (92.00–102.00) |
| Curative dose | 81.33 $\pm$ 4.13<br>80.50 (76.00–87.00) | 127.00 $\pm$ 1.90$^{***}$<br>127.00 (124.00–129.00) |
| High dose | 119.67 $\pm$ 4.93<br>119.50 (113.00–126.00) | 156.50 $\pm$ 3.62$^{***}$<br>157.00 (151.00–161.00) |

$^{***}P<0.001$.

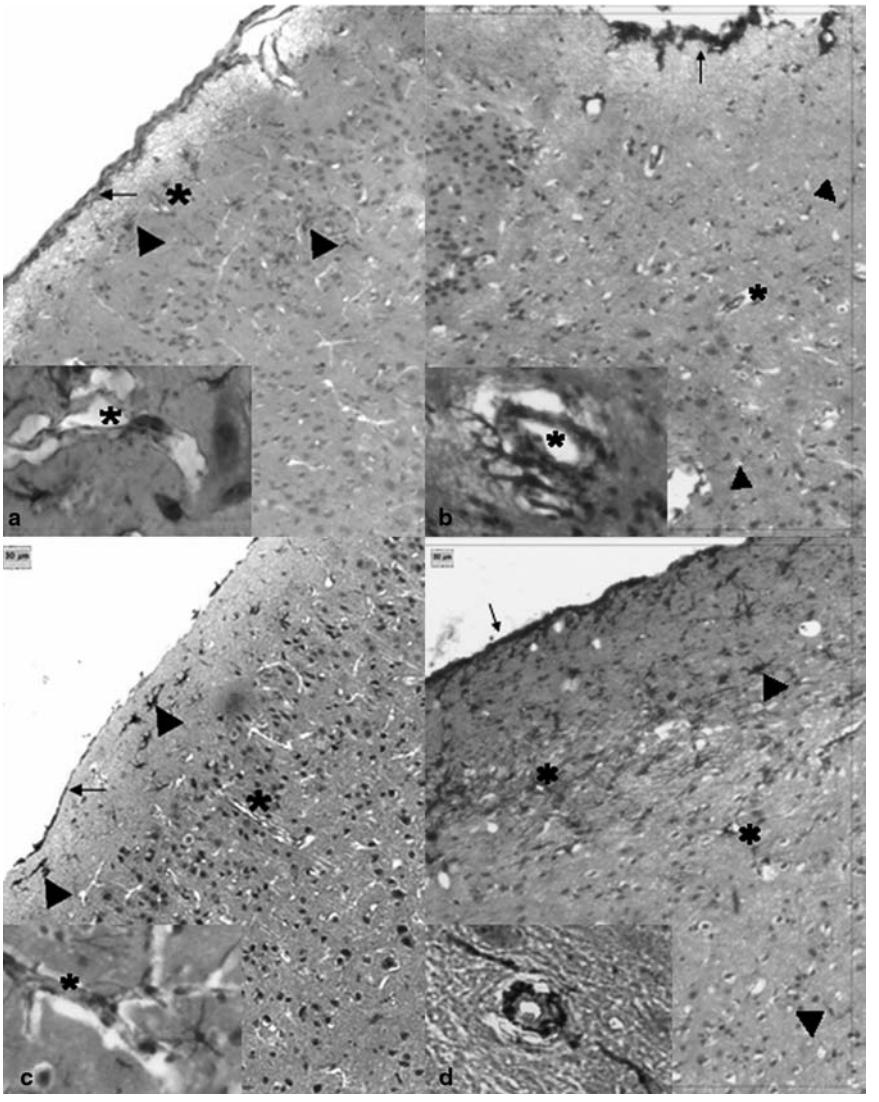


Figure 1. GFAP immunoreactivity in control (a), low dose (b), curative dose (c) and high dose (d) Ritalin treated prefrontal cortex. Immunoreactivity in pial surface (➔), astrocytes (◗) of superficial and deepest layer of cortex, capillary (★) were seen (immunoperoxidase–haematoxylin ×100).

of GFAP (+) astrocytes processes were seen under the pial membrane. A group forming GFAP (+) astrocytes network was also detected in the deepest layer of cortex. The surrounding capillaries exhibited no prominent GFAP immunoreactivity (Figure 1a).

In the low dose treated group (5 mg/kg/day), especially molecular layer of the cortex showed an increased number of GFAP (+) astrocytes with intense reaction compared to the control group. GFAP (+) cells were observed remarkably in pia-glial membrane as an unfragmanted line (Figure 1b).

In the curative dose treated group (10 mg/kg per day), GFAP (+) cells showed a very strong reactivity and were localized in piaglial membrane. The piaglial membrane was observed as a thick GFAP (+) line. The molecular layer was also detected as a GFAP (+) area and astrocytes immunoreactivity was similar to the low dose treated group but also in this group, GFAP (+) astrocytes with intense immunoreactivity were observed in the surrounding tissue of the capillary walls (Figure 1c).

In the high dose treated group (20 mg/kg per day), GFAP (+) cells and their immunoreactivity displayed a marked increase. The pia-glial membrane GFAP (+) astrocytes of strong reactivity was similar to curative dose treated group but positive cell number was also increased in molecular layer and deepest cortex layer of this group. GFAP (+) astrocytes of the molecular layer were in close relation with the pia-glial membrane. Besides, in the deepest layer of the cortex, GFAP (+) astrocytes displayed a close relation pattern and were forming a network. They were also forming a line around the capillary walls like curative dose treated group (Figure 1d). A dose-related

Table II. Comparison of the GFAP (+) cell number of the dose groups in cerebrum and cerebellum.

| | Mean ± standard deviation<br>Median | | | |
|---|---|---|---|---|
| | Control | Low | Curative | High |
| Cerebellum $n=6$ | $48.83 \pm 0.75^{***}$<br>49.00 (48.00–50.00) | $56.33 \pm 5.79^{***}$<br>54 (50.00–64.00) | $81.33 \pm 4.13^{***}$<br>80.50 (76.00–87.00) | $119.67 \pm 4.93^{***}$<br>119.50 (113.00–126.00) |
| Cerebrum $n=6$ | $84.50 \pm 1.05^{***}$<br>84.50 (83.00–86.00) | $96.50 \pm 3.62^{***}$<br>96.00 (92.00–102.00) | $127.00 \pm 1.90^{***}$<br>127.00 (124.00–129.00) | $156.50 \pm 3.62^{***}$<br>157.00 (151.00–161.00) |

$^{***}P<0.001$.

statistically meaningful increase of the GFAP (+) cells was observed ($P<0.001$) (Table II).

The histological evaluation of the cerebellar vermis showed that; in the control group, GFAP (+) astrocytes were localized prominently in granule cell layer and in medulla with their cytoplasmic prolongations surrounding capillaries (Figure 2a). In the low dose treated group, especially granular layer astrocytes number was increased with intense immunoreactivity (Figure 2b). In the curative and high dose treated groups, additionally to medulla and granular layer of the cortex, GFAP (+) astrocytes cytoplasmic prolongations were present evidently in molecular layer. Also, the immunoreactive cell number and their labelling level was increased (Figure 2c–d). These findings were found to be statistically meaningful ($P<0.001$) (Table II).

*D2*

In the cerebrum, dopamine-2 (D2) immunoreactivity was particularly observed in prefrontal cortex (PfC) and ventral tegmental area (VTA) where dopaminergic neurons were abundantly localized. In the control group; D2 immunoreactive neurons were dispersed in the PfC region forming regular groups. All dopaminergic neurons show moderate to intense cytoplasmic immunoreactivity. No immunostaining was observed in capillary wall and in glial cells of this region (Figures 3a and 4a). In the low dose treated group; D2 immunoreactivity was similar to control group (Figures 3b and 4b). In the curative dose treated group; D2 immunoreactive neuron were more widespread localized with intense immunoreactivity compared to control group and the capillary dilatation was also present in this group

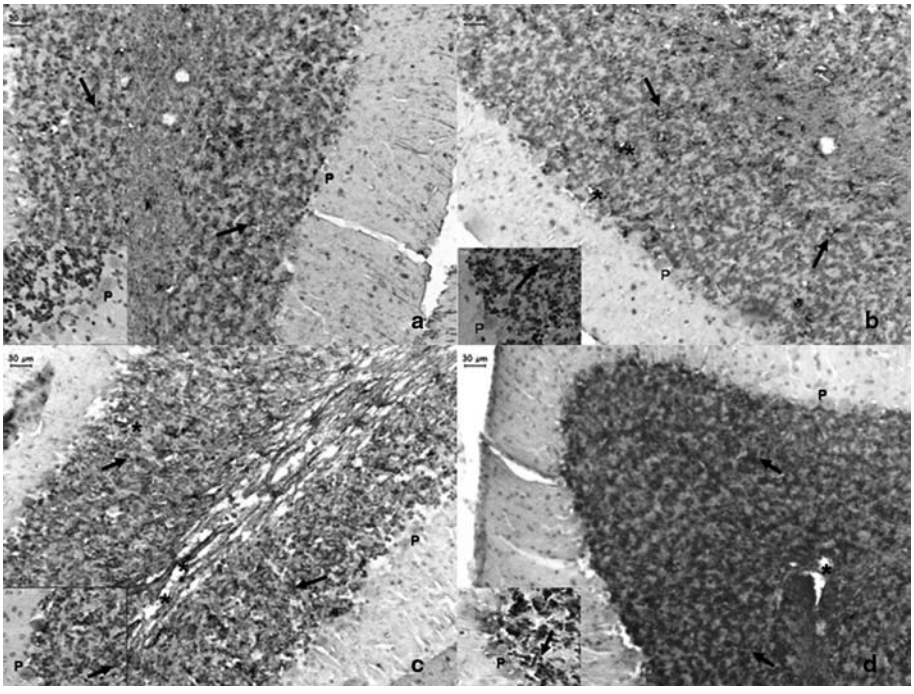


Figure 2. GFAP immunoreactivity in control (a), low dose (b), curative dose (c) and high dose (d) Ritalin treated cerebellar vermis; Purkinje cells (P), immunoreactive astrocytes/astrocytes prolongations in granular layer (➔), immunoreactive astrocytes prolongations in perivascular regions (✱) were seen (immunoperoxidase–haematoxylin ×100).

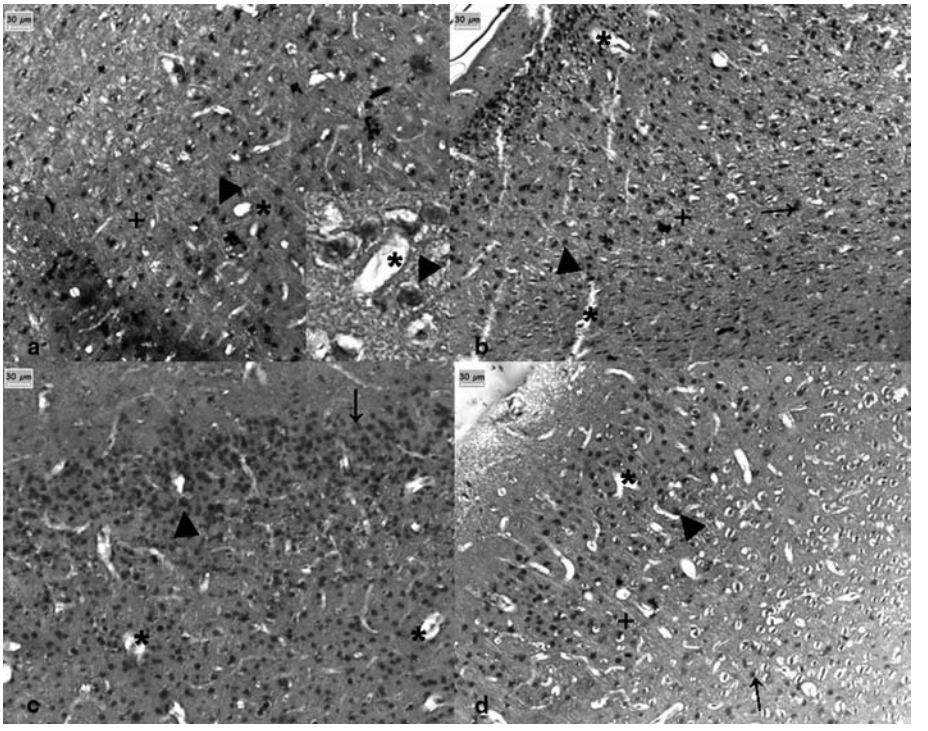


Figure 3. Dopamine D2 receptor immunoreactive regions in control (a), low dose (b), curative dose (c) and high dose (d) Ritalin treated prefrontal cortex; D2DR positive neurons (▶), glial cells (+), and capillaries (★) and immunoreactive neurons (➔) were seen (immunoperoxidase–haematoxylin ×100).

(Figures 3c and 4c). In the high dose treated group; most evident findings were the capillary dilatation and regional D2 receptor activity of the axons. The neurons of this group showed intense D2 immunoreactivity compared to other groups (Figure 3d and 4d). These findings were found to be statistically meaningful ($P<0.001$) (Table III).

The evaluation of the control group showed that the most widespread and intense D2 receptor immunoreactivity was displayed in the ventral tegmental area (VTA). The dopaminergic neurons were organized as groups showing mostly cytoplasmic intense reactivity. The non-dopaminergic neurons were distinguished with their blue–purple colours. No sign of labelling was observed in capillary wall and in glial cells (Figure 5a). In the low dose treated group; D2 receptor immunoreactivity of the VTA was similar to control group like PfC region (Figure 5b). In the curative dose treated group; D2 receptor immunoreactive neuron number of the VTA was increased with intense staining. These findings were similar to PfC results of the same dose. But interestingly, axons of the VTA showed also immunoreactivity along with the neurons (Figure 5c). In the high dose treated group; most evident finding was the intense labelling of the axons additional to intense immunoreactive neurons (Figure 5d).

In the control group of the cerebellum, all layers showed normal structure and dopamine receptor immunoreactivity was minimally observed in cellular level. The dopamine receptor immunoreactivity was present in widespread of the tissue in neuronal fibres. The most intense labelling was distinguished in medullary layer (Figure 6a). In high magnification, Purkinje cells displayed a weak D2 receptor immunoreactivity (Figure 6a inset). In the low dose treated group, most evident changes were the capillary dilatation in the molecular layer. Also, the disruption of the Purkinje cell layer was observed in some areas. As in the control group, dopamine-2 receptor immunostaining was present in neuronal fibres of the medulla but also reactivity around the purkinje cells was increased compared to control group (Figure 6b). In high magnification, purkinje cells showed moderate membranous D2 immunoreactivity (Figure 6b inset). In the curative dose treated group; especially capillary dilatation of the molecular layer was very clear with disruption of the Purkinje cell layer similar to the low dose treated group. The dopamine-2 receptor reactivity evaluation displayed intense neuronal fibre immunoreactivity in some area of the molecular layer apart to other groups. The cerebellar medullary reactivity was also increased

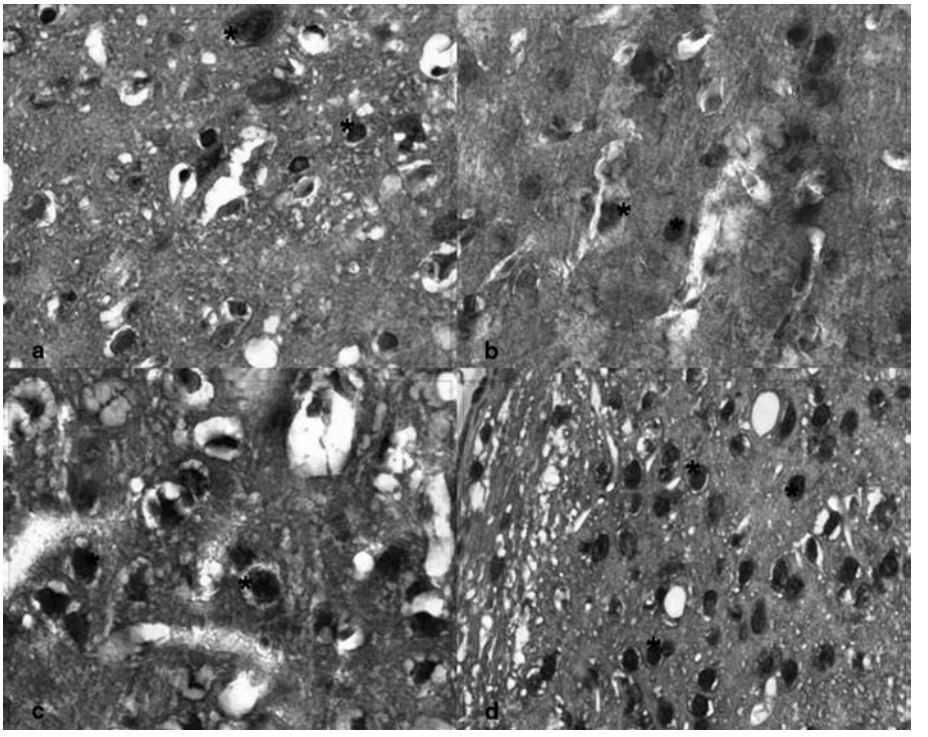


Figure 4. Dopamine D2 receptor immunoreactive regions in control (a), low dose (b), curative dose (c) and high dose (d) Ritalin treated prefrontal cortex; D2DR positive neurons(★) were seen in high magnification (immunoperoxidase–haematoxylin ×400).

(Figure 6c). In high magnification, Purkinje cells displayed weak to moderate reactivity (Figure 6c inset). In the high dose treated group; capillary dilatation was very obvious. The D2 reactivity of the molecular layer neuronal fibre and the medulla was similar to previous group (Figure 6d). In high magnification, Purkinje cell staining was increased and showed intense labelling compared to other groups. The proximal part of the neuronal fibres exhibited also intense reactivity in this group (Figure 6d inset). Our immunohistochemical results were in accordance with HSCORE findings and they were statistically meaningful ($P<0.001$) (Tables IV and V).

### *Ultrastructure*

In the control group, structural evaluation of the prefrontal cortex displayed capillary walls, neurons and myelinated nerves in their normal structure (data not shown). In the low dose treated group; most evident changes observed in PfC, was the pericellular and perivascular oedema. The neuron nuclei showed eucromatic structure with minimal

Table III. HSCORE of the cerebrum pyramidal neuron dopamine receptor immunoreactivity.

| | Mean ± standard deviation<br>Median<br>$n=6$ | | | | |
|---|---|---|---|---|---|
| | Control group | Low dose group | Curative dose group | High dose group | *P* |
| Weak | $51.4286 \pm 25.44836^{a}$<br>60.0000 | $53.3333 \pm 24.22120^{a\#}$<br>50.0000 | $102.8571 \pm 40.70802^{b\#}$<br>100.0000 | $174.2857 \pm 37.07135^{c\#}$<br>180.0000 | <0.001 |
| Medium | $67.1429 \pm 35.45621^{a}$<br>60.0000 | $85.0000 \pm 22.58318^{a¥}$<br>90.0000 | $201.4286 \pm 37.60699^{b¥}$<br>210.0000 | $270.0000 \pm 42.42641^{c¥}$<br>270.0000 | <0.001 |
| Strong | $177.1429 \pm 39.03600^{a€}$<br>160.0000 | $220.0000 \pm 41.95235^{a€}$<br>220.0000 | $280.000 \pm 76.59417^{b€}$<br>280.0000 | $425.7143 \pm 59.68170^{c€}$<br>420.0000 | <0.001 |
| *P* | <0.01 | <0.01 | <0.01 | <0.001 | |

Different symbols display differences between groups and intensity.

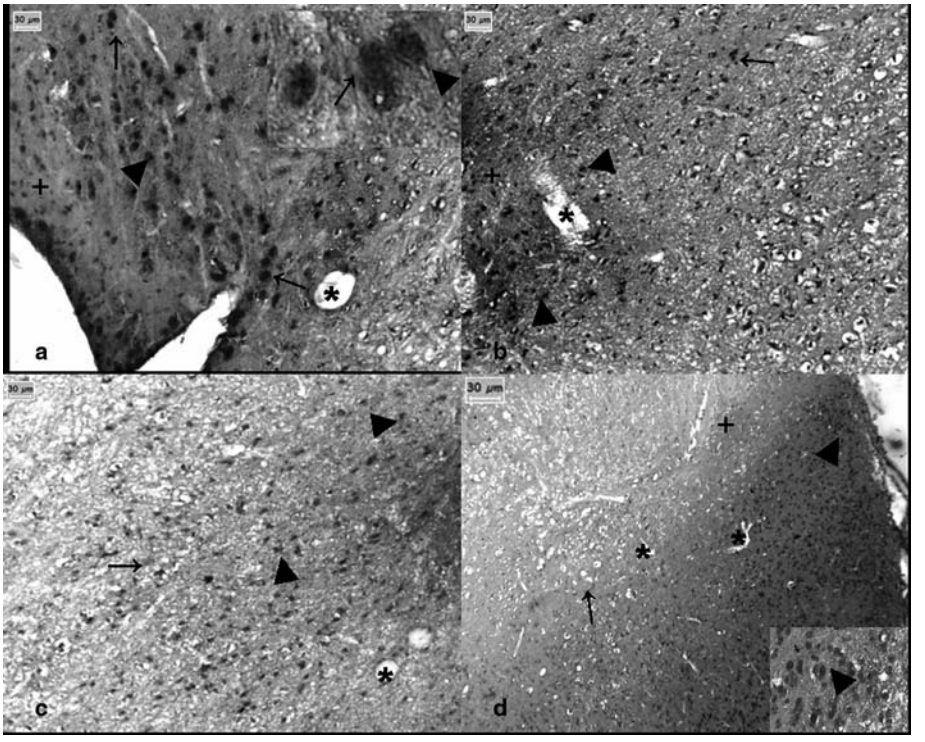


Figure 5. Dopamine D2 receptor immunoreactive regions in control(a), low dose (b), curative dose (c) and high dose (d) applied ventral tegmental area; D2DR positive neurons (◗), glial cells (+), and capillaries (✱) and immunoreactive neurons (➔) were seen (immunoperoxidase–haematoxylin ×100).

invagination while swelling of the mitochondrions and especially in these ones, minimal crystolysis was seen. The rough endoplasmic reticulum cisternae (rER) were active. A few number of pinostatic

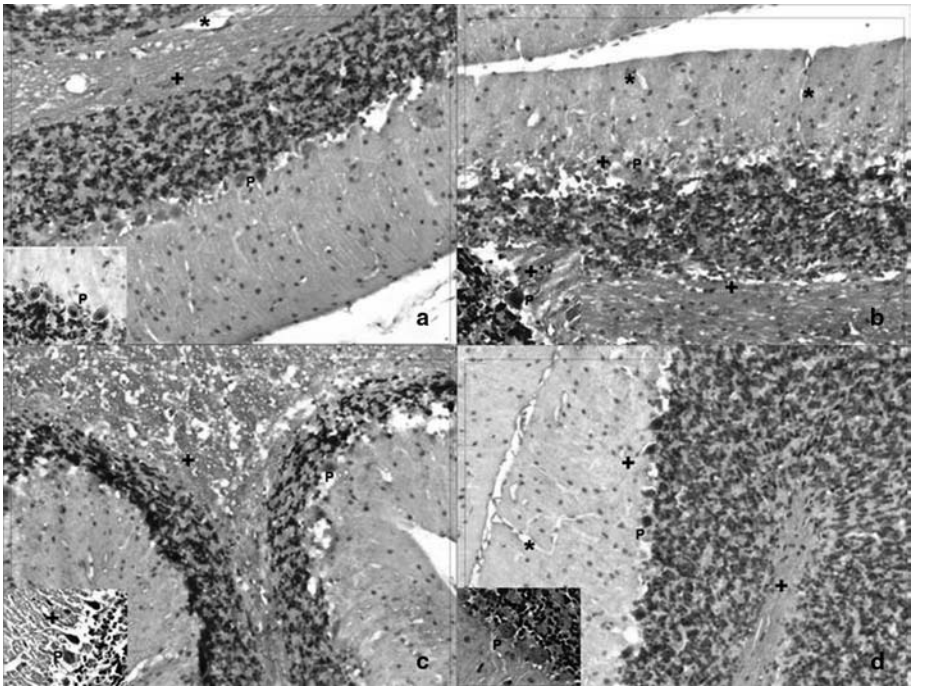


Figure 6. Dopamine D2 receptor immunoreactive regions in control (a), low dose (b), curative dose (c) and high dose (d) Ritalin treated cerebellar vermis; Purkinje cells (P), immunoreactive neuronal process (✚), capillaries (✱) were seen (immunoperoxidase–haematoxylin ×100).

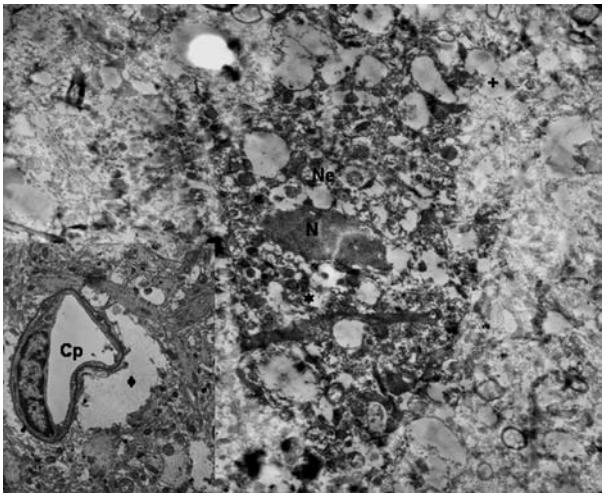


Figure 7. Ultrastructural evaluations in high dose Ritalin treated prefrontal cortex; shrinking neurons (Ne), degenerated cytoplasmic organelles (✸), heterochromatic and shrinking nucleus (N), capillaries (Cp), pericellular (+) and perivascular (◆) oedema were seen (uranyl acetate–lead citrate ×4400).

vesicle inside the endothelium cells with normal basal membrane of the capillary wall and normal cytoplasmic organelle composition were observed (data not shown).

In the curative dose treated group; the pericellular and perivascular oedema were increased. The neurons displayed normal nuclei structure and rER cisternae were active. While, swelling of the mitochondrions with loss of cristae were observed in some cells. In this group, the most prominent change was, additionally to pericellular oedema, cytoplasmic diminution related to cellular shrinking. The evaluation of the capillary wall revealed that the condensation of the endothelium cell nuclei was increased in some area with large mitochondrions and pinostatic vesicle ratio was similar to the low dose treated group but basal membrane was thicker (data not shown).

In the high dose treated group; pericellular oedema was similar to curative dose but perivascular oedema was increased dramatically. The evaluation of the neurons revealed that due to their ultrastructural degeneration, their organelles were not identified clearly and their nucleus was smaller (Figure 7). But, capillary endothelial cell nuclear chromatin was increased especially under nuclear membrane with minimal intracytoplasmic oedema and interestingly increased perivascular oedema. Also, the basal membrane was clearly thicker (Figure 7 inset).

In the control group, structural evaluation of the cerebellum displayed Purkinje cells, neuronal fibres and capillary in their normal structure (data not shown). In the low dose treated group; nucleus and nucleolus of the Purkinje cells displayed normal structure with crystolysis and swelling of the some mitochondrions. The rough endoplasmic reticulum cisternae were active. The pericellular oedema was not observed. The evaluation of the neuronal fibres exhibited existence of the normal structure in small-sized fibres but myelin sheaths disruption and axonal dystrophy of the large-sized fibres (Figure 8a). In the curative dose treated group; shrinking of the Purkinje cells with pericellular oedema was first to notice. In cell nucleus, chromatin accumulation under the membrane was observed. The cytoplasm

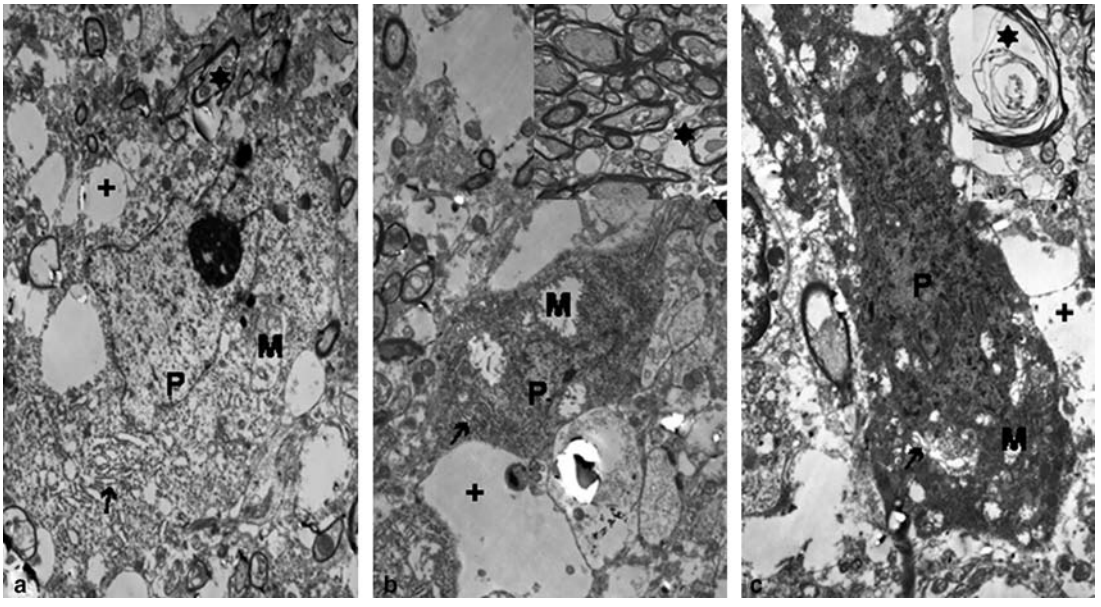


Figure 8. Ultrastructural evaluations in low dose (a), curative dose (b) and high dose (c) Ritalin treated cerebellar vermis. P, dose-related increase of cytoplasmic density and condensation of the chromatin in Purkinje cells; M, dose-related increase of the crystolysis and swelling of the mitochondrions (➔) (uranyl acetate–lead citrate ×4400).

Table IV. HSCORE of the cerebellum neuron dopamine receptor immunoreactivity.

| | Mean ± standard deviation<br>Median<br>$n = 6$ | | | | |
|---|---|---|---|---|---|
| | Control | Low | Curative | High | *P* |
| Weak | $79.7143 \pm 5.58911^{a\#}$<br>80.0000 | $30.0000 \pm 9.86577^{b\#}$<br>34.0000 | $11.7143 \pm 6.67618^{c\#}$<br>12.0000 | $13.4286 \pm 5.74042^{c\#}$<br>14.0000 | <0.001 |
| Medium | $7.28657 \pm 2.36039^{a¥}$<br>9.0000 | $25.2857 \pm 10.65699^{b¥}$<br>27.0000 | $36.4286 \pm 7.82852^{c¥}$<br>39.0000 | $32.5714 \pm 8.90425^{c¥}$<br>33.0000 | <0.001 |
| Strong | $11.7143 \pm 3.35233^{a€}$<br>12.0000 | $46.2857 \pm 8.28079^{b€}$<br>46.0000 | $100.8571 \pm 12.26687^{c€}$<br>102.0000 | $304.0000 \pm 15.51344^{d€}$<br>311.0000 | <0.001 |
| *P* | <0.001 | <0.001 | <0.001 | <0.001 | |

Different symbols display differences between groups and intensity.

was electron dense with the appearance of the gigantic mitochondrions. The crystolysis of the mitochondrions was also seen. Compared to the low dose treated group, rER were more active with a high number of ribosome dispersed in the cytoplasm. The evaluation of the neuronal fibres revealed the evident myelin sheaths disruption in all size fibres with axonal dystrophy (Figure 8b). In the high dose treated group; shrinking of the Purkinje cells with pericellular oedema was similar to the curative dose group. The invagination of the cell nucleus and condensation of the chromatin was also noticed. The cytoplasm was highly electron dense and crystolysis of the mitochondrions was very prominent. Other cytoplasmic organelles were not observed related to the cytoplasmic density. In this group, neuronal fibre degeneration was increased. The small-sized fibres showed similar characteristic features like the curative dose while large-sized fibres showed duplication of the myelin sheath (Figure 8c).

## Discussion

Practice-dependent plasticity underlies motor learning in everyday life and motor relearning after lesions of the nervous system. Previous studies showed that practice-dependent plasticity is modifiable by neuromodulating transmitters such as norepinephrine (NE), dopamine (DA) or acetylcholine (ACh) (Meintzschel and Ziemann 2006). Dopamine is an important neurotransmitter of the central nervous system controlling the various organ activities depending on the activation of different receptors (Cavallotti et al. 2002).

Consistent with the dopaminergic hypothesis of ADHD, methylphenidate blocks dopamine transporter (DAT), thereby elevating extracellular dopamine levels in various limbic, striatal, cortical and cerebellar terminal fields (Krain and Castellanos 2006; Sellings et al. 2006; Wooters et al. 2006). The agonists of neuromodulating transmitters systems (e.g., methylphenidate) enhanced practice-dependent plasticity, while the antagonists reduced it (Meintzschel and Ziemann 2006). Several researchers studied possible consequences of long-term exposure to methylphenidate and found no increase in dopamine levels in the nucleus accumbens and no sensitized locomotor responses in adolescent rats (Kuczenski and Segal 2002; Volkow and Insel 2003). Feron et al. (2005) reported also that, in single-photon emission computed tomography study of five children; methylphenidate does not lead to permanent damage of the nigrostriatal dopaminergic pathways. While, Yang et al. (2006b) demonstrated that the extracellular DA activates the motor nuclei and suppresses incoming peripheral information

Table V. HSCORE of the cerebellum Purkinje cells dopamine receptor immunoreactivity.

| | Mean ± standard deviation Median $n = 6$ | | | | |
|---|---|---|---|---|---|
| | Control group | Low dose group | Curative dose group | High dose group | *P* |
| Weak | $8.5714 \pm 3.59894^{a}$<br>8.0000 | $10.2557 \pm 3.90360^{a\#}$<br>10.0000 | $16.5714 \pm 4.42734^{b}$<br>16.0000 | $33.7143 \pm 7.25062^{c}$<br>36.0000 | <0.001 |
| Medium | $9.000 \pm 3.46410^{a}$<br>9.0000 | $18.4286 \pm 4.03556^{b¥}$<br>18.0000 | $17.14429 \pm 4.48808^{b}$<br>18.0000 | $27.8571 \pm 5.92814^{c¥}$<br>27.0000 | <0.001 |
| Strong | $8.5714 \pm 5.85540^{a}$<br>8.0000 | $14.5414 \pm 5.74042^{b€}$<br>12.0000 | $28.5714 \pm 5.38074^{c€}$<br>28.0000 | $42.5714 \pm 5.96817^{d}$<br>42.0000 | <0.001 |
| *P* | 1.000 | <0.05 | <0.01 | <0.05 | |

Different symbols display differences between groups and intensity.

arriving to the VTA and NAc and this suppression is intensified following chronic MPH application which may be the indication of the development of electrophysiological sensitization. Easton et al. (2007) indicated that MPH has a weaker effect at inhibiting vesicular accumulation of DA and NA compared to its potent synaptosomal effects. Mague et al. (2005) reported evidence that early adolescent exposure to MPH in rats causes complex behavioural adaptations that endure into adulthood. Lagace et al. (2006) studied the neuronal consequences of early-life exposure to MPH and suggested that decreased adult neurogenesis is an enduring consequence of MPH exposure which inhibits the survival of adult-generated neurons in the temporal hippocampus and may reduce progenitor sensitivity to corticosterone-induced decreases in proliferation. Thanos et al. (2007) pointed out that MPH effect of D2R expression in the striatum are sensitive not only to length of treatment but also to the developmental stage at which treatment is given. Consequently, they reported that further studies are needed to evaluate different treatment regimens for assessing optimal duration to minimize adverse effects on the propensity for drug self administration (Thanos et al. 2007).

While, neurobiological theories of ADHD have mainly focused on the prefrontal cortex and basal ganglia, some researchers have also found that children of both sex with ADHD have smaller posterior-inferior lobules of the cerebellar vermis than healthy children (Castellanos et al. 1996; Berquin et al. 1998; Mostofsky et al. 1998; Castellanos et al. 2001; Anderson et al. 2002; Glaser et al. 2006; Krain and Castellanos 2006). The cerebellum is associated with coordination of motor movements but is also known to be involved in non-motor functions such as timing and attentional shifting through connections with frontal regions (Krain and Castellanos 2006). Researches pointed out several compelling reasons for the involvement of cerebellar vermis in both the pathophysiology of ADHD and therapeutic response to stimulant medications (Krain and Castellanos 2006). They reported that cerebellum receives dopamine projections from the ventral tegmental area (granule layer of crus 1 and paraflocculus) and the posterior-inferior lobules of the cerebellar vermis have the highest concentration of DAT (Ikai et al. 1992, 1994; Melchitzky and Lewis 2000). Also, there are reciprocal loops that interconnect a large and diverse set of cerebral cortical areas, including the prefrontal cortex, with the basal ganglia and cerebellum by way of the pons, dentate nucleus, and thalamus (Anderson et al. 2002). The vermis, through its fastigial projections to the ventral tegmental area and locus ceruleus, exerts strong effects on the turnover of dopamine and noradrenaline in the caudate and nucleus accumbens (Anderson et al. 2002). So, the vermis has been shown to modulate forebrain dopamine systems, to influence locomotor activity, and to contain a significant density of dopamine transporters but cerebellum's influence on cortico-striatal-thalamo-cortical circuits, which choose, initiate, and carry out complex motor and cognitive responses still need to be explored (Anderson et al. 2002; Krain and Castellanos 2006).

We believe that Ritalin is causes dose-related activation of the the dopaminergic neurons in brain regions especially in ventral tegmental area. Strong activity of the VTA axons related to increased doses and the close relation of the VTA with dopaminergic path suggest the possibility that Ritalin is activating this dopaminergic pathway.

GFAP is well known to be a good marker for reactive astrocytes in response to the central nervous system injury, due to specificity of it in astrocytes (Eng and DeArmond 1986; Lin et al. 1993). Increased astroglial reaction in the striatum was found by several researchers after MPTP treatment, a well known selective neurotoxicant to deplete striatal dopamine which causes neuronal degeneration of the nigrostriatal pathway (Stromberg et al. 1986; Francis et al. 1995; Araki et al. 2001). Araki et al. (2001) suggested that increases in GFAP staining produced by MPTP in the striatum are linked to decrements in TH staining suggested that factors originating in the damaged dopamine neurons initiated the astrocytes reaction to MPTP.

Our findings shows that the increased dose of Ritalin is inducing astrocytes hypertrophy especially astrogliosis in pia-glial membrane and this is the result of the degenerative changes in prefrontal cortex region due to high dose methylphenidate administration. This astrogliosis related to the degeneration was also prominent in the medullary region of the cerebellum.

Our ultrastructural results show that Ritalin is causing prominent extracellular oedema in prefrontal cortex especially in perivascular area with increased doses. Besides, dose-related basal membrane thickness and augmentation of the pinostatic vesicle in the endothelial cells revealed the result that Ritalin is negatively affecting substance transport. Also, Purkinje cell and neuronal fibres degeneration occurring in the cerebellum is revealing the possibility that Ritalin has negative effect on non-motor functions, in accordance with recent literature findings.

In the light of all our data, we believe that methylphenidate is causes dose-related activation of the dopaminergic system in several brain regions and also causing neuronal degeneration and capillary wall

structural changes in ultrastructural level. The dose-related accumulation of the astrocytes in capillary wall might well be a consequence of the need for nutrition of the neuronal tissue, due to transport mechanism deficiency related to neuronal and vascular degeneration. Thus, we believe that the therapeutic dose of methylphenidate must be kept to the minimum level to prevent ultrastructural changes.

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## Statement of interest

None.

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**ORIGINAL INVESTIGATION**

# Association of monoamine oxidase A (MAOA) polymorphisms and clinical subgroups of major depressive disorders in the Han Chinese population

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**Abstract**

It has been proposed that an MAOA abnormality may be an important factor in the development of major depressive disorder (MDD).Various polymorphisms of the *MAOA* gene have been investigated for possible associations with mood disorders, but results have been inconsistent. The goal of the present study was to investigate whether polymorphisms of the *MAOA* gene are associated with MDD or alternatively with different clinical subgroups of MDD. A total of 590 Han Chinese subjects in Taiwan (312 controls and 278 MDD patients) were recruited. Among the males, there were no associations with MAOA polymorphisms. Among the females, an association was found between *MAOA* polymorphisms and *severe* MDD ($P = 0.041$ for *uVNTR* and 0.017 for *EcoRV* (rs1137070), respectively). However, in analyses of haplotype frequencies and multiple logistic regression, *MAOA* polymorphisms were not associated with either MDD or its subgroups. The results suggest that *MAOA* polymorphisms do not play a major role in the pathogenesis of MDD or its subgroups. However, a potential role for a minor association with some specific subgroups and with different ethnic samples needs to be explored further.

**Key words:** *MAOA gene, major depression, clinical subgroup, association study*

## Introduction

Major depressive disorder (MDD) is a common and disabling disorder that may result in impairment of daily functioning sufferers (Kessler et al. 2003). Several lines of evidence suggest that the heritability of MDD is about 38%. In twin studies, the genetic correlation of the susceptibility to MDD in men and women is estimated to be 0.55–0.63 (Kendler et al. 2006). We can therefore conclude that genetic factors play a role in MDD.

Over a period of 40 years, the monoamine depletion hypothesis of depression came to be widely accepted (Schildkraut and Kety 1967). Although there are different mechanisms of monoamine actions, many studies have shown that serotonin, norepinephrine and dopamine play important roles in the pathogenesis of MDD. Monoamines are mainly metabolized by monoamine oxidase (MAO). There are two isoforms of MAO (MAOA and MAOB) which can be differentiated based on their substrate and inhibitor specificities. MAOA preferentially metabolizes serotonin, and norepinephrine; MAOB prefers β-phenylethylamine and benzylamine as substrates. Dopamine and tryptamine are metabolized equally by both isoforms (Shih 1991). It is well-accepted that patients with major depression have abnormal MAO activity (Schildkraut 1965; von Knorring et al. 1985). A recent study which suggests that elevated MAOA density is the primary monoamine lowering process during major depression (Meyer et al. 2006) is in line with the hypothesis that MAOA is an important factor in major depression.

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Fibroblasts from monozygotic twins had levels of MAOA activity that were highly concordant (Breakefield et al. 1980), indicating that the level of MAOA activity is genetically controlled. The possibility that point mutations in MAOA or lack of the MAOA gene might result in mental retardation and behavioural changes (Brunner et al. 1993; Cases et al. 1995) was suggested. Along these lines, we propose that the expression of the MAOA gene is associated with the pathogenesis of MDD.

In 1998, Sabol et al. identified a functional polymorphism of a variable number tandem repeat in the promoter region of the MAOA gene (*uVNTR*). They found in cell lines that alleles of 3.5 or 4 repeats had 2–10 times more catalytic efficiency than those with alleles of 3 or 5 repeats (Sabol et al. 1998). Another study found in postmortem males that 3 repeat alleles of the MAOA promoter had low transcription activity (Denney et al. 1999). Therefore, our study of the associations of the MAOA gene with major depression focused on the MAOA promoter region. Despite the biological evidence of the involvement of the MAOA gene variants in the etiology of symptoms of depression, association studies contrasting patients with major depressive disorders and control subjects have not showed consistent results. Kunugi et al. (1999) found no significant association between *MAOA-uVNTR* and unipolar depression. Further studies have shown similar results (Cusin et al. 2002; Lin et al. 2000; Syagailo et al. 2001), whereas other have found a significant association (Schulze et al. 2000; Yu et al. 2005). There are several possible explanations for these conflicting results. These include different clinical phenotypes in major depressive disorder, different frequencies of the MAO gene in different ethnic groups, varying definitions of healthy controls, and the fact that some reports only used a single polymorphism, which may be less powerful than using the haplotype to define the role of the MAOA gene in bipolar disorder.

In our study, we investigated whether the promoter (*uVNTR*) and *EcoRV* (rs1137070) variants of the *MAOA* gene are associated with major depressive disorder in a Taiwan Han Chinese population. To reduce the clinical heterogeneity within major depressive disorder, we examined clinical subgroups of major depressive disorders separately. Individuals with major depressive disorder were classified into several subgroups according to clinical variables including family history of major affective disorder and moderate vs. severe major depression.

## Materials and methods

### *Subjects and clinical assessments*

The protocol of this study was approved by the Institutional Review Board for the Protection of Human Subjects at Tri-Service General Hospital (TSGH), a medical-teaching hospital affiliated with the National Defense Medical Center in Taipei, Taiwan. Written informed consent was obtained from all participants following a full explanation of the study procedure. To minimize the effects of ethnic differences on gene frequencies, all 590 individuals were recruited from the Han Chinese population in northern Taiwan. All participants were unrelated and were matched for ethnicity and geographic origin.

The patient group consisted of 278 patients with major depression (108 males and 170 females) who were recruited from clinical settings. Each patient was initially evaluated by an attending psychiatrist (SYH) and then interviewed by a well-trained psychologist using the Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L) (Endicott and Spitzer 1978; Merikangas et al. 1998) and DSM-IV criteria (American Psychiatric Association 1994). The inter-rater reliability $\kappa$ values of the Chinese Version of the Modified SADS-L were good-to-excellent for major depression, bipolar disorder, anxiety disorder, schizophrenia, and alcohol abuse and dependence (Huang et al. 2004). All patients in this study met DSM-IV criteria for major depressive disorder on the basis of an interview and a best-estimate procedure that used all available information, including clinical observations, medical records and family information. Subjects were recruited either in their first episode or in a recurrent episode. The severity of the episode was assessed with a 21-item Hamilton depression rating scale (HAM-D). Only subjects with a minimum score of 18 on the HAM-D entered the study. Individuals with a history of substance dependence, severe medical illness, organic brain disease, or any concomitant major psychiatric disorders were excluded from the study. Further, patients were classified into four clinical subgroups: major depression with family history, major depression without family history, moderate major depression, and severe major depression. A positive family history here indicates that one or more first-degree relatives had either bipolar disorder or major depression. HAM-D scores between 18 and 24 were defined as moderate major depression; HAM-D scores $>24$ were defined as severe major depression.

The normal control group included 312 healthy volunteers (201 males and 111 females) recruited

from the community. The Chinese Version of the SADS-L (Endicott and Spitzer 1978; Merikangas et al. 1998) was used to screen out psychiatric conditions in the control group. Control subjects were free of past or present major or minor mental illness including affective disorder, schizophrenia, anxiety disorder, personality disorder and substance use disorders and those with family history of such disorders were excluded.

*Genotyping for MAOA gene*

The 30-bp repeat polymorphism of the *MAOA-uVNTR* gene (variable number of tandem repeats located upstream of the promoter region) was investigated using a modification of the polymerase chain reaction (PCR) method described by Zhu et al. (1992). The *EcoRV* (1460C > T) polymorphism (rs1137070) in exon 14 of *MAOA* gene were detected using the modified PCR-RFLP (restriction fragment length polymorphism) method described by Hotamisligil and Breakefield (1991). The *MAOA EcoRV* (−) polymorphism remained intact and was 703 bp long, whereas the *MAOA EcoRV* (+) polymorphism was cut into two DNA fragments of 340 and 363 bp by the *EcoRV* restriction enzymes.

*Statistical analyses*

Independent-samples *t*-tests were employed to determine differences in mean age between patients with major depression and normal controls. Pearson $\chi^2$ analysis was used to compare gender difference between the patient and control groups. Hardy–Weinberg equilibrium was assessed for each group, and the frequencies of genotype and allele were also compared in patients versus controls using Pearson $\chi^2$ analyses. Fisher exact tests were substituted for the Pearson $\chi^2$-test when sample sizes were smaller than expected (less than five subjects). Multiple logistic regression analysis was applied to correct for effects of possible covariates such as age, gender and other *MAOA* polymorphisms on the risk of major depression. SPSS (version 11.5, SPSS, Taipei, Taiwan) statistical software was used for all analyses and a probability value $P<0.05$ was considered statistically significant.

Haplotype frequencies, linkage disequilibrium coefficients, and standardized linkage disequilibrium coefficients between *uVNTR* and *EcoRV* polymorphisms in the *MAOA* gene were estimated by using two computer programs: (1) estimating haplotypes and (2) permutation and model free analysis. Examination of frequencies of haplotypes used Fisher's exact test when small cell sizes were encountered (Zhao and Sham 2002). Power analyses were performed with the use of G-Power computer software and effect size conventions were determined according to the method of Erdfelder et al. (1996). All tests were two-tailed; $\alpha$ was set at 0.05.

## Results

Data for gender and age are shown in Table I. The difference in mean age between controls and subjects with major depression (or subgroups) was not significantly different, except for the subgroup of patients without a positive family history for affective disorder ($P=0.04$). The gender difference between controls and total MDD and its subgroups was significantly different ($P<0.001$).

The five repeat polymorphism of the *MAOA uVNTR* gene was not found. The 2 repeat allele was rare in our study (four normal males and one MDD male). Therefore, we did not include subjects with the 2 repeat polymorphism of the *MAOA-uVNTR*. For data analysis of halplotype (between *MAOA-uVNTR* and *MAOA-EcoRV*) and single *MAOA-uVNTR*, the study included 277 major depression patients (107 males, 170 females) and 308 normal controls (197 males, 111 females). For data analysis of *MAOA-EcoRV*, the study included 278 major depression patients (108 males, 170 females) and 312 normal controls (201 males, 111 females).

Table I. Comparison of the mean age and gender between patients and controls and between controls and patient subgroups.

| Group | Sample Size (*N*) | Mean age (± SD)(years) | *P*[c] | Sex (%) | | *P*[c] |
|---|---|---|---|---|---|---|
| | | | | Male | Female | |
| Total MD[a] | 278 | 39.51(14.706) | 0.098 | 108(38.8) | 170(61.2) | <0.001 |
| MD, positive FH[a] | 72 | 37.79(14.674) | 0.949 | 25(34.7) | 47(65.3) | <0.001 |
| MD, negative FH | 206 | 40.11(14.706) | 0.040 | 83(40.3) | 123(59.7) | <0.001 |
| MD, moderate[b] | 81 | 39.69(15.207) | 0.206 | 33(40.7) | 48(59.3) | <0.001 |
| MD, severe[b] | 197 | 39.43(14.534) | 0.141 | 75(38.1) | 122(61.9) | <0.001 |
| Healthy control subjects | 312 | 37.69(11.972) | | 201(64.4) | 111(35.6) | |

[a]MD, major depressive disorder; FH, family history.
[b]Moderate MD indicates HAM-D score 18–24; Severe MD indicates HAM-D score >24.
[c]Compared with the control group.

The allele frequencies of *MAOA-uVNTR* and *EcoRV* polymorphisms in total MDD patients and the total control group (females and males) were not significantly different ($P>0.1$, data not shown). Because the MAOA gene is located on the X chromosome, we analysed males and females separately. Therefore, these results for males and females are not influenced by gender. Among males, neither the *MAOA-uVNTR* nor the *EcoRV* polymorphisms showed a significant difference in the allele frequencies between MDD patients and controls, even if they were divided into the different subgroups of major depression ($P>0.1$, Table II). Among female subjects, the *MAOA-uVNTR* genotype frequencies showed weak associations between severe MDD subjects versus controls ($P=0.041$) (Table II), but the allele frequency was not significantly different between severe MDD subjects and controls ($P>0.1$, data not shown). Regarding genotype frequencies of the *MAOA-EcoRV* polymorphism in female subjects, there were significant associations between total MDD versus controls ($P=0.049$), between MDD with family history versus controls ($P=0.047$), and between severe MDD versus controls ($P=0.017$) (Table II). But the allele frequency of *MAOA-EcoRV* was not different between controls and patients with MDD or between controls and MDD subgroups among female subjects (data not shown). Among female subjects, the genotype distributions of *uVNTR* and *EcoRV* polymorphisms of the *MAOA* gene were in the Hardy–Weinberg equilibrium, both in the healthy control subjects and total female patients with MDD ($P>0.05$).

Although we found that some subgroups of major depression are associated with the genotypes of the *MAOA* gene in female subjects (Table II), the most allele frequency did not show this association. To rule out associations by chance, we used haplotype analysis because it is more powerful and can provide more information in an association study. The haplotype frequencies of 3 repeat/$-$ and 4 repeat/$+$ were less than 10% in each of the 10 study groups. A strong linkage disequilibrium between the *uVNTR* and *EcoRV* of the *MAOA* gene was also evident. There were no significant differences in the haplotype frequencies of the MAOA gene between controls and total MDD patients, and between the subgroups of MDD patients versus controls ($P>0.05$, data not shown). In addition, we used multiple logistic regression analysis and corrected for age, and gene to gene interactions for *MAOA-uVNTR* and *EcoRV* polymorphisms in the risk of major depression. There were no significant differences between controls and major depression patients or its subgroups both in male and female subjects (Table III).

The study power was approximately 0.42–0.66 to detect a small effect, and 0.99 to detect a medium and large effect in the haplotype and allele frequencies of total males and females respectively. As for the patient subgroups, the statistical power was considerably lower because of the limited sample size. In the present power analysis, effect size conventions were determined according to the method of Erdfelder et al. (1996) as follows: small effect size $=0.10$, medium effect size $=0.30$, large effect size $=0.50$ ($\alpha=0.05$).

## Discussion

Variable tandem repeat alleles of the *MAOA* promoter gene had different transcription activities in cell lines (Hotamisligil and Breakefield 1991; Sabol et al. 1998) and it was thus selected as a good candidate for genetic studies of major depression. In spite of the strong rationale for the present study, however, the results showed that the genotype frequencies of the *MAOA* gene were not associated with major depressive disorder or its subgroups after multiple logistic regression analyses (Table III). Moreover, the haplotype frequencies of the *MAOA* gene did not have a significant association with major depression either in females or in males.

There are some limitations in our study. First, our total sample size was enough to find an effect of MAOA polymorphisms, but after division of subjects into different gender groups or MDD subgroups, the sample size of some subgroups was small. The results should therefore be interpreted carefully, and further research will be needed to rule out possible associations. Second, our study design was a cross-sectional approach, where we employed single time-point observations to subgroups of major depression, which may not corresponded to a lifetime perspective.

This result supports some previous studies (Kunugi et al. 1999; Syagailo et al. 2001; Gutierrez et al. 2004), but whilst contradicting others (Schulze et al. 2000; Yu et al. 2005). In a study by Yu et al. (2005), it was found that 4 repeat alleles of the *MAOA uVNTR* gene was higher in major depression patients than in controls, and a second study also reported increasing frequencies of the 4 repeat allele in major depression in German patients (Schulze et al. 2000). One of the explanations for these controversial results is ethnic stratification. The frequency of the 3 repeat allele in our control subjects was 0.617 for males and 0.586 for females, and in Japanese subjects the frequency was 0.60 concurrent with the current study population (Kunugi et al. 1999). In Caucasian populations, the 3 repeat allele frequencies were around 0.35–0.42 (Sabol et al. 1998; Cusin et al. 2002) and showed

Table II. Genotype frequencies of the promoter *uVNTR* and exon 14 *Eco*RV polymorphisms in the *MAO-A* gene for patients with major depression or its clinical subtypes and for healthy controls.

| | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *uVNTR* in females (%) | | | | | | *uVNTR* in males (%) | | | |
| Group | Females ($N^a$) | 3R/3R | 3R/4R | 4R/4R | $x^2$ | $P^b$ | Males ($N^a$) | 3R | 4R | $x^2$ | $P^b$ |
| Total MD[a] | 170 | 69(40.6) | 69(40.6) | 32(18.8) | 3.691 | 0.158 | 107 | 62(57.9) | 45(42.1) | 0.730 | 0.393 |
| MD, positive FH[a] | 47 | 18(38.3) | 21(44.7) | 8(17.0) | 0.772 | 0.680 | 25 | 14(56.0) | 11(44.0) | 0.455 | 0.500 |
| MD, negative FH | 123 | 51(41.5) | 48(39.0) | 24(19.5) | 3.880 | 0.144 | 82 | 48(58.5) | 34(41.5) | 0.476 | 0.490 |
| MD, moderate | 48 | 13(27.1) | 25(52.1) | 10(20.8) | 0.912 | 0.634 | 33 | 17(51.5) | 16(48.5) | 1.556 | 0.212 |
| MD, severe | 122 | 56(45.9) | 44(36.1) | 22(18.0) | 6.405 | 0.041 | 74 | 45(60.8) | 29(39.2)) | 0.104 | 0.747 |
| Healthy control subjects | 111 | 36(32.4) | 58(52.3) | 17(15.3) | | | 197 | 124(62.9) | 73(37.1) | | |
| | | *Eco*RV in female | | | | | | *Eco*RV in male | | | |
| | Females ($N^a$) | +/+ | +/− | −/− | $x^2$ | $P^b$ | Males ($N^a$) | + | − | $x^2$ | $P^b$ |
| Total MD[a] | 170 | 67(39.4) | 72(42.4) | 31(18.2) | 6.041 | 0.049 | 108 | 61(56.5) | 47(43.5) | 0.401 | 0.527 |
| MD, positive FH[a] | 47 | 21(44.7) | 17(36.2) | 9(19.2) | 6.119 | 0.047 | 25 | 12(48.0) | 13(52.0) | 1.366 | 0.242 |
| MD, negative FH | 123 | 46(37.4) | 55(44.7) | 22(17.9) | 3.623 | 0.163 | 83 | 49(59.0) | 34(41.0) | 0.033 | 0.856 |
| MD, moderate | 48 | 14(29.2) | 24(50.0) | 10(20.8) | 0.741 | 0.691 | 33 | 17(51.5) | 16(48.5) | 0.883 | 0.347 |
| MD, severe | 122 | 53(43.4) | 48(39.3) | 21(17.2) | 8.130 | 0.017 | 75 | 44(58.7) | 31(41.3) | 0.053 | 0.817 |
| Healthy control subjects | 111 | 30(27.0) | 63(56.8) | 18(16.2) | | | 201 | 121(60.2) | 80(39.8) | | |

[a]MD, major depressive disorder; FH, family history; N, sample size.
[b]Compared with the control group.

Table III. Logistic regression analysis of the *MAO-A* gene (promoter and *Eco*RV) for risk of major depression, and for risk of its clinical subtypes in female patients.

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| | Major depression ($n=170$) | | | | | |
| Variable | Odds ratio | | 95% CI | | *P* value | |
| 3/3 repeat | 0.396 | | 0.084–1.875 | | 0.243 | |
| 3/4 repeat | 0.565 | | 0.166–1.922 | | 0.361 | |
| +/+ | 2.997 | | 0.617–14.561 | | 0.174 | |
| +/− | 1.110 | | 0.328–3.753 | | 0.867 | |
| Age | 1.006 | | 0.989–1.024 | | 0.465 | |
| | MD, positive FH ($n=47$) | | | MD, negative FH ($n=123$) | | |
| Variable | Odds ratio | 95% CI | *P* value | Odds ratio | 95% CI | *P* value |
| 3/3 repeat | 0.236 | 0.026–2.124 | 0.198 | 0.529 | 0.103–2.704 | 0.444 |
| 3/4 repeat | 0.937 | 0.209–4.209 | 0.933 | 0.467 | 0.129–1.689 | 0.246 |
| +/+ | 5.095 | 0.610–42.529 | 0.133 | 2.283 | 0.421–12.393 | 0.339 |
| +/− | 0.619 | 0.144–2.653 | 0.518 | 1.432 | 0.393–5.221 | 0.587 |
| Age | 0.997 | 0.972–1.022 | 0.788 | 1.011 | 0.993–1.030 | 0.232 |
| | MD, moderate ($n=48$) | | | MD, severe ($n=122$) | | |
| Variable | Odds ratio | 95% CI | *P* value | Odds ratio | 95% CI | *P* value |
| 3/3 repeat | 0.204 | 0.022–1.876 | 0.160 | 0.556 | 0.119–2.603 | 0.456 |
| 3/4 repeat | 0.719 | 0.163–3.177 | 0.664 | 0.548 | 0.163–1.847 | 0.332 |
| + / + | 3.464 | 0.380–31.608 | 0.271 | 2.508 | 0.516–12.185 | 0.254 |
| + / − | 0.978 | 0.223–4.282 | 0.976 | 1.060 | 0.315–3.571 | 0.925 |
| Age | 1.011 | 0.987–1.035 | 0.378 | 1.004 | 0.986–1.024 | 0.642 |

FH, family history; MD, major depression.
Reference group is 4/4 repeat, −/− and female, respectively.
The *P* values and odds ratios of early-onset major depression ($n=17$) have not been shown in this table, but the risk of the *MAO-A* gene for this group was not significantly different, $P>0.1$.
There were no significant differences between controls and patients with major depression or male patient subgroups.

lower values than Asian populations (0.58–0.65) (Kunugi et al. 1999; Yu et al. 2005**)**. This difference in allele frequency may be partially responsible for the divergent association results. The ethnicity difference may influence population gene frequencies and may produce a false-positive or false-negative result by chance rather than revealing a direct relationship between an allele and a disease.

However, among subjects with the same ethnicity and the same allele frequency, which was the case in our study, the negative results need to be more carefully explained. Our results still contrasted with previous reports that showed significant associations between *MAOA-uVNTR* and MDD in a Han Chinese population (Yu et al. 2005). In the study of Yu et al. (2005), only a single polymorphism of the MAOA gene in total major depression was detected. The statistical power of a single polymorphism is weaker than haplotype analysis (Kidd. 1993). Thus, we measured two polymorphisms, the promoter *uVNTR* and exonic *EcoRV* in the *MAOA* gene, to assess the association between the MDD group and controls. The haplotype frequencies were analysed to increase statistical power. On the other hand, Yu et al. (2005) used medical staff for controls, whereas the controls in our study were from the community and all had the same diagnostic interview as patients, conducted by a psychiatrist using the SADS-L to rule out psychiatric disorders. Un-screened control subjects could influence the result. Therefore, our results may carry more weight and the probability of a negative finding by chance was relatively low.

The MAOA gene may have a gender-specific effect. In our study, the association of the *MAOA-uVNTR* and *EcoRV* polymorphisms with MDD was only in female subjects. Healthy females with a 4R *MAOA-uVNTR* allele had higher 5-hydroxyindoleacetic acid and homovanillic acid levels in their cerebrospinal fluid than subjects with a 3R allele (Jonsson et al. 2000). Deckert et al. (1999) found that there was a significant excess of the 4R allele of *MAOA-uVNTR* in female patients with panic disorder. Stronger differences in genotype frequency of the *MAOA* gene were found in female patients with obsessive compulsive disorder (OCD) than in males

with OCD (Camarena et al. 2001). The 3R *uVNTR* allele of the *MAOA* gene in MDD females had a better antidepressant response, but the 3R *uVNTR* allele of the *MAOA* gene in MDD males did not (Yu et al. 2005). Therefore, the *MAOA* gene may have gender effects for some aspects of clinical psychiatry. One of the possible explanations is that the *MAOA* gene is located on the X chromosome, and female subjects have two X chromosomes. Another consideration is the hormone effect. Estrogen treatment can suppress the expression of MAOA mRNA (Gundlah et al. 2002; Smith et al. 2004) and hence modulate monoamine transcription and mood symptoms.

Phenotype definition is an important issue in candidate gene association studies of complex disorders such as major depression. The use of a narrowly defined phenotype or a refined quantitative phenotype can lead to a dramatic increase in power (Rao and Province 2001). Thus, the use of a narrow, specific subtype of major depression may be powerful enough to detect an association between major depression and the MAOA gene. Although, previous Caucasian studies used some variables to create subtypes of MDD (Schulze et al. 2000), they also used only one polymorphism of the MAOA gene and the association was relatively small ($P = 0.055$). The strength of our study is that we controlled for the heterogeneity of major depressive disorder by dividing all MDD subjects into different sex and clinical subgroups. Furthermore, we used a more powerful haplotype analysis and multiple logistic regression analysis for correcting age and gene–gene interactions. Therefore, our study results might be more powerful than those of previous studies (Schulze et al. 2000; Yu et al. 2005).

However, environmental factors also play an important role in the aetiology and pathogenesis of major depression and influence some clinical aspects of the disease (Silberg et al. 2001; Eley et al. 2004a). In a study by Eley et al. (2004b,) a gene–environment interaction was found for the serotonin transporter gene in depressed adolescent females. This result suggests that we should consider possible interactions between the MAOA gene and environmental factors in major depression patients. Therefore, further replication studies with stricter methods and with a larger sample size are needed to validate whether different MAOA gene polymorphisms confer different risks for major depression.

In conclusion, we found some female groups within MDD are associated with the *MAOA-uVNTR* and *EcoRV* genotypes, respectively. However in haplotype analysis and in multiple logistic regression analysis for risk of MDD, we could not confirm the association of a single SNP in MDD patients. Therefore, we suggest that the *MAOA* gene does not play a major role in increasing susceptibility to major depression. Nevertheless, since major depression is a heterogeneous disorder, this gene may have a gender specific effect or gene–gene interaction effect in the pathogenesis of MDD.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**ORIGINAL INVESTIGATION**

# The neural substrates of affective face recognition in patients with Hwa-Byung and healthy individuals in Korea

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**Abstract**

Hwa-Byung (HB) is a Korean culture-bound psychiatric syndrome caused by the suppression of anger. HB patients have various psychological and somatic symptoms, such as chest discomfort, a sensation of heat, and the sensation of having an epigastric mass. In this study, we measured brain activity in HB patients and healthy individuals in response to affective facial stimuli. Using functional magnetic resonance imaging (fMRI), the current study measured neural responses to neutral, sad, and angry facial stimuli in 12 healthy individuals and 12 patients with HB. In response to all types of facial stimuli, HB patients showed increased activations in the lingual gyrus and fusiform gyrus compared with healthy persons, but they showed relatively lower activation in the thalamus. We also found that patients with HB showed lower activity in response to the neutral condition in the right ACC than healthy controls. The current study indicates that the suppression of affect results in aberrant function of the brain regions of the visual pathway, and functional impairment in the ACC may contribute to the pathophysiology of HB.

**Key words:** *Hwa-Byung, functional magnetic resonance imaging (fMRI), sad, anger, visual pathway*

**Abbreviations:** *BOLD, blood oxygen level dependent, HB, Hwa-Byung, LH, left hemisphere, RH, right hemisphere, TH, thalamus, LG, lingual gyrus, IOG, inferior occipital gyrus, FG, fusiform gyrus.*

## Introduction

Hwa-Byung (HB) is a common Korean culture-bound syndrome, and could be translated as "anger syndrome" (American Psychiatric Association 2000). HB is more prevalent in women and in older individuals (Park et al. 2001). A community-based survey revealed that the prevalence of HB was 4.95% among Korean women 41–65 years old (Park et al. 2001). The cause of HB has been suggested as being the suppression of anger or the projection of anger into the body in reaction to suffering an injustice (Lin 1983; Min 1989). Usually, HB patients have various psychological and somatic symptoms. Somatic symptoms such as pushing-up in the chest, heaviness in the head and chest tightness are typical features of HB. HB is also frequently combined with depression and anxiety symptoms (Park et al. 2002). Generally, women with HB have lived a life of suffering, as a result of their conflict-laden relationships with their spouses (Park et al. 2002). Suffering individuals are not allowed to express their emotions directly, due to certain repressive aspects of Korean culture (Lin 1983; Park et al. 2002). In addition, HB patients usually have difficulty with interpersonal conflicts and anger (Lin 1983).

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Several neuroimaging studies have investigated the brain regions involved in processing angry facial expressions (Sprengelmeyer et al. 1998; Blair et al. 1999; Phillips et al. 1999; Damasio et al. 2000; Whalen et al. 2001; Yang et al. 2002; Adams et al. 2003; Strauss et al. 2005). The anterior cingulate cortex (ACC) and amygdala have been the most frequently reported areas that become activated in response to angry facial expressions (Strauss et al. 2005).

To our knowledge, the pathophysiology of HB has not been investigated, and there have been no neuroimaging studies of HB patients. Based on previous evidence of the neural substrates of anger, and the fact that HB is an anger syndrome, we hypothesized that HB patients would have aberrant function with neural substrates of anger and that they would show a tendency for increased sensitization to human facial stimuli.

Previous neuroimaging studies in humans identified several occipitotemporal cortical regions that are preferentially activated in response to faces (Sergent et al. 1992; Kanwisher et al. 1997). In addition, Felleman and Van Essen (1991) proposed that the face-sensitive neurons of the inferior occipital gyrus (IOG) are responsible for recognizing facial features, whereas the fusiform gyrus is responsible for processing whole facial identities (Haxby et al. 2000). In the structural aspect of the visual system, the pathway can be traced to the early stage of the thalamus and primary visual cortex. The lateral geniculate nucleus (LGN) is part of the thalamus, which is the primary processor of visual information received from the retina, in the central nervous system. In addition, it receives many strong feedback connections from the primary visual cortex. The LGN sends projections directly to the primary visual cortex. With incoming facial information from the LGN, the primary cortex can submits the signal into the inferior occipital gyrus.

Thus, the regulated information of facial stimuli with an affective state in the thalamus would flow into the primary visual cortex or lingual gyrus, and then is sent to the inferior occipital cortex and fusiform gyrus. If HB patients are over-sensitized to the facial information on the perceptual level, they should show a larger response to facial stimuli in the primary cortex and the locus for later processing compared with healthy persons. However, if their dysfunction is due to flaws on the cognitive level or later stage, we should observe increased activation only in the fusiform gyrus.

The aim of the study was to investigate brain activation patterns using functional magnetic resonance imaging (fMRI) in patients with HB and healthy subjects presented with affective facial stimuli. We tested two hypotheses. First, supposing that an affective problem is a primary component of HB, patients with HB would exhibit increased activation in the brain regions that produce emotions, such as the amygdala and OFC, and decreased activation in brain regions that regulate emotions, such as the ACC, in response to sad and angry facial stimuli, as compared to healthy individuals. Second, if patients with HB have problems regulating incoming visual information, which could involve emotional control in the amygdala and ACC, there might be increased activation in response to affective facial stimuli in the visual system, such as the inferior occipital cortex, and lingual and fusiform gyrus of patients with HB, as compared to healthy controls. More specifically, if the regulating signal influences an earlier stage of visual processing, increased activations will be found only in the fusiform gyrus. Alternatively, if it affects a later stage of visual processing, higher activations will be observed in the earlier structures in the visual pathway, such as the lingual gyrus and IOG.

## Material and methods

### *Subjects and clinical assessment*

Twelve patients with HB (mean age $48.4 \pm 6.4$) and 12 healthy persons (mean age $45.5 \pm 4.4$) from the Hangang Sacred Heart Hospital and Kyoung-hee University Hospital in Seoul, participated in the current experiment. All subjects were female and age range for study inclusion was 40–55 years. Demographic and clinical characteristics are presented in Table I. The degree of depressive symptoms of the patients was assessed by the Hamilton Depression Rating Scale (HDRS) (Hamilton 1967). There was no significant difference in age between the groups ($P > 0.1$). The diagnosis of HB was established using the Hwa-Byung Diagnostic Interview Schedule (HBDIS; Kim et al. 2004) by two trained psychiatrists. The HBDIS is a semi-structured interview for diagnosing HB, consisting 16 items with seven dimensions. The 16 items include four core items (chest discomfort, a sensation of heat, pushing-up sensation in the chest, a sensation of epigastric mass), four related to somatic symptoms (dry mouth, headache, insomnia, palpitation), two other core items (feel victimized, deep sorrow), three related psychological symptoms (anger, helplessness, fear), one about impaired psychosocial functioning, one about stress, and one regarding medical illness. The validity and reliability of HBDIS have been reported to be high in Korean populations (Kim et al. 2004). Exclusion criteria were a current diagnosis of an Axis-I disorder requiring psychotropic

Table I. Demographic and clinical characteristics.

| | Hwa-Byung patients ($n=12$) | | Healthy subjects ($n=12$) | |
|---|---|---|---|---|
| Demographic and clinical variables | Mean | SD | Mean | SD |
| Age (years) | 48.4 | 6.4 | 45.5 | 4.4 |
| 17-item HDRS Score | 19.8 | 4.5 | NA | |
| Duration of Illness | 17.7 | 16.2 | NA | |
| Number of previous episodes | 2.1 | 0.928 | NA | |

HDRS, Hamilton Depression Rating Scale; SD, standard deviation; NA, not applicable.

medication, as identified by the Korean version of the Structured Clinical Interview for DSM-IV (SCID-IV) (Han et al. 2000) except HB, and a DSM-IV Axis-II diagnosis of antisocial or borderline personality disorder. All study subjects were drug-free for at least 2 weeks at the time of scanning. The mean duration of illness in this group was 12.9 months (SD = 5.0). All participants were right-handed according to the Edinburgh Handedness Test (Oldfield 1971). The study protocol was approved by the Institutional Review Board at the Ethical Committee of the Hangang Sacred Heart Hospital. After the subjects were given a complete description of the study, written informed consent was obtained.

### *Stimulus presentation*

Because it is difficult to know if over-sensitization to face stimuli is specific to a given affective stimulus, affective stimulation was induced by the presentation of facial stimuli that included a range of affective valences: neutrality, anger and sadness. We used 18 grayscale faces (six anger, six sad and six neutral) for this study. These were derived from standardized Korean faces from Lee et al. (2004). Based on large-sample valence rating studies, a rank between 1 and 6 was previously assigned to each of these pictures according to their valence. Negative (valence scores 1–2), neutral (valence scores 3–4), and positive (valence scores 5–6) facial stimuli were selected for presentation. Each affective condition contained the same number of pictures.

A trial was started by displaying a facial stimulus using a projector; the image was presented for 1.5 s with a 0.5-s inter-stimulus interval. After a series of stimuli were presented, the fixation spot consisting of a single cross on a blank screen was turned on for 12 s. Considering hemodynamic parameters, each run consisted of three alternating 24-s blocks of neutral (N), angry (A), and sad (S) pictures, bracketed by 12-s blocks of fixation. The procedure and an example of pictures are summarized in Figure 1. All participants experienced two runs (3 min 46 s per run). Within a run, each block was ordered in a pseudo-random manner to counterbalance the order of conditions (for example, neutral–angry–sad). Before scanning, subjects were instructed not to move and to fixate on the presented faces (e.g. passive viewing). None of the participants admitted having seen the experimental faces before. After each session, participants were asked to describe and then rate the faces according to the dimensions of arousal (low to high, 1–6) and emotional valence (negative to positive, 1–6) measures. A repeated-measures analysis of variance was used to assess group and facial stimuli (neutral, angry and sad) interactions for both valence and arousal ratings.

### *Acquisition of MR images and statistical analysis*

A 1.5-Tesla Siemens Sonata whole-body imaging system (Siemens Medical Systems, Iselin, NJ, USA) was used to acquire scan images. To prevent motion of the participants' heads, padding was placed around their heads. After an anatomical scan was performed (1120 ms TR, 2.73 ms TE, 240 mm FOV, $256 \times 256$ matrix size, 24 axial slices without gap, $1.875 \times 1.875 \times 5.0$ mm$^3$ voxel), T2*-weighted functional images were acquired at the same slice locations using an interleaved EPI gradient echo sequence ($64 \times 64$ matrix size, 40 ms TE, 2000 ms TR, $3.75 \times 3.75 \times 5.0$ mm$^3$ voxel). Functional data were corrected against motion artifacts, which were followed by spatial normalization at $4 \times 4 \times 4$ mm$^3$ resolution and smoothing using a Gaussian kernel of FWHM 7 mm. This procedure was performed using the SPM2 (Wellcome Department of Cognitive Neurology, London, UK), which was used for voxelwise univariate parameter estimation based on a general linear model. In the current study, regions of interest (ROIs) were acquired using MarsBar ROI toolbox for SPM2 (http://marsbar.sourceforge.net) (Brett et al. 2002) for the bilateral amygdalae, rostral ACC, thalami, lingual gyri, inferior occipital gyri, and fusiform gyri. With a height threshold of $P < 0.01$ (uncorrected for multiple comparisons) and a cluster size of 6 voxels, we determined ROIs by activated voxels within the predefined masked regions in any group across all affective conditions.

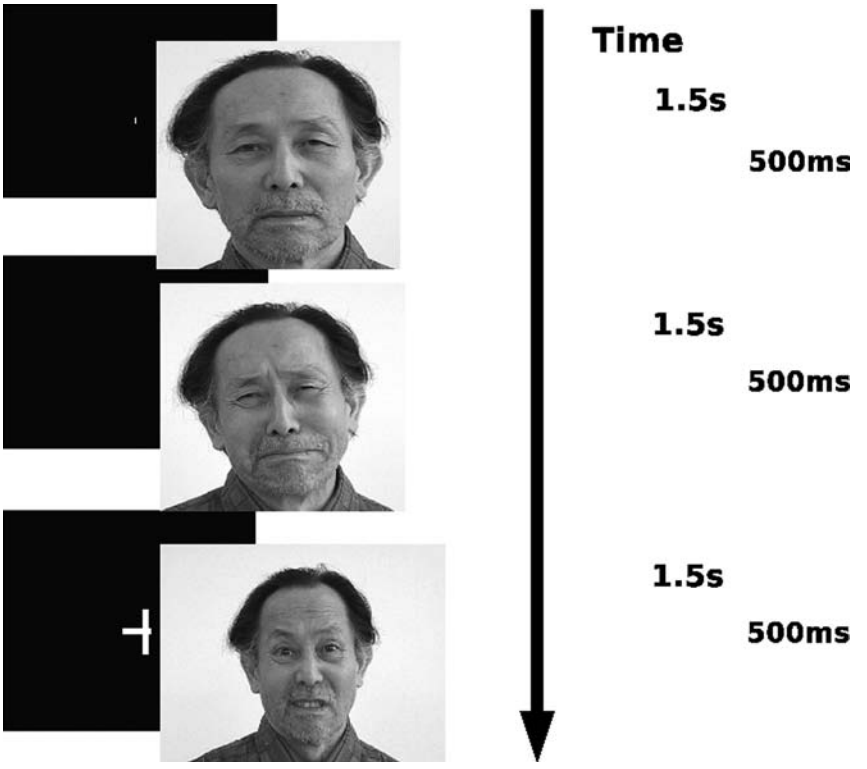


Figure 1. Example of stimuli and time parameters. The upper face is an example of the neutral condition. The face in the middle row represents the condition of sadness, and the lower picture represents the condition of anger. In each block, the fixation mark was displayed for 1.5 s, and then the facial stimulus was presented for 0.5 s. The affective face conditions were ordered in a pseudo-random manner. The sequence was repeated four times, and then a resting period followed for 12 s.

The specific coordinates of the ROIs found to have significantly activated clusters are listed in Table II. These ROIs are known as loci of emotional or affective information processing (Seminowicz et al. 2004) and face recognition (Murphy et al. 2003). For each ROI, the mean effect size was calculated for each affective condition for each participant, and then the mean activations were compared between HB and healthy groups and facial stimuli (neutral, angry, sad). Significance was tested with analysis of variance or Student's *t*-test ($P<0.05$, FDR, corrected for multiple comparisons).

Among the ROIs, it is very difficult to detect activation in the LGN because it is a very small body in terms of volume. Due to the limited spatial resolution of fMRI, we considered the thalamus, including the LGN, as a single ROI, which is known to be the locus for regulating incoming visual information on the visual pathway for facial recognition.

## Results

### *Subjective experience of facial stimuli*

The average subjective ratings for arousal/valence of neutral, sad and angry facial pictures were reported by HB patients (mean = 3.13/2.52, 3.59/1.26 and 4.27/1.64, respectively) and the healthy control subjects (mean = 2.83/3.30, 4.20/2.28 and 4.38/1.72 respectively). There was only a significant difference in subject rating for arousal of sad facial pictures between the two groups ($t(22)=5.025$, $P<0.001$). Both HB and healthy comparison groups gave higher ratings of arousal for the negative condition compared to the neutral condition

### *fMRI results*

After examining the activated coordinates in each ROI, the amygdala and insular were dropped from the analysis because no activated voxels were found in these ROIs. The specific coordinates of the ROIs are described in Table II. We found main effects only for the group and the affective condition. Group effects were found in the right medial OFC ($F(1,22)=14.625$, $P<0.01$), the bilateral ACC (left: $F(1,22)=12.036$, $P<0.025$; right: $F(1,22)=11.641$, $P<0.025$), the thalami (left: $F(1,22)=12.121$, $P<0.025$; right: $F(1,22)=13.509$, $P<0.001$), the fusiform gyri (left: $F(1,22)=18.439$, $P<0.001$; right: $F(1,22)=14.633$, $P<0.01$), the inferior occipital cortices (left: $F(1, 22)=11.421$, $P<0.025$; right: $F(1,22)=10.115$, $P<0.025$), and

Table II. From each ROI, specific coordinate on the MNI space and its mean signal change ratios on exposure to each emotional condition are presented. The standard error is indicated within brackets. The specific signal change ratio was calculated using SPM2. In the table, *k* represents the cluster size in terms of the number of voxels.

| | | | Coordinates | | | Hwa-Byung patients ($N=12$) | | | Healthy individuals ($N=12$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ROI | Side | *k* | *x* | *y* | *z* | Neutral | Anger | Sad | Neutral | Anger | Sad |
| ACC | L | 128 | 0 | 40 | 4 | −0.123 | -0.090 | -0.104 | -0.036 | -0.038 | -0.018 |
| | | | | | | (0.028) | (0.031) | (0.023) | (0.020) | (0.021) | (0.027) |
| | R | 9 | 8 | 40 | 4 | -0.112 | −0.097 | −0.096 | −0.046 | −0.040 | −0.033 |
| | | | | | | (0.031) | (0.029) | (0.018) | (0.015) | (0.022) | (0.024) |
| mOFC | R | 12 | 4 | 44 | –16 | −0.145 | −0.121 | −0.092 | −0.088 | −0.021 | −0.028 |
| | | | | | | (0.035) | (0.033) | (0.029) | (0.024) | (0.034) | (0.024) |
| Thalamus | L | 16 | –12 | –32 | 4 | −0.046 | −0.040 | −0.043 | 0.043 | 0.003 | −0.031 |
| | | | | | | (0.027) | (0.020) | (0.011) | (0.010) | (0.015) | (0.017) |
| | R | 8 | 16 | –32 | 4 | −0.016 | −0.042 | −0.037 | 0.058 | −0.001 | −0.024 |
| | | | | | | (0.028) | (0.018) | (0.015) | (0.006) | (0.016) | (0.018) |
| Lingual gyrus | L | 29 | –8 | –80 | –12 | 0.244 | 0.206 | 0.181 | 0.104 | 0.021 | 0.062 |
| | | | | | | (0.025) | (0.032) | (0.023) | (0.028) | (0.044) | (0.020) |
| | R | 21 | 12 | –84 | –16 | 0.423 | 0.426 | 0.412 | 0.187 | 0.136 | 0.172 |
| | | | | | | (0.060) | (0.064) | (0.067) | (0.040) | (0.058) | (0.044) |
| IOG | L | 20 | –48 | –72 | –8 | 0.262 | 0.232 | 0.259 | 0.088 | 0.124 | 0.127 |
| | | | | | | (0.029) | (0.041) | (0.046) | (0.022) | (0.028) | (0.016) |
| | R | 25 | 44 | –76 | –12 | 0.286 | 0.258 | 0.299 | 0.117 | 0.153 | 0.172 |
| | | | | | | (0.049) | (0.045) | (0.046) | (0.024) | (0.019) | (0.026) |
| Fusiform gyrus | L | 37 | –40 | –52 | –20 | 0.298 | 0.241 | 0.253 | 0.110 | 0.054 | 0.072 |
| | | | | | | (0.036) | (0.056) | (0.035) | (0.022) | (0.027) | (0.022) |
| | R | 34 | 36 | –52 | –16 | 0.248 | 0.254 | 0.240 | 0.138 | 0.078 | 0.070 |
| | | | | | | (0.035) | (0.035) | (0.049) | (0.019) | (0.021) | (0.014) |

ACC, anterior cingulate cortex; mOFC, medial orbitofrontal cortex; IOG, inferior occipital gyrus.

the lingual gyri (left: $F(1,22)=21.858$, $P<0.005$; right: $F(1,22)=12.382$, $P<0.025$).

To identify the exact locus of the effects among the affective conditions, we segregated the contrast into sadness over the neutral condition and anger over the neutral condition. First, the group effect in the left ACC was attributable primarily to the higher activity in healthy participants than in patients with HB under the neutral condition ($t(22)=2.57$, $P<0.05$) and the sad condition ($t(22)=2.42$, $P<0.05$). Second, individual differences in the bilateral thalami seemed to be due to the effect under the neutral condition (left: $t(22)=3.09$, $P<0.025$; right: $t(22)=2.59$, $P<0.05$), in which healthy controls showed higher activity than patients with HB. Third, the group effect in the left lingual gyrus showed significant differences under all affective conditions (sad: $t(22)=3.72$, $P<0.01$; angry: $t(22)=3.41$, $P<0.025$; neutral: $t(22)=3.89$, $P<0.01$), for which patients with HB showed higher responses than healthy controls. The differences in the right lingual gyrus also resulted from higher activation in patients with HB than in healthy controls under all affective conditions (sad: $t(22)=3.28$, $P<0.025$; angry: $t(22)=3.35$, $P<0.025$; neutral: $t(22)=3.00$, $P<0.025$). Fourth, in the left inferior occipital cortex, we observed significantly larger responses to neutral and sad facial pictures in the patients with HB than in healthy controls (neutral: $t(22)=4.80$, $P<0.005$; sad: $t(22)=2.71$, $P<0.05$). In the right inferior occipital cortex, the pattern observed in the left area was replicated (neutral: $t(22)=3.09$, $P<0.025$; sad: $t(22)=2.38$, $P<0.05$). Finally, the main effect in the left fusiform gyrus originated from a higher level of blood oxygenation level-dependent (BOLD) response in patients with HB than in healthy controls under all affective conditions (neutral: $t(22)=4.45$, $P<0.005$; angry: $t(22)=3.01$, $P<0.025$; sad: $t(22)=4.36$, $P<0.005$). Activation in the right fusiform gyrus also replicated the pattern in the left (neutral: $t(22)=2.74$, $P<0.05$; angry: $t(22)=4.27$, $P<0.005$; sad: $t(22)=3.34$, $P<0.025$) (Figure 2).

Although group effects were evident for all ROIs, no main effects for the affective conditions were found.

## Discussion

In the current study, we found increased activation in response to angry facial stimuli in the bilateral lingual gyri and fusiform gyri of patients with HB compared to healthy controls (Figure 2). Higher activation in HB in response to sad facial stimuli was found in the bilateral lingual gyri, inferior occipital cortices, and fusiform gyri compared to healthy individuals. We also observed higher activation in response to neutral facial pictures in the bilateral lingual gyri, inferior occipital cortices, and fusiform

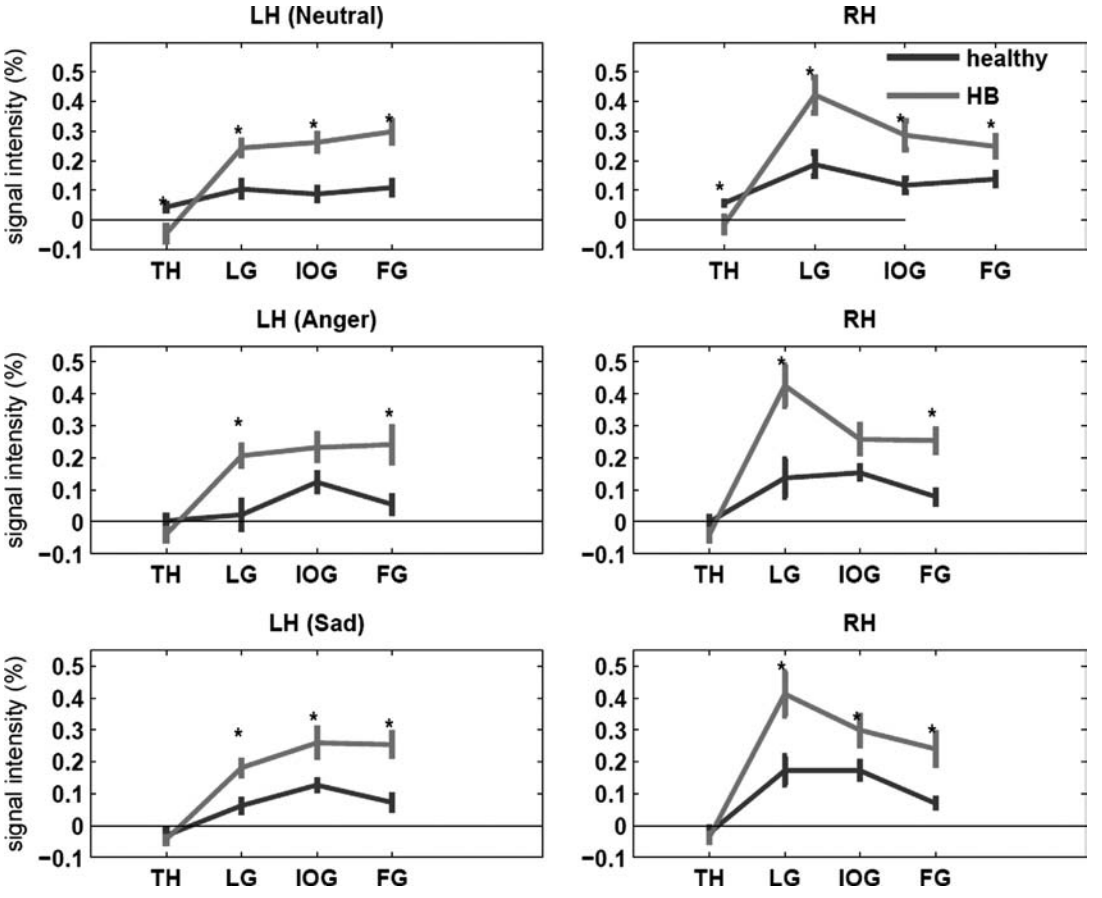


Figure 2. The BOLD signal intensity on the visual pathway in the HB patients and healthy controls. The upper figures show the signal intensity at each ROI on the visual pathway in the HB patients (red) and healthy controls (blue) in the neutral condition. The middle figures demonstrate the same contrast in the anger condition. The lower figures reveal that the signal intensity from the lingual gyri is higher in HB patients than in healthy controls. In each row, the left figures represent the results in the left hemisphere and right figures in the right hemisphere.The labels on x axis represent the structure of visual pathway, thalamus, lingual gyrus, inferior occipital gyrus, and fusiform gyrus, respectively. The symbols +, *, ** indicate that the difference in the ROI is significant at *P* values of 0.1, 0.05, 0.01, respectively.

gyri in patients with HB than in healthy controls, whereas healthy controls showed higher activation in the bilateral thalami than patients with HB.

The most interesting finding was that patients with HB showed higher activation in response to all affective facial stimuli in the lingual gyri. This suggests that patients with HB may be over-sensitized to facial stimuli, which may be due to a problem at an earlier stage in face recognition. If this tendency is attributable only to a holistic perception from face recognition, group differences in activation regions should have been found in the fusiform gyrus, rather than in the primary visual cortex.

Similarly, activation patterns in the thalamus of healthy controls revealed higher activity and were different from those in the other regions on the visual pathway for face recognition. This fact could be explained by assuming that the feedback from the primary visual cortex and other areas influences the activity in the thalamus (Wang et al. 2006). As described above, the LGN in the thalamus regulates information flowing through the thalamus and also organizes information that flows into the thalamus. If the primary visual cortex and/or fusiform gyrus of the HB patients fail to submit the regulating signal into the LGN or thalamus, unorganized or unregulated signals from the thalamus will be submitted into the primary visual cortex. Furthermore, the current study did not reveal the activation pattern specific to each affective condition, which suggests that HB patients generalize over-sensitization to every affective expression. This explanation suggests that the individual differences on the perceptual level of face recognition between HB patients and healthy controls might result from a flaw in the feedback system from the visual primary cortex to thalamus.

We also found that patients with HB showed lower activity in response to the neutral condition in the right ACC than healthy controls. The ACC plays an important role in many cognitive and affective processes. ACC dysfunction has been implicated in

many psychiatric disorders, including depression (Frodl et al. 2007; Lee et al. 2007), posttraumatic stress disorder (Kim et al. 2007), schizophrenia (Sanders et al. 2002), and obsessive–compulsive disorder (Rauch et al. 2004). Thus, the ACC is thought to be a vulnerable part of an important and common pathway involved in the cognitive and emotional regulation of behavior (Yucel et al. 2003). Our finding is consistent with these implications.

However, we failed to find increased amygdala activation to negative emotional facial stimuli. Previous fMRI studies have reported increased activation in the amygdala in patients with depression (Whalen et al. 2002) and anxiety disorders (Rauch et al. 2003; Pfleiderer et al. 2007). Our results support our suggestion that patients with HB have abnormalities in an earlier stage of face recognition in the feedback system from the visual primary cortex to the thalamus. Further research is needed to test this hypothesis.

Our study has certain limitations that should be considered when interpreting the findings. Although we mentioned the activation in the thalamus, the LGN was not explored separately. The LGN is an important part of the thalamus in the visual system, but the actual size is too small to be observed by fMRI. In the future, more elaborate imaging techniques will specify the visual mechanism of face recognition in HB patients. Second, we did not cover every affective condition, but rather we included only neutral and negative affective conditions such as sadness and anger. We could not include positive affective conditions such as happiness because the negative picture presentation could affect the response to subsequently presented positive pictures, and positive pictures could also affect the response to neutral pictures (Ekman et al. 1980).

In conclusion, this study provides evidence for overactivities of the lingual gyrus, inferior occipital cortex, and fusiform gyrus in patients with HB in response to certain affective stimuli, and dysfunction in the ACC. These findings suggest that the suppression of affect leads to a dysregulated feedback system from the visual primary cortex to the thalamus, resulting in aberrant function of the brain regions of the visual pathway, and that this functional impairment in the ACC might contribute to the pathophysiology of HB.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**ORIGINAL INVESTIGATION**

# Depressive status does not alter renal oxidative and immunological parameters during early diabetic nephropathy in rats

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**Abstract**

Depression is frequently observed among patients with diabetes and depressive status has been associated to activation of inflammatory processes, suggesting a role of depression in the inflammatory events observed in diabetes. To test that proposal, it was studied the effect of depression induced by forced swimming test (FST) on the evolution of early diabetic nephropathy. Diabetes was induced by streptozotocin injection. Rats were submitted to FST for 15 days. Struggle time was determined during FST and motor activity previously to FST. Nitric oxide, malondialdehyde, reduced glutathione and catalase activity were measured in kidney homogenates by enzymatic and biochemical methods. Superoxide anion, monocyte/macrophage (ED-1 positive cells) and RAGE were determined by hystochemical and immunohistochemical methods. Diabetic rats had decreased struggle time and locomotor activity at day 1 of FST. Both control and diabetic rats had those parameters decreased at day 15. Renal oxidative stress, RAGE expression and ED-1 cells were observed increased in diabetic animals. Those parameters were not significantly altered by FST. The depressive status does not alter oxidative and immune parameters during the early renal changes of diabetic nephropathy.

**Key words:** *Diabetes, nephropathy, depression, macrophages, oxidative stress*

## Introduction

The forced swimming test (FST) represents a stress situation capable of inducing a state of depressive behavioral, reflected in the immobility induced in animals (Porsolt et al. 1977). Depression has been linked to diabetes. In this regard, depression is a common feature among patients with diabetes, and patients with depression have high risk of developing diabetes (Eaton et al. 1996; Anderson et al. 2001; Sahmoun et al. 2007). In addition, depression has been implicated in the modulation of immune system. A bidirectional relation between depression and natural immunity has been identified; depressive episodes are associated with immunodeficiency; conversely, inflammatory activity has been implicated in the pathophysiology of depression suggesting a dual effect on immune system. In this regard, decreased number and activity of NK lymphocyte have been observed in patients with depression. However, depression can be accompanied by an inflammatory state mediated by proinflammatory cytokines (IL-6, TNF and IL-1), increased expression of ICAM-1 and oxidative stress (Lespérance et al. 2004; Kendall-Tackett 2007; Volchegorskiĭ and Mester 2007; Adler et al. 2008).

Monocyte-macrophages, advanced glycation end products receptors (RAGE) and oxidative stress are central mediators of renal inflammation during diabetic nephropathy (Furuta et al. 1993; Sassy-Prigent et al. 2000; Kanauchi et al. 2002; Wendt et al. 2003; Chow et al. 2004; Galkina and Ley 2006; Ohtake et al. 2007; Pan et al. 2007). Since depression has been linked to diabetes and to inflammatory and oxidative events, the aim of the present study was to determine the effect of stress/depression induced by FST in the progression of experimental diabetic nephropathy.

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## Methods

### *Streptozotocin-induced diabetes*

Male Sprague–Dawley rats weighing 100–150 g (Instituto Venezolano de Investigaciones Cientificas, Venezuela) were used. Diabetes was induced by intravenous injection of streptozotocin (STZ) (55 mg/kg in 0.2 M sodium citrate, pH 4.0; Sigma-Aldrich, MO, USA). Control rats were injected with vehicle. Animals were tested for blood glucose levels 48 h later and several times to confirm the diabetic status using a glucometer (Lifescan, Milpitas, CA, USA). Rats having a blood glucose level of $>200$ mg/dl were considered to be diabetic. Diabetic rats were randomly divided into four groups: control, control subjected to FST, diabetic rats and diabetic rats subjected to FST. Groups were sacrificed at weeks 4 and 8 of diabetes (these periods included 15 days of FST). Total urine protein was measured using sulphosalicylic acid. Presence of glucose in urine was determined by urine test strips. Experimental procedures followed the ethical guidelines of the committee of bioethical and biosecurity of FONACIT (Caracas, Venezuela).

### *Forced swimming test*

FST was performed with modifications of the Porsolt's procedure (Porsolt et al. 1977). Briefly, rats were placed individually in cylindrical tanks containing water (25°C) at a depth of 30 cm. FST was performed daily for 15 days and struggling time was measured during a 30-min test. A rat was judged to be immobile when it remained floating in the water and made only small movements to keep its head above water. Struggle occurred when rats were diving, jumping, strongly moving all four limbs or scratching the walls. Measures of motor activity and weight gain were performed to determine if changes observed in the FST were associated which changes in those parameters. Animals were tested for motor activity in an optical digital animal activity monitor (Opto-Varimex-Minor, Columbus Instruments Co., OH, USA), which records motor activity values (pulses) automatically. Prior to FST, total horizontal activity and ambulatory and stereotypic movements were obtained. At day 16, kidneys from controls and depressive rats were removed after perfusion with 20 ml of saline solution under ether anaesthesia. Slices of kidneys were embedded in OCT compound (Tissue Tek, Miles Inc. IL, USA), frozen in dry ice and acetone and stored at $-70$°C or homogenized for biochemical or enzymatic studies.

### *Determination of renal oxidative metabolism*

Renal superoxide anion production was determined at cellular levels by a previously described cytochemical method (Briggs et al. 1986). In the glomerulus results were expressed as positive cells per glomerular cross section and in the tubulointerstitial area as positive cells per 0.025 $mm^2$. Nitric oxide (accumulation of nitrite and nitrate) in the renal homogenates was detected by the Griess reaction (Green et al. 1982) and expressed as μmol/mg of protein. Renal malondialdehyde (MDA) content was assessed by the thiobarbituric acid assay (Ohkawa et al. 1979), and expressed as nmol per mg of protein. Catalase activity was determined by a method described by Aebi (1982) and results were expressed as k per mg of proteins. Content of reduced glutathione (GSH) was determined as previously described (Beutler et al. 1963) and expressed as nmol per mg of renal protein. Total protein content was measured in the renal homogenates by the method of Lowry.

### *Immunohistochemical studies*

Renal monocyte/macrophage infiltration and RAGE expression were determined by indirect immunofluorescence using an anti-rat ED1 monoclonal antibody (Accurate Chemical & Scientific Corporation, NY, USA) and a rabbit anti-rat RAGE polyclonal antibody (Sigma–Aldrich. MO, USA), respectively. As negative controls a no relevant

Table I. Effect of FST on blood, urine and corporal control and diabetic parameters

| | Blood glucose (mg/dl) | Total urine protein (mg/24 h) | Urine glucose | Weight gain (g) | Kw/bw (g) |
|---|---|---|---|---|---|
| Control ($n=10$) | 127.5±21.8 | 2.13±1.23 | − | 287.9±39.2 | 3.5±0.46 |
| Control FST ($n=10$) | 121±18.5 | 2.91±1.53 | − | 264.6±33.3 | 3.67±0.56 |
| Diabetes 4w ($n=5$) | 355.2±36.11* | 3.53±2.04 | + | 41.31±27.6* | ND |
| Diabetes 4w FST ($n=5$) | 252.5±50.56* | 1.44±1.57 | + | 31.52±11.77* | ND |
| Diabetes 8w ($n=8$) | 306.4±84.66* | 2.54±1.21 | + | 179.4±82* | 5.45±2.14* |
| Diabetes 8w FST ($n=8$) | 330.5±79.52* | 2.16±1.52 | + | 114.6±49.7* | 6.11±1.62* |

4w, 4 weeks; 8 w, 8 weeks; FST, forced swimming test; Kw, kidney weight (g); bw, body weight (Kg); *$P<0.05$ vs. control and control FST. ND, not done.

monoclonal antibody or rabbit IgG were used. At least 20 glomeruli and 20 randomly selected tubulointerstitial fields were examined for each renal section and results are expressed as positive cells per glomerular cross-section (GCS) and per 0.025 $mm^2$ of tubulointerstitial area.

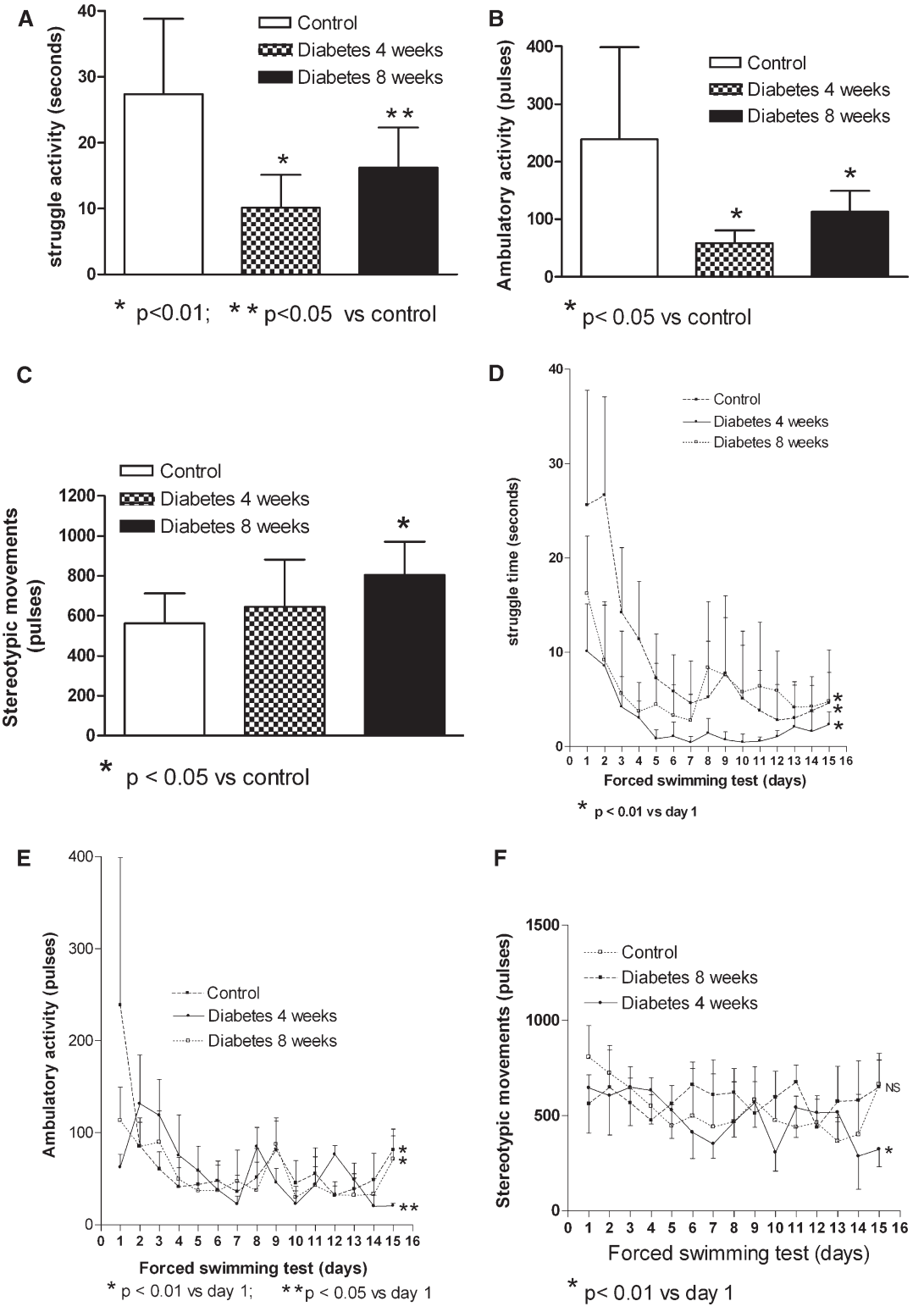


Figure 1. Basal depressive mood is observed in diabetic rats. Decreased struggle and ambulatory activities (A and B) were observed in diabetic rats at day 1. (C) Increased stereotypic activity in diabetic animals at week 8. After 15 days of FST control and diabetic animals had decreased struggle and ambulatory activities when compare to day 1 (D and E). Decreased stereotypic activity was observed in diabetic animals at week 4 (F).

*Statistical analysis*

Data were expressed as mean ± standard deviation. To evaluate variations in data, analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test were used. Significance was assumed to be at $P < 0.05$.

## Results

*Biochemical parameters*

The biochemical parameters indicated that the STZ injection produced diabetic animals with high levels of blood glucose and glucose excretion (Table I). There were no differences between values of total urine proteins in diabetic and control animals (Table I). Decreased weight gain and increased kidney weight were observed in diabetic animals. FST did not significantly alter those values in diabetic animals (Table I).

*Induction of depression*

Initial analysis (first day) using FST showed decreased struggle time and ambulatory activity in diabetic animals (Figures 1A and 1B). Stereotypic activity was elevated in diabetic animals at week 8 (Figure 1C). A significant reduction of those

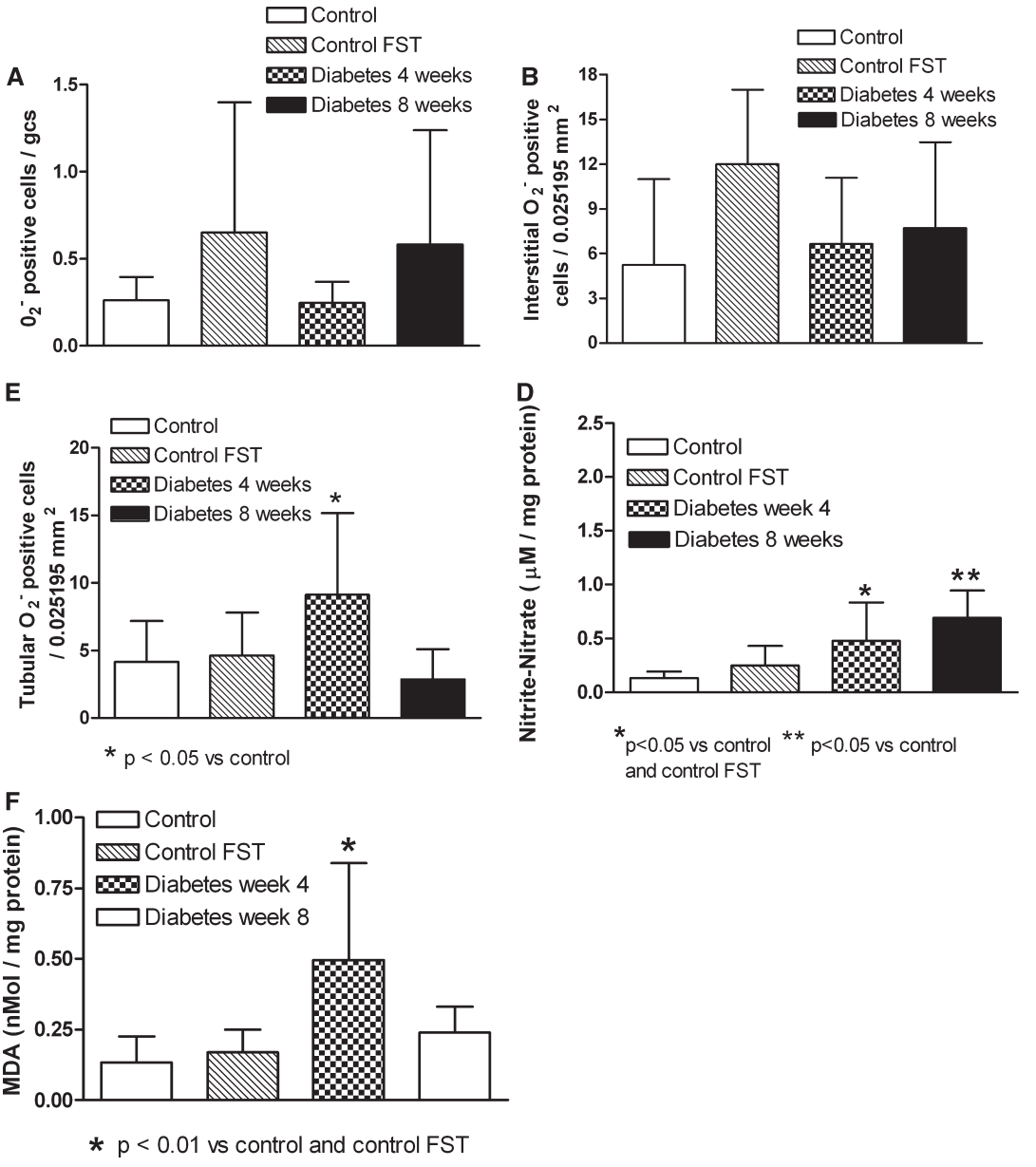


Figure 2. Oxidative status in control and diabetic animals. Significant increased renal tubular production of superoxide anion ($O_2^-$) was observed in diabetic animals at week 4 (C). Renal nitrite-nitrates (NO) production was observed increased in diabetic animals at weeks 4 and 8 (D). These alterations were accompanied by increase of thiobarbituric acid reactive substances in diabetic animals at week 4 (E).

parameters was observed in control and diabetic animals when day 1 and day 15 values were compared. No significant differences were observed between controls and diabetic animals at day 15. Diabetic animals (week 4) showed decreased stereotypic activity at day 15 (Figures 1D, 1E and 1F).

*Oxidative and immunological parameters*

Increased tubular superoxide anion (week 4) and nitric oxide productions (weeks 4 and 8) (Figures 2C and 2D) accompanied with increased lipid peroxidation (week 4) (Figure 2E) were observed in the kidney of STZ-treated animals. Catalase activity and reduced GSH content were elevated in kidney homogenates at 4 and 8 weeks of diabetic nephropathy (Figures 3A and 3B). Renal macrophages (Figures 3C and 3D) and expression of RAGE (Figures 3E and 3F) were increased in diabetic animals at week 8. FST did not induce significant differences in renal oxidative stress, leukocyte infiltration and RAGE expression; only increased tubular superoxide production was observed in FST-diabetic animals at week 8 (Table II).

## Discussion

Depression could increase the risk of developing diabetes and it is common in patients with this disease (Eaton et al. 1996; Freedland 2004;

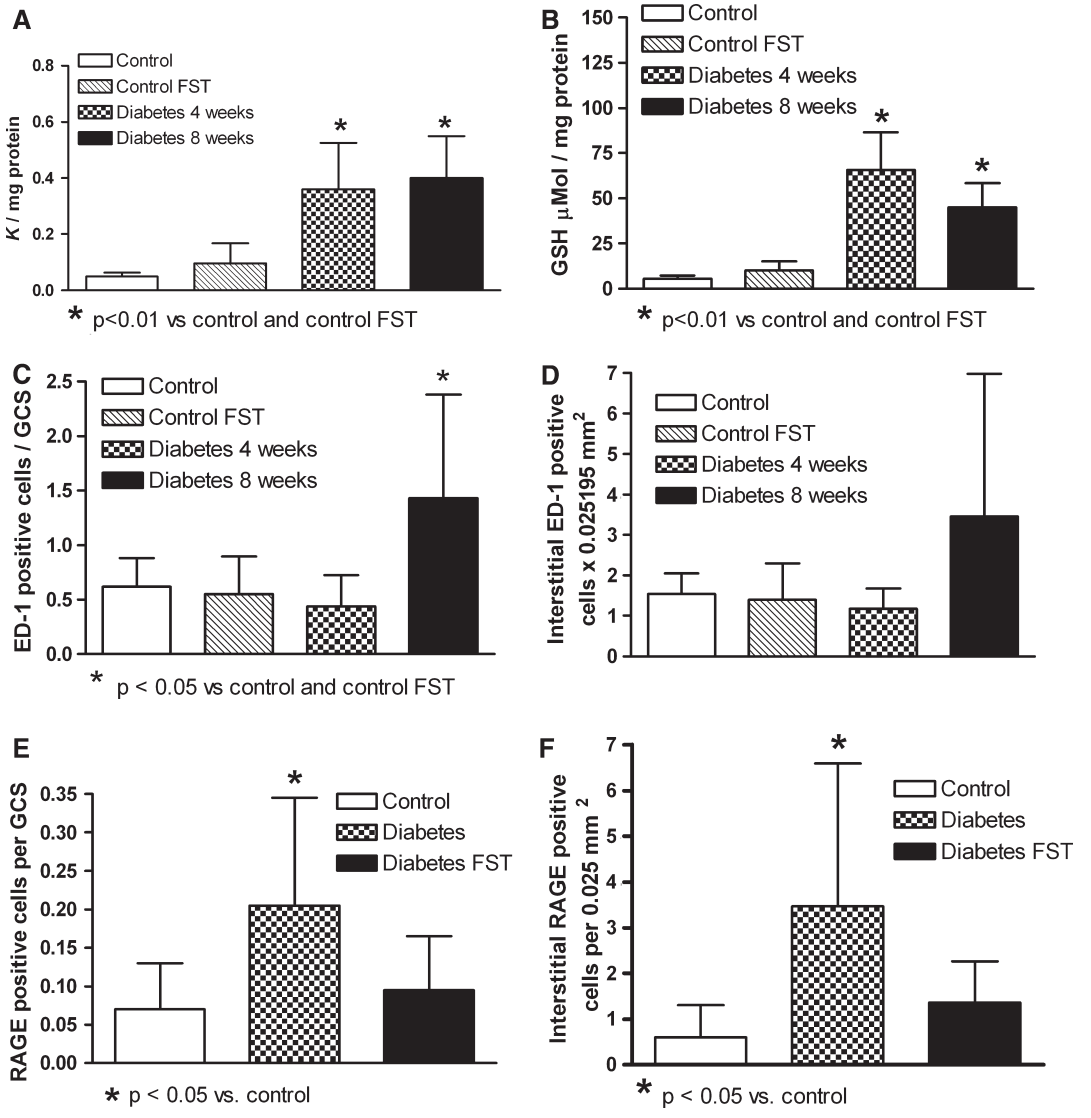


Figure 3. Renal anti-oxidant status, monocyte/macrophage infiltration and RAGE expression in diabetes. Catalase activity (A) and concentration of reduced glutathione (B) were found incremented in diabetic animals. Increased glomerular ED-1 positive cells were observed at week 8 in diabetic animals (C); however, there was no statistical significance in the interstitial areas (D). Glomerular (E) and interstitial (F) advanced glycation end products receptor (RAGE) expression were found increased in diabetic animals (week 8). FST did not alter significantly values of RAGE.

Table II. Immunological and oxidative renal parameters in rats with diabetes subjected or not to FST

| Parameters | Diabetes (4w) | Diabetes FST (4w) | Diabetes (8w) | Diabetes FST (8w) |
|---|---|---|---|---|
| Superoxide anion | | | | |
| Glomerulus | 0.25±0.12 | 0.42±0.37 | 0.58±0.66 | 0.77±0.54 |
| Interstitium | 6.66±4.42 | 9.92±5.61 | 7.72±5.74 | 8.45± 4.75 |
| Tubules | 9.13±6.05 | 5.16±4.57 | 2.87± 2.24 | 17.01±12.91* |
| Nitrite/nitrate | 0.48±0.35 | 0.25± 0.07 | 0.69±0.25 | 0.63±0.34 |
| Catalase | 0.36±0.17 | 0.28±0.11 | 0.40±0.15 | 0.30±0.10 |
| Reduced glutathione | 65.64±20.94 | 43.24±20.73 | 45.13±13.36 | 39.93±18.08 |
| Malondialdehyde | 0.50±0.34 | 0.32±0.10 | 0.24±0.09 | 0.56±0.82 |
| Macrophages | | | | |
| Glomerulus | 0.44±0.28 | 0.26±0.16 | 1.43±0.95 | 1.18±0.74 |
| Interstitium | 1.17±0.51 | 0.96±0.77 | 3.46±3.43 | 1.09±0.70 |

*$P < 0.01$ vs. diabetes 8w. Glomerulus (glomerular cross-section), intertitium and tubules (per 0.025 $mm^2$); nitrite–nitrate: μM/mg protein; catalase: K/mg protein; reduced glutathione: μM/mg protein; malondialdehyde: nMol/mg protein.

Sahmoun et al. 2007; Kendall-Tackett 2007), suggesting that depression is involved in diabetes. Our results showed that diabetic animals had basal depressive behaviour as shown by the decreased struggle time and ambulatory activity observed in these animals. This finding agrees with the clinical depression observed in diabetic humans. This depressive mood increased during the 15 days of FST in both diabetic and control rats, suggesting that chronic experimental depression were development in all FST groups. FST has been considered as one of the most used tools for screening antidepressants (Lucki 1997; Petit-Demouliere et al. 2005; de Paulis 2007; Fereidoni et al. 2007) and as one of the experimental depression inducer (Takao et al. 1995; Carrizo et al. 1997). Depressive parameters observed in FST need to be confirmed with some spontaneous locomotor activity studies (Petit-Demouliere et al. 2005). According with this, decreased locomotor activities were observed in diabetic rats.

Diabetic nephropathy is a common inflammatory consequence of diabetes, where monocytes/macrophages, expression of RAGE and oxidative stress are involved in the progression of this disease (Sugimoto et al. 1997; Kanauchi et al. 2003; Wendt et al. 2003; Ohtake et al. 2007; Pan et al. 2007). In our study, and consistent with previous reports, increased expression of glomerular and interstitial RAGE, infiltration of ED-1 positive cells and oxidative stress were observed in the kidney of diabetic animals, suggesting the presence of effectors capable of inducing renal damage. Depressive status could be implicated in some of the events during the course of diabetic nephropathy. In this regard, increased expression of ICAM-1 and oxidative stress have been reported in depressed patients (Petit-Demouliere et al. 2005; Volchegorskiĭ and Mester 2007). However, attempts to determine the effect of FST-induced depression in the cellular and oxidative parameters during the diabetic nephropathy were unsuccessful. No significant differences in renal function, hyperglycaemia, weight gain, monocyte/macrophage infiltration, RAGE expression and oxidant and antioxidant parameters between diabetic depressed and non depressed rats were observed. These data suggest that the inflammatory effect found during the depressive status (Petit-Demouliere et al. 2005; Kendall-Tackett 2007; Volchegorskiĭ and Mester 2007) was not capable of accelerating the diabetic nephropathy. This study was designed to obtain optimal experimental conditions to determine the modulator effect of depression on kidney. Thus, experiments were performed in two different periods of the diabetic nephropathy (weeks 4 and 8) and under a chronic swim stress condition that it seems to be effective to induce brain biological modifications (Takao et al. 1995). This experiment was performed during initial events of diabetic nephropathy in order to determine how the proinflammatory effects of depression could accelerate renal damage, however, we cannot rule out the possibility that inflammatory effect of depression could have a role in later periods of the diabetic nephropathy; therefore, further investigation is required to demonstrate that proposal.

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## Statement of Interest

None

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**BRIEF REPORT**

# Functional magnetic resonance imaging of tics and tic suppression in Gilles de la Tourette syndrome

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**Abstract**

Tics are defined as involuntary, quick, sudden, and stereotypical movements or phonic productions. Despite the fact that tic suppression plays an important role for the patient's ability to cope with tic disorders, investigations of the underlying neural correlates using functional imaging focused on tic generation rather than tic suppression. We examined a patient with Gilles de la Tourette syndrome with regard to neural mechanisms of tic generation and tic suppression using fMRI. Three different conditions were compared: "tics", "tics suppressed", and "tics imitated". The comparisons of "tics" to tics suppressed" and of "tics" to "tics imitated" showed similar activation in the anterior cingulate cortex. This leads to distinct suggestions concerning the neural network involved in tic suppression. Similar mechanisms may be involved in tic suppression via mental efforts or active movements.

**Key words:** *Anterior cingulate cortex, fMRI, Gilles de la Tourette syndrome, tic, tic suppression*

## Introduction

Tics are involuntary, quick, sudden, and stereotypical movements or phonic productions that often lead to severe impairment of motor behaviour and to social stigmatization of the affected person. Regarding the high burden of the patients, it is of major importance to reveal the underlying neurobiology of tics and to develop strategies for their control. Tics have been described as "isolated disinhibited fragments of normal motor or vocal behaviours" (Leckman et al. 1999). This accentuates the importance of inhibition or control for the occurrence of tics.

The feeling which arises before the occurrence of many tics is described as "pressure" or "urge" by patients. Premonitory urges that are reported to occur before the execution of a tic have been interpreted as the involuntary component (Kwak et al. 2003), with the performance of the tic itself as a voluntary response to this urge (Lang 1991). Tics can be suppressed to a certain degree, they worsen under conditions of distress, emotional involvement and when the patient feels unobserved (Kawohl et al. 2007). The suppression of tics is reported to be more or less stressful and resource consuming by many patients. It is, next to the social stigmatization by the tics itself, one of the most straining features of tic disorders. Many patients with tic disorders highlight their ability to control or suppress their tics when describing the impairment caused by tics in everyday live. However, research efforts have usually focused on tic generation instead of tic suppression: the neural mechanism of tic generation have been subject of various functional imaging studies (for a review see Adams et al. (2004)). There is, to our knowledge, only one functional magnetic resonance imaging (fMRI) study investigating tic suppression (Peterson et al. 1998). However, that study did not take into account the role of active movements in tic suppression. The absence of tics during voluntary motor tasks coinciding with supplementary motor activation has been reported for Gilles de la Tourette syndrome (Fattapposta et al. 2005). This leads to the assumption that tic suppression may be linked to the performance of other movements. Nevertheless, tics can be suppressed by mental efforts, as reported by many patients. The aim of our investigation was

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to uncover mechanisms underlying tic generation and tic suppression.

## Methods

### *Patient*

A 28-year-old, right handed male with Gilles de la Tourette syndrome according to DSM-IV (307.22) participated in the investigation after giving written informed consent. At the time of the fMRI acquisition, the patient had been unmedicated for 2 weeks, after having taken aripiprazole for 17 months. After suffering from severe periods of the disorder with pronounced and frequent motor and vocal tics in the past, the patient reported the preponderance of a simple motor tic of the thumbs (right $\gg$ left) at the time of the investigation. He showed good tic control and was able to easily suppress the tics by mere mental effort. However, tic frequency and interference worsened under distress and when alone. He scored 26 points on the Yale Global Tic Severity Scale (YGTSS) (Leckman et al. 1989).

### *Task*

While being scanned with fMRI, the patient exerted three conditions, each lasting 3 min in a block design. In the first condition, "tics", the patient gave free rein to the tics. In the second condition, "tics suppressed", he mentally suppressed the tics. In the third condition, "tics imitated", he imitated the tics by actively and voluntarily moving the right thumb in the way it would be done by the tic. Due to the frequent occurrence of tic at rest no real resting condition was possible. The time in between the different conditions was approximately 1 min.

### *FMRI acquisition and analysis*

Imaging was performed with a 3.0-Tesla General Electrics Signa HD whole body scanner (GE Health Care, Munich, Germany) equipped with a head coil. Initially, high-resolution three-dimensional T1* weighted anatomical volumes were acquired (TR/TE 9.4/2.1 ms; matrix size $256 \times 256$; $1 \times 1 \times 1$ mm resolution, axial orientation) for later coregistration with the fMRI. T2* weighted functional MR images were obtained using echoplanar imaging (EPI, TR/TE 1980/32 ms, flip angle 70°, 22 sequential axial slices covering the whole brain, slice thickness 5 mm, 0.5 mm gap, matrix $64 \times 64$ pixels, FOV 220 mm, resulting voxel size $3.4 \times 3.4 \times 5.5$ mm). Altogether 270 volumes were obtained, 90 volumes per condition. During the session, the patient watched a neutral blank screen via digital video goggles (Resonance Technology Inc., Northridge, USA).

FMRI data were analyzed using BrainVoyager QX 1.8 (Brain Innovation, Maastricht, The Netherlands). Preprocessing of the functional scans included motion correction (translation and rotation did not exceed 3 mm), slice scan time correction, high frequency temporal filtering, and removal of linear trends. The three different conditions were compared by building contrasts within the block design.

## Results

An increased signal in the "tic" condition compared to the condition "tics suppressed" was observed in the left anterior cingulate cortex (ACC) (Talairach coordinates $-11/24/26$, BA 24, 135 voxels of 1 $mm^3$ voxel size, $P < 0.001$, Bonferroni-corrected; Figure 1a). For the same area, a similarly increased signal in the "tic" condition compared to the condition "tics imitated" was observed (Talairach coordinates $-9/23/28$, BA 24, 357 voxels; $P < 0.001$, Bonferroni-corrected; Figure 1b). No statistically significant difference was observed between the signal changes during the conditions "tics suppressed" and "tics imitated".

## Discussion

The "tics" condition was associated with an activation in the left middle anterior cingulate cortex (ACC) when compared to both other conditions, "tics suppressed" (by mental effort) and "tics imitated" (by voluntary action). This area matches the rostral cingulate motor area (CMA, BA24c (Vogt and Vogt 2003)). In the first gaze this similarity between tic suppression and imitation appears to be counterintuitive, but it may be explained within the frame of feedback models. Earlier, electrical stimulation in the cingulate motor area (CMA) was reported to produce involuntary movements and complex gestures (Vogt and Vogt 2003), which could be modified or resisted. Moreover, it is part of a network of paralimbic areas which are activated before tic onset (Bohlhalter et al. 2006), which is in accordance with our results. The mediating role of the CMA between emotional and motivational influences on the one hand, and motor control on the other hand may correspond to the differential influences on tic severity by various emotional states (Wood et al. 2003). Within the frame of our conservative statistical threshold, the ACC was the one area more active during the involuntary happening of tics compared to the suppressed state. We assume that there may be either a dysfunction of the CMA causing continuous pathological activity propagating the tics, or an afferent dysfunctional input

to the CMA. The latter might arise mainly from the basal ganglia or the thalamus (Leckman et al. 2006) and cause a continuous pathological discrepancy between the actual and the desired state. The ACC detects this discrepancy and accordingly initiates the correcting movement in form of a tic. This view would fit with the role of the ACC, especially the caudal or midcingulate area, as an error or conflict detector (Carter et al. 1998; van Veen and Carter 2002).

In our design we cannot determine whether a pathological intrinsic activity is generated in the CMA itself or if it would activate the CMA via afferences from the striatum or the thalamus, because the afferent areas would be active continuously and would not present themselves in any behavioural comparison. An influence by "higher" cortical areas during suppression of tics is also conceivable: The ACC has a strong bidirectional connection with the prefrontal cortex, especially the dorsolateral prefrontal cortex (DLPFC) (Bates and Goldman-Rakic 1993; Devinsky et al. 1995; Paus 2001), which is involved in important processes of cognitive and executive control (Brass et al. 2005; Egner and Hirsch 2005). The motor action of tics is assumed to be caused by a propagation of the ACC

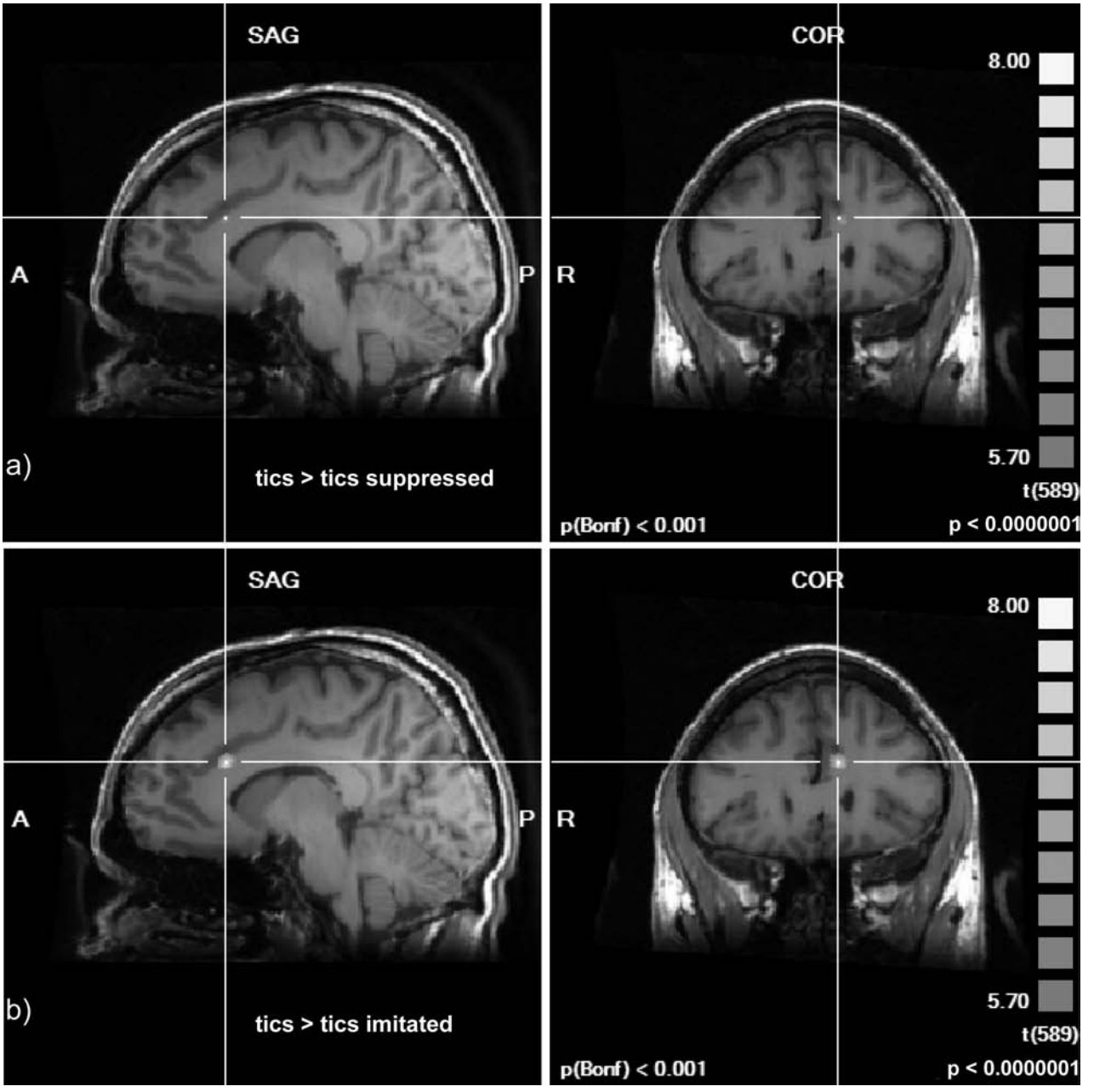


Figure 1. (a) Comparison of the conditions "tics" and "tics suppressed", (b) comparison of the conditions "tics" and "tics imitated".

information to the DLPFC and subsequently to premotor (PMC) and supplementary motor area (SMA), and finally to the motor cortex (MC). Our findings would further fit with the assumption of a voluntarily induced suppression of the dysfunctional ACC signal by top-down control from the DLPFC (MacDonald et al. 2000; Paus et al. 2001). Accordingly, in the suppression condition compared with the native tics condition the activity of the ACC would be reduced. During "tics imitated" the subject made the same movements he normally did involuntarily now by actively and voluntarily moving the thumb. We assume in this condition an executive signal by the DLPFC towards the PMC, SMA and MC to initiate a moving of the thumb. Now, the SMA, which is cytoarchitectonally similar to the CMA (BA 24c–BA 6a (Vogt and Vogt 2003)), may be the source of a so called efferential copy of this information to the ACC. This leads to a reduction of the discrepancy between actual and target state. Alternatively, the efferential copy could also arise from the MC (Morecraft and Van Hoesen 1992). Thus, the decreased activity of the ACC of both conditions, "tics suppressed" and "tics imitated" compared to the resting condition "tics" would explain the activation shown in Figure 1.

This is also in accordance to clinical descriptions of patients about tic control by voluntary movements and is used in habit reversal training, a behaviour therapy using voluntary movements to control tics. This leads to the conclusion that similar mechanisms are likely to be involved in tic suppression via mental efforts or active movements.

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## Statement of interest

The authors declare that they have no conflict of interest concerning commercial or financial involvements.

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**BRIEF REPORT**

# Parental psychiatric hospitalisation and offspring schizophrenia

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**Abstract**

The risk of schizophrenia has been linked with a family history of schizophrenia and less strongly with other psychiatric disorders in family members. Using data from the Copenhagen Perinatal Cohort and from the Danish Psychiatric Case Register, we studied the relationship between offspring risk of schizophrenia and a range of psychotic and non-psychotic psychiatric diagnoses in parents. Psychiatric admission data after 1969 were available for 7047 cohort members born between 1959 and 1961, and for 7006 mothers and 6993 fathers. Univariate analysis showed that neurosis, alcohol and substance dependence in both parents were associated with elevated risk of offspring schizophrenia; in addition, maternal schizophrenia, affective disorder and personality disorder were associated with elevated risk. Controlling for parental age, parental social status, and parental psychiatric co-diagnosis, offspring risk of schizophrenia was associated with maternal schizophrenia (OR = 15.41 with 95% CI 5.96–39.81) and, independently, with paternal hospitalisation with neurosis (OR = 5.90 with 95% CI 2.23–15.62). The risk of schizophrenia associated with paternal neurosis remained significant after excluding offspring of parents with non-affective psychosis from the sample. These findings suggest that genetic and family studies should not only focus on parental history of schizophrenia since the simple distinction between positive and negative family history could not accurately describe offspring risk in this sample.

**Key words:** *Schizophrenia, parental psychiatric history, offspring risk of schizophrenia, neurosis and schizophrenia, Copenhagen Perinatal Cohort*

## Introduction

It is well established that schizophrenia aggregates in families (Gottesman 1991). Relatives of patients with schizophrenia not only have higher rates of disorders related to schizophrenia such as schizoaffective disorder, schizotypal personality disorder, and paranoid personality disorder (Kendler and Diehl 1993; Kendler et al. 1995; Ingraham and Kety 2000; Kringlen 2000; Tsuang et al. 2001). Other psychiatric disorders may also aggregate in relatives of schizophrenic patients. A large register-based study found the risk of schizophrenia to be associated with a range of psychiatric diagnoses in family members (Byrne et al. 2002).

It is important to further explore and possibly replicate these findings. Prospective studies linking parental psychiatric hospitalisation history with the offspring risk of hospitalisation with schizophrenia may illuminate whether psychiatric disorders outside the schizophrenia spectrum aggregate in the families of schizophrenic patients. We undertook the present register-based study linking information from the Copenhagen Perinatal Cohort (Zachau-Christiansen and Ross 1975) with the Danish Psychiatric Register (Munk-Jørgensen and Mortensen 1997). Previous analyses of this cohort have shown that maternal hospitalisation with schizophrenia predicts offspring risk of schizophrenia, while paternal hospitalisation with schizophrenia is a weaker predictor (Sørensen et al. 2004). We hypothesised that some parental

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psychiatric disorders outside the schizophrenia spectrum might be associated with offspring risk of schizophrenia.

The aim of the study was to investigate the potential associations between a range of parental psychiatric hospital diagnoses and offspring risk of schizophrenia.

## Methods

The Copenhagen Perinatal Cohort consists of 9125 individuals delivered by 8949 pregnant women from October 1959 to December 1961 at the maternity department of the Copenhagen University Hospital, Rigshospitalet. At the establishment of this cohort, demographic, socioeconomic, prenatal, and perinatal medical data were recorded prospectively (Villumsen 1970; Reinisch et al. 1993). Previous studies based on this cohort demonstrated associations between parental social status and offspring risk of schizophrenia (Sørensen et al. 2003).

Written approval to conduct a register-based psychiatric follow-up was obtained from the regional scientific and ethics committee. The Danish Psychiatric Register has been computerised since 1 April 1969. It contains data on all admissions to Danish psychiatric inpatient facilities. The diagnostic system in use when The Danish Psychiatric Register was computerised was the *International Classification of Diseases, 8th Revision* (ICD-8).

A total of 8400 infants survived the first month after birth. A total of 7047 cohort members, 7006 of their mothers, and 6993 of their fathers were registered Danish citizens by 1 April 1969. The cohort members and their parents were followed in The Danish Psychiatric Central Register to identify all admissions with a diagnosis of schizophrenia (ICD-8 code 295) among the cohort members and all admissions with a diagnosis of schizophrenia (ICD-8 code 295), affective psychosis (ICD-8 code 296), paranoid psychosis (ICD-8 code 297), reactive psychosis (ICD-8 code 298), neurosis (ICD-8 code 300), personality disorder (ICD-8 code 301), alcohol abuse/dependence (ICD-8 code 303), and other substance abuse/dependence (ICD-8 code 304) in either of the parents. The cohort members and their parents were followed in the register until 1994 when ICD-10 diagnoses were implemented in Denmark.

### *Statistical analysis*

The cumulative incidences of the above mentioned ICD-8 diagnostic categories between 1 April 1969 and 1994 were calculated for both parents. Univariate analysis compared the proportion of offspring who developed schizophrenia in relation to maternal and paternal ICD-8 diagnostic categories, and unadjusted odds ratios (with 95% CI) were estimated in a logistic regression model. Multivariate logistic regression analysis estimated the odds ratio of offspring developing schizophrenia in relation to each maternal and paternal ICD-8 category with adjustment for parental social status, maternal and paternal age (the covariates were entered as continuous variables). The second adjustment (full model) adjusted for these covariates and for all maternal and paternal ICD-8 diagnostic categories. The analysis was performed using SPSS version 12.0 for windows.

The outputs of the full model represent the independent contribution of each parental ICD-8 diagnostic category to the prediction of offspring risk of schizophrenia without assuming a diagnostic hierarchy. Inherently, the model takes into account concomitant psychiatric diagnoses (some mothers and fathers attracted more than one ICD-8 diagnosis through the lifespan).

### *Ethics*

The study is purely register-based study and consequently, it is not necessary to obtain informed consent according to Danish rules and the Helsinki Declaration.

## Results

Table I shows the cumulative incidence of hospitalisation with various ICD-8 diagnoses in mothers and fathers of the cohort members from 1 April 1969 to the end of 1993. The low cumulative incidence of paternal schizophrenia may be related to low reproduction in male schizophrenia and attrition before 1969.

Table II shows the offspring risk of schizophrenia in relation to maternal and paternal history of psychiatric hospitalisation with a range of ICD-8

Table I. Cumulative incidence of hospitalisation with psychiatric disorders in 7006 mothers and 6993 fathers in the Copenhagen Perinatal Cohort.

| ICD-8 category | Mothers (*n*/sample *N*) | Fathers (*n*/sample *N*) |
|---|---|---|
| Schizophrenia | 0.8% (55/7006) | 0.4% (30/6993) |
| Paranoid psychosis | 0.8% (56/7006) | 0.3% (20/6993) |
| Reactive psychosis | 2.7% (190/7006) | 1.4% (100/6993) |
| Affective disorder | 2.1% (147/7006) | 1.1% (80/6993) |
| Neurotic disorder | 4.9% (346/7006) | 1.5% (108/6993) |
| Personality disorder | 6.1% (432/7006) | 4.5% (316/6993) |
| Alcohol dependence | 3.5% (246/7006) | 6.4% (448/6993) |
| Other substance dependence | 2.6% (186/7006) | 1.7% (120/6993) |

Table II. Schizophrenia in offspring with and without parental history of psychiatric hospitalisation with a range of ICD-8 diagnoses.[a]

| Parental psychiatric hospitalisation ICD-8 diagnostic category | % in offspring with parental diagnosis who developed schizophrenia | % in offspring without parental diaganosis who developed schizophrenia | Odds ratio (95% CI) |
|---|---|---|---|
| Maternal schizophrenia ($n=55$) | 12.7 (7/55) | 0.9 (64/6951) | 15.69 (6.84–36.00)* |
| Maternal paranoid psychosis ($n=56$) | 0 (0/56) | 0.8 (71/6950) | 0 |
| Maternal reactive psychosis ($n=190$) | 1.6 (3/190) | 1.0 (68/6816) | 1.59 (0.50–5.10) |
| Maternal affective disorder ($n=147$) | 3.4 (5/147) | 1.0 (66/6859) | 3.62 (1.44–9.13)* |
| Maternal neurotic disorder ($n=346$) | 2.9 (10/346) | 0.9 (61/6660) | 3.22 (1.64–6.34)* |
| Maternal personality disorder ($n=432$ | 3.2 (14/432) | 0.9 (57/6574) | 3.83(2.12–6.93)* |
| Maternal alcohol dependence ($n=246$) | 2.9 (7/246) | 1.0 (64/6760) | 3.06 (1.39–6.76)* |
| Maternal substance dependence ($n=186$) | 3.2 (6/186) | 1.0 (65/6820) | 3.46 (1.48–8.10)* |
| Paternal schizophrenia ($n=30$) | 3.3 (1/30) | 1.0 (70/6963) | 3.40 (0.46–25.28) |
| Paternal paranoid psychosis ($n=20$) | 5.0 (1/20) | 1.0 (70/6973) | 5.19 (0.69–39.31) |
| Paternal reactive psychosis ($n=100$) | 3.0 (3/100) | 1.0 (68/6893) | 3.10 (0.96–10.04) |
| Paternal affective disorder ($n=80$) | 2.5 (2/80) | 1.0 (69/6913) | 2.54 (0.61–10.56) |
| Paternal neurotic disorder ($n=108$) | 6.5 (7/108) | 0.9 (64/6885) | 7.39 (3.20–16.52)* |
| Paternal personality disorder ($n=316$) | 1.6 (5/316) | 1.0 (66/6677) | 1.61 (0.64–4.03) |
| Paternal alcohol dependence ($n=448$) | 2.0 (9/448) | 1.0 (62/6545) | 2.14 (1.64–4.34)* |
| Paternal substance dependence ($n=120$) | 3.3 (4/120) | 1.0 (67/6873) | 3.50 (1.26–9.77* |

[a]Based on 7006 mothers and 6993 fathers.

diagnostic categories. A total of 71 cases with ICD-8 schizophrenia were identified (cumulative incidence of 1.0%). Significant predictors of offspring schizophrenia included the following maternal diagnostic categories: schizophrenia, affective disorder, neurotic disorder, personality disorder, alcohol dependence, other substance dependence; and the following paternal diagnostic categories: neurotic disorder, alcohol dependence and other substance dependence.

Table III shows the results of multivariate logistic regression analysis estimating offspring risk of schizophrenia in relation to the included maternal and paternal ICD-8 diagnostic categories with adjustment for parental social status, maternal and paternal age. The effect of adjusting not only for the covariates but, in addition, for the entire range of parental ICD-8 diagnostic categories, is shown in the second column. In this model, the only significant predictors of offspring risk of schizophrenia was maternal schizophrenia with an adjusted odds ratio of 15.41 with 95% CI 5.96–39.81 and paternal neurotic disorder with an adjusted odds ratio of 5.90 with 95% CI 2.23–15.62.

We examined the distribution of co-diagnoses among the fathers with a hospital diagnosis of

Table III. Risk of offspring schizophrenia in relation to parental history of psychiatric hospitalisation.[a]

| | Odds ratio (95% CI) | |
|---|---|---|
| | First model | Second model (Full model)[a] |
| Parental psychiatric hospitalisation ICD-8 diagnostic category | | |
| Maternal schizophrenia | 15.38 (6.68–35.42)* | 15.41 (5.96–39.81)* |
| Maternal reactive psychosis | 1.59 (0.49–5.09) | 0.36 (0.09–1.36) |
| Maternal affective disorder | 3.68 (1.46–9.29)* | 2.03 (0.74–5.60) |
| Maternal neurotic disorder | 3.17 (1.61–6.25)* | 1.69 (0.74–3.85) |
| Maternal personality disorder | 3.83 (2.10–6.98)* | 2.17 (0.97–4.86) |
| Maternal alcohol dependence | 3.05 (1.38–6.74)* | 1.28 (0.48–3.37) |
| Maternal substance dependence | 3.37 (1.44–7.90)* | 1.22 (0.42–3.60) |
| Paternal schizophrenia | 3.10 (0.41–23.24) | 1.41 (0.13–15.56) |
| Paternal paranoid psychosis | 4.91 (0.65–37.42) | 2.81 (0.26–30.39) |
| Paternal reactive psychosis | 3.14 (0.97–10.19) | 1.90 (0.49–7.41) |
| Paternal affective disorder | 2.60 (0.63–10.81) | 0.91 (0.18–4.68) |
| Paternal neurotic disorder | 7.25 (3.24–16.24)* | 5.90 (2.23–15.62)* |
| Paternal personality disorder | 1.55 (0.62–3.92) | 0.43 (0.13–1.42) |
| Paternal alcohol dependence | 2.08 (1.02–4.26)* | 1.36 (0.53–3.47) |
| Paternal substance dependence | 3.46 (1.23–9.72)* | 2.12 (0.56–8.02) |

[a]Risk of schizophrenia associated with parental history of psychiatric hospitalisation after adjustment for maternal and paternal age and household social status. The second model adjusted for these covariates and all parental ICD-8 diagnostic categories. This analysis included 6993 subjects.

neurotic disorder. Out of the 108 fathers, 50 had an ICD-8 co-diagnosis of personality disorder, 44 had a co-diagnosis of alcoholism, 19 a co-diagnosis of reactive psychosis, 18 a co-diagnosis of affective disorder, three were registered with a co-diagnosis of paranoid psychosis, and one with an ICD-8 co-diagnosis of schizophrenia. In 1994–1999, only seven of the 108 fathers were hospitalized and received ICD-10 diagnoses (none in the schizophrenia spectrum).

*Post-hoc* analyses were also conducted on a restricted sample comprising 6585 cohort members (3318 men and 3267 women) with psychiatric hospitalisation data on both parents and excluding offspring of parents with a lifetime diagnosis of ICD-10 code F20-29 or ICD-8 code 295, 297, or 298. In this restricted sample 56 cases (0.9%) had been registered with schizophrenia according to ICD-8. Adjusted for parental social status, maternal and paternal age, paternal hospitalisation with neurosis remained a significant predictor of offspring risk of schizophrenia (OR = 8.30 with 95% CI 3.21–21.42). Further analyses indicated that offspring risk of schizophrenia was more strongly associated with certain sub-categories of paternal neurosis than others. As outlined in Appendix 1, there are nine sub-categories of neurosis according to ICD-8. Hysterical neurosis (code 300.19) and asthenic neurosis (code 300.59) were significant predictors (with clearly elevated odds ratios), while anxiety neurosis (code 300.09), phobic neurosis (code 300.29) and obsessive–compulsive neurosis (code 300.39) were not.

## Discussion

The risk of schizophrenia was significantly increased in offspring whose mothers had been hospitalised with schizophrenia while paternal hospitalisation with schizophrenia was not significantly associated with offspring risk of the disorder. However, the risk of schizophrenia was increased more than 6-fold in offspring whose fathers had been hospitalised with a diagnosis of neurosis. This unexpected finding may reflect genetic associations between schizophrenia and other mental disorders leading to psychiatric admission.

It is possible that paternal neurosis in some cases was associated with schizophrenia in second-degree relatives and the wider family circle, but this potential explanation of the elevated risk also assumes a link between neurosis and schizophrenia. A diagnostic hierarchy could have been used to handle co-diagnoses in the parents, but a diagnostic hierarchy was not considered appropriate when detailed clinical information was unavailable. In addition, the analyses adjusting for covariates, but not for co-diagnoses, correspond to an analysis with each diagnostic category at the top of a diagnostic hierarchy (Table III, first column). Furthermore, the results of the analysis based on the restricted sample without offspring of parents with non-affective psychosis strongly suggest that psychotic co-diagnoses do not explain or mediate the association between paternal neurosis and offspring risk of schizophrenia. Finally, the clinical hospital diagnoses in the Danish Psychiatric Central Register may be unreliable, but it is likely that misclassification of the 'exposure' (neurosis) would have introduced noise and biased the results towards null findings.

The relationship between ICD-8 and the contemporary ICD-10 diagnostic classification system is complex and cannot be discussed in detail in this brief report. However, the important question in the context of the present findings is the possible relationship between ICD-8 diagnosis of neurosis and ICD-10 diagnosis of schizophrenia. Few studies have used ICD-10 to evaluate the long-term outcome of the ICD-8 diagnosis of neurosis, but we did not observe a single case of ICD-10 schizophrenia among the fathers having previously received an ICD-8 diagnosis of neurosis. This finding should obviously be interpreted with caution because of the relatively small sample size and because the median age of the fathers with neurotic disorder was 60 years in 1994 when the ICD-10 classification was implemented in Denmark.

In spite of the above mentioned limitations concerning family history information and the fact that detailed clinical information was not available, we believe that our results support the register-based study by Byrne et al. (2002) demonstrating that a simple distinction between schizophrenia positive and schizophrenia negative family history could not accurately describe the offspring risk of schizophrenia. Some independent predictive effect of parental non-psychotic diagnosis appears to exist, and personality traits associated with paternal diagnosis of neurosis may play a role in pathways leading to increased offspring risk of schizophrenia. The broad personality dimension of neuroticism reflects emotional instability (McCrae and Costa 1997) and appears to be partly genetically determined (Bouchard 1994). It includes trait anxiety and depression, and high neuroticism appears to be a broad vulnerability factor for mental disorders, particularly internalising disorders (Khan et al. 2005).

*Post-hoc* analyses of the Perinatal Cohort suggested that offspring schizophrenia was associated with parental hysterical, asthenic and hypochondric neurosis. It is possible that these conditions are more

deviant in males than anxiety neurosis and related conditions, and that the fathers diagnosed with neurosis severe enough to be admitted to a psychiatric department manifested a range of vulnerabilities including emotional instability, chronic distress, poor coping skills and poor social competence. These characteristics may adversely influence the climate of upbringing and interact with genetic predisposition to increase the risk of schizophrenia.

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**Statement of interest**

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**Appendix 1**

300.09 neurosis anxiosa; 300.19 neurosis hysterica; 300.29 neurosis phobica; 300.39 neurosis obsessiva compulsiva; 300.49 neurosis depressiva; 300.59 neurosis asthenica; 300.69 neurosis cum syndroma depersonalisationis; 300.79 neurosis hypochondica; 300.89 neurosis alia and 300.99 neurosis. The ICD-8 classification of personality disorder consisted of 10 sub-categories: 301.09 disordo personalitatis, typus paranoides; 301.19 disordo personalitatis typus affectivus; 301.29 disorder personalitatis, typus schizoides; 301.39 disordo personalitatis typus explosivus; 301.49 neurosis characterogenes typus anankasticus; 301.59 neuroses characterogenes typus hystericus; 301.69 neurosis characterogenes typus asthenicus; 301.79 disordo personalitatis typus antisocialis; 301.8 neurosis characterogenes alia/insufficientia characteris/ casus limitares pseudoneuroticae sive pseudopsychopathicae.

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**BRIEF REPORT**

# Winter/summer seasonal changes in malondialdehyde formation as a source of variance in oxidative stress schizophrenia research

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**Abstract**

*Background.* Malondialdehyde (MDA), an oxidative stress biological marker, is one of the most frequently used markers of lipid peroxidation in schizophrenia research. Data regarding MDA levels in schizophrenia are controversial. Our aim is to study the existence of winter/summer seasonal changes in serum MDA levels in schizophrenic patients. *Methods.* Twenty-three clinically stable treated chronic paranoid schizophrenic outpatients were studied in summer and winter. Blood was sampled between 08:30 and 09:00 h. Serum MDA was determined by the thiobarbituric acid reactive substances technique. The clinical state was assessed by means of the Clinical Global Impression (CGI) scale. *Results.* Mean serum MDA levels were significantly higher in summer than winter ($2.49 \pm 0.25$ vs. $1.86 \pm 0.11$ nmol/ml, $P < 0.03$). Summer MDA was increased by a 33.9% compared to winter MDA. Age, gender, smoking status, body mass index, psychopharmacological treatment, illness duration, age of illness onset and CGI did not affect significantly MDA levels. *Conclusion.* Our results show that serum MDA presents a winter/summer rhythm of formation, with higher levels in summer than winter. It is strongly advisable to take into account the summer/winter variation in MDA levels when researching into this field.

**Key words:** *Lipid peroxidation, oxidative stress, schizophrenia, seasonality*

## Introduction

There is accumulative evidence that free radicals play a role in schizophrenia, probably mediated by membrane pathology (Reddy and Yao 1996; Schmidt et al. 2005). Lipid peroxidation is one of the consequences of oxidative stress and the level of malondialdehyde (MDA) is an usual marker of lipid peroxidation (Gawel et al. 2004).

Data regarding MDA levels in schizophrenic patients are controversial. In a recent meta-analysis on MDA levels in schizophrenia (Grignon and Chianetta 2007), an effect size meta-analysis has been reported, confirming the existence of an increase in MDA levels in patients with schizophrenia, but a very large heterogeneity of the results was also reported. Increased levels (McCreadie et al. 1995; Mahadik et al. 1998; Akyol et al. 2002; Khan et al. 2002; Kuloglu et al. 2002; Arvindakshan et al. 2003a, Dakhale et al. 2004; Gama et al. 2006; Zhang et al. 2006), no alterations (Bindoli et al. 1987; Brown et al. 1998; SSRG 2000; Ranjekar et al. 2003) or even a decrease in MDA levels (Arvindakshan et al. 2003b) have been reported.

Seasonal variations in several biological markers have been described (Wirz-Justice and Richter 1979). This seasonal variation may have acted as a source of variance that may help explaining part of the heterogeneity in the results of MDA levels in schizophrenic patients.

The objective of this research is to study the existence of winter/summer seasonal changes in serum MDA levels in a sample of clinically stable treated paranoid schizophrenic outpatients.

## Methods

The study was carried out in two phases. The first phase took place in July 2005 while the second was carried out in January 2006. Throughout this paper the terms January/Winter and July/Summer will be used interchangeably. Patients were selected from an outpatient health setting in La Vera Mental Health

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Center in Puerto de la Cruz (Tenerife, Spain). Exclusion criteria were: (1) concomitant physical illness, (2) consumption of alcohol or illegal drugs, (3) pregnancy, (4) mental retardation, (5) being on a special diet (vegetarian, to lose weight, etc.), and (6) consumption of vitamin supplements. Inclusion criteria were: (1) DSM-IV criteria for paranoid schizophrenia, (2) being on a stable psychopharmacological treatment at least 1 year before the start of the study, and (3) the treatment must be kept stable during the follow-up. Patients were asked to keep to their usual eating habits during the study. Diagnostic interviews were carried out by the same psychiatrist, according to the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al. 1998). The study was carried out in accordance with the Helsinki Declaration and all subjects gave written informed consent before inclusion in the study.

The initial sample (July 2005) was comprised by 30 subjects. Seven subjects dropped out from the study: two due to abnormal transaminase levels, one had a urinary infection, two refused to participate in the follow-up and two did not come to the laboratory on the day of the blood testing. The final sample was comprised by 23 clinically stable chronic schizophrenic outpatients.

Blood samples were extracted and introduced into BD vacutainer SST II advance tubes, without anticoagulant, between 08:30 and 09:00 h, after fasting for one night. The same routine was followed during the two experimental sessions. After each extraction, blood samples were centrifuged at 3000 rpm for 10 min, and serum was separated and frozen at $-70^{\circ}$C until assayed for MDA. To avoid the possible dispersion of serum MDA analysis, all the samples were analyzed at the same time, at the end of the study.

Serum MDA levels, referred to as thiobarbituric acid reactive substances (TBARS), were measured according to the method described by Kikugawa et al. (1992). The pink complex coloured samples were placed in a 96-well plate and read at 535 nm in a microplate spectrophotometer reader (Benchmark Plus, Bio-Rad, Hercules, CA, USA). The intra- and inter-assay coefficients of variation were 1.82 and 4.01%, respectively.

Clinical global improvement and severity of illness state was assessed in summer and winter by means of the Clinical Global Impression (CGI) scale (Guy 1976).

Data were analyzed using the 13th version of the SPSS statistical package (Illinois, USA). Wilcoxon paired *t*-tests were applied to analyze differences between summer and winter. The effect of clinical and sociodemographic variables on summer and winter MDA levels was studied by a multivariate General Linear Model (GLM). Multivariate GLM allowed us to carry out a regression analysis and variance analysis for several dependent variables using several covariates and several factor variables. Summer and winter MDA levels acted as dependent variables, while gender (male/female), smoking status (smokers/non-smokers) and treatment (typical/atypical antipsychotics) acted as factors. Quantitative variables, age, body mass index (BMI), age at illness onset, illness duration and CGI scores, acted as covariates in this model. The biochemist was blind with regard to the samples belonging to summer or winter season.

## Results

Sociodemographic and clinical characteristics of the sample are shown in Table I. Our sample is comprised of medium age chronic patients with long disease duration, on a stable psychopharmacologic treatment and with a stable clinical picture. Seven patients were on fluphenazine decanoate (considered as a typical antipsychotic) and 16 on long-acting risperidone (considered as an atypical antipsychotic).

Mean MDA levels were significantly higher in summer than winter ($2.49 \pm 0.25$ vs. $1.86 \pm 0.11$ nmol/ml, $P < 0.03$). MDA levels increased by a 33.9% in summer with respect to winter MDA levels (% summer MDA-% winter MDA).

Table II shows the results of the multivariate GLM. None of the factors, gender (male/female), smoking status (smoker/non-smoker) and antipsychotic treatment (typical/atypical) produced a significant effect on summer or winter MDA levels. The interaction between gender and smoking status and gender and treatment neither produce significant effects. Age, BMI, age at illness onset, illness duration and CGI scores did not produce significant effects on MDA levels in summer and in winter.

Table I. Sociodemographic and clinical characteristics of the sample.

| Variables | |
|---|---|
| Age, mean $\pm$ SD (min/max) | $47.0 \pm 16.4$ (18/70) |
| Sex (male/female) | 15/8 |
| Civil status (single/married) | 17/6 |
| Smokers/non-smokers | 12/11 |
| Illness onset, mean $\pm$ SD (min/max) | $23.9 \pm 8.7$ (14/44) |
| Illness duration, mean $\pm$ SD (min/max) | $23.5 \pm 14.4$ (2/49) |
| Treatment (typical/atypical antipsychotics) | 7/16 |
| CGI global improvement summer/winter, mean $\pm$ SD (*P* value) | $3.4 \pm 0.8/3.3 \pm 0.8$ (0.2) |
| CGI severity of illness summer/winter, mean $\pm$ SD (*P* value) | $2.3 \pm 1.4/2.5 \pm 1.5$ (0.2) |

Table II. Effect of clinical and sociodemographic variables on summer and winter MDA levels.

| Source | Dependent variable | Sum of squares | df | Mean square | *F* | *P* |
|---|---|---|---|---|---|---|
| BMI | MDAS | 1.252 | 1 | 1.252 | 0.492 | 0.503 |
| | MDAW | 0.024 | 1 | 0.024 | 0.150 | 0.708 |
| Age | MDAS | 0.668 | 1 | 0.668 | 0.262 | 0.622 |
| | MDAW | 0.055 | 1 | 0.055 | 0.340 | 0.576 |
| Illness onset | MDAS | 0.981 | 1 | 0.981 | 0.386 | 0.552 |
| | MDAW | 0.178 | 1 | 0.178 | 1.112 | 0.322 |
| Illness duration | MDAS | 0.782 | 1 | 0.782 | 0.307 | 0.595 |
| | MDAW | 0.100 | 1 | 0.100 | 0.625 | 0.452 |
| CGISIS | MDAS | 1.279 | 1 | 1.279 | 0.503 | 0.498 |
| | MDAW | 0.096 | 1 | 0.096 | 0.600 | 0.461 |
| CGIGIS | MDAS | 0.856 | 1 | 0.856 | 0.337 | 0.578 |
| | MDAW | 0.036 | 1 | 0.036 | 0.222 | 0.650 |
| CGISIW | MDAS | 2.016 | 1 | 2.016 | 0.792 | 0.399 |
| | MDAW | 0.029 | 1 | 0.029 | 0.178 | 0.684 |
| CGIGIW | MDAS | 0.985 | 1 | 0.985 | 0.387 | 0.551 |
| | MDAW | 0.052 | 1 | 0.052 | 0.322 | 0.586 |
| Gender | MDAS | 0.301 | 1 | 0.301 | 0.118 | 0.740 |
| | MDAW | 0.226 | 1 | 0.226 | 1.410 | 0.269 |
| Smoking status | MDAS | 1.701 | 1 | 1.701 | 0.668 | 0.437 |
| | MDAW | 0.449 | 1 | 0.449 | 2.798 | 0.133 |
| Treatment | MDAS | 0.317 | 1 | 0.317 | 0.125 | 0.733 |
| | MDAW | 0.119 | 1 | 0.119 | 0.740 | 0.415 |
| Gender × Smoking status | MDAS | 0.045 | 1 | 0.045 | 0.018 | 0.897 |
| | MDAW | 0.003 | 1 | 0.003 | 0.019 | 0.894 |
| Gender × Treatment | MDAS | 0.814 | 1 | 0.814 | 0.320 | 0.587 |
| | MDAW | 0.084 | 1 | 0.084 | 0.523 | 0.490 |

BMI, Body Mass Index; CGISIS, Clinical Global Impression Severity of Illness in Summer; CGIGIS, Clinical Global Impression Global Improvement in Summer; CGISIW, Clinical Global Impression Severity of Illness in Winter; CGIGIW, Clinical Global Impression Global Improvement in Winter.

## Discussion

As far as we know, our results show for the first time that serum MDA levels in chronic stable treated paranoid schizophrenic outpatients have a winter/summer pattern of formation, with 33.9% higher MDA levels in summer than winter. No paper published until now (Bindoli et al. 1987; McCreadie et al. 1995; Brown et al. 1998; Mahadik et al. 1998; SSRG 2000; Akyol et al. 2002; Khan et al. 2002; Kuloglu et al. 2002; Arvindakshan et al. 2003a, b, Ranjekar et al. 2003; Dakhale et al. 2004; Gama et al. 2006; Zhang et al. 2006) has taken into account this effect. The reasons for such fluctuations are unknown, but seasonal variations in several biological parameters have been reported (Wirtz-Justice and Richter 1979). Seasonal variations of melatonin synthesis, with higher levels in winter than summer, have also been reported (Morera and Abreu 2006). MDA is an end product of lipid peroxidation, while melatonin has antioxidant properties (Reiter et al. 1995). The relation between antioxidants and prooxidants is in a continuous dynamic equilibrium (Halliwell et al. 1996) and an inverse relationship between melatonin and MDA has been reported (Morera and Abreu 2007). Therefore, though speculative, an inverse relationship between MDA and melatonin may be a possible explanation. In summer, when melatonin levels are lower, MDA levels are higher. On the other hand, in winter, it is just the opposite: when melatonin levels are higher, MDA levels are lower. Animal experimental data also seem to support this hypothesis. Tunez et al. (2003) have shown that light stimulation, the main inhibitor of melatonin secretion, increases MDA formation, and in summer humans are exposed to more hours of sunlight and more intense light than in winter, when daytime is shorter and light is less intense.

Anticoagulant type (Knight et al. 1987), smoking (Block et al. 2002), gender (Knight et al. 1987; Kharb and Ghalaut 2003) and age (Knight et al. 1987; Kharb et al. 2003) have been described as affecting MDA levels in healthy subjects.

Knight et al. (1987) reported that in healthy donors EDTA plasma MDA levels were lower than heparin, sodium citrate and CPDA plasma MDA ones. Four studies (SSRG 2000; Khan et al. 2002; Arvindakshan et al. 2003; Ranjekar et al. 2003a) used EDTA vacutainer tubes to collect blood. In two papers there were no differences in TBARS levels

between schizophrenic patients and healthy controls (SSRG 2000; Ranjekar et al. 2003), while in the other two papers (Khan et al. 2002; Arvindakshan et al. 2003a), schizophrenics had higher levels of MDA than the controls. As in the four studies, patients and controls blood samples were collected in tubes containing EDTA, the same bias is present in both samples of subjects. The fact that we used tubes without anticoagulant for collecting blood has to be taken into account. Thus, we used tubes without anticoagulant, so our results are free of this kind of bias.

Smoking has also been reported to affect MDA levels. Smokers have higher MDA blood levels than non-smokers (Block et al. 2002). We did not find any effect of smoking on MDA levels. Our results are in accordance with the results of Akyol et al. (2002) and Kropp et al. (2005), but are contrary to the results of the SSRG (2000) study which that found that MDA levels were higher in smokers than non-smoker patients, although this difference was not present in the control group. Methodological differences may explain this lack of coherence of results. While Block et al. (2002) measured the level of cotinine, the metabolite of nicotine, the rest of the studies "measured" smoking by questioning the subjects whether or not they smoked.

The effect of the gender on MDA level is quite controversial. Knight et al. (1987) studied 230 male and 148 female healthy subjects, finding that men had significantly higher levels of MDA than women. On the other hand, Kharb (2003) studied MDA blood level in 80 male and 120 female volunteers. They found that women had significantly higher MDA levels than men. We did not find any effect of gender on MDA level. Our results are in accordance with the results of Mahadik et al. (1998), Akyol et al. (2002), Arvindakshan et al. (2003a) and Kropp et al. (2005). Zhang et al. (2006) found no effect of gender on MDA levels in the control group but female schizophrenics had higher MDA level than male schizophrenics.

Older healthy subjects had higher blood MDA levels than younger subjects (Knight et al. 1987; Kharb et al. 2003). We did not find any effect of age on MDA levels in accordance with the results of Mahadik et al. (1998) and Arvindakshan et al. (2003). Sample size may explain these differences. While in the studies of Knight et al. (1987) and Kharb et al. (2003) the samples sizes were 378 and 200 subjects, respectively, the samples of the other studies were comparatively lower, 23 patients in our study, 26 patients in the study of Mahadik et al. (1998) and 52 patients in the study of Arvindakshan et al. (2003a). However, Block et al. (2002) did not find an association between age and MDA levels in a sample of 292 healthy subjects. So the relation between age and MDA is not as simple as we initially believed.

The clinical type of schizophrenia and the type of treatment are among the variables that have been reported affecting MDA levels. Akyol et al. (2002) studies the TBARS levels in a sample of disorganized, paranoid and residual schizophrenics. Only the TBARS level of the residual schizophrenics was significantly higher than the healthy controls. Zhang et al. (2006) reported that paranoid, disorganized and residual schizophrenic patients had higher MDA level than healthy controls, but there were no significant differences in MDA levels among the schizophrenic subgroups. Our sample was comprised of only paranoid schizophrenics, so our data were not subjected to this possible bias.

It has been reported that schizophrenic patients on clozapine had higher MDA level than those on haloperidol (Gama et al. 2006). However, Zhang et al. (2006) found no difference in MDA levels among patients on clozapine, risperidone and typical antipsychotics. Our results are in accordance with those of the Zhang et al. (2006) study, we did not find differences in MDA levels between patients on typical or atypical antipsychotic.

Illness duration (Arvindakhan et al. 2005; Kropp et al. 2005) and age at onset (Krop et al. 2005) do not affect MDA levels. Our results are in consonance with the previous research results.

The most plausible explanation as to why our results were not affected by the previously mentioned variables is due to the methodological design, an intra-subject design; thus all subjects' comparisons were made against themselves.

The major limitation of our study is the small sample size. Although we obtained significant differences in MDA levels between winter and summer, a type I error cannot be completely excluded. The lack of a control group may be argued as a weakness of our study, although according to our objective it was unnecessary. The possible effect of eating habits on our results cannot be completely excluded, despite the fact that we asked the patients not to change their eating habits. The absence of changes in BMI during the study points to the lack of dramatic changes in our patients' diet. On the other hand, the fact that our research has been carried out using an intra-subject design, with repeated measures, in subjects on a stable treatment, points to internal consistency in our results.

To conclude, our results show that MDA presents a winter/summer rhythm of formation, with higher levels in summer and lower levels in winter. The variation in seasonal MDA levels should be taken into account when researching into this field.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**BRIEF REPORT**

# Development of 5-HT transporter density and long-term effects of methylphenidate in an animal model of ADHD

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**Abstract**

Although stimulants as the treatment of choice are widely prescribed in ADHD, little is known about their long-term neurobiological effects. Hence, for the first time the present study examined the long-term effect of chronic methylphenidate (MPH) administration on striatal 5-hydroxytryptamine transporter (5-HTT) densities in an animal model of ADHD. First, it compared the normal development of striatal 5-HTT densities of spontaneously hypertensive rats (SHR) as an animal model of ADHD and Wistar Kyoto (WKY) rats as controls; binding of the highly selective ligand of 5-HTT [$^3$H]paroxetine was determined on membrane preparations of the striatum of SHR and WKY rats on postnatal days 25, 50, and 90, i.e. from the time of weaning until adulthood. Second, the long-term effects of chronic administration of 2 mg/kg per day MPH at two different developmental stages (days 25–39 or 50–64) on the striatal 5-HTT density was examined in both rat strains at day 90. Long-term effects of MPH treatment on striatal 5-HTT density in adulthood could be ruled out in both healthy (WKY) and "ADHD" rats (SHR). But a higher striatal 5-HTT density in older SHR versus WKY rats might indicate ADHD specific changes in the 5-HT system that needs further investigation not only in animals.

**Key words:** *ADHD, serotonin transporter, methylphenidate, spontaneously hypertensive rat*

## Introduction

Although methylphenidate (MPH) is the most prescribed therapeutic agent for attention-deficit/hyperactivity disorder (ADHD) and affected children may be treated with MPH for years (Kollins and March 2007), little is known about the long-term neurobiological adaptations resulting from MPH exposure during this highly plastic and sensitive period of brain maturation (Grund et al. 2006). Its positive effects seem to be mediated mainly by increasing dopamine levels while blocking dopamine transporter (DAT) (Pliszka 2005). Hence, we followed the suggestion of Volkow and Insel (2003) to study animal models that mimic ADHD in order to determine the extent to which synaptic changes might take place when MPH is given. In spontaneously hypertensive rats (SHR) as the best studied animal model of ADHD we found an increased DAT density in the striatum at different developmental stages that was decreased in adult SHR by prepubertal treatment with MPH for 2 weeks (Roessner et al. 2008). But possible long-term changes of MPH treatment in the serotonergic system are also of high interest because several human genetic (Albayrak et al. 2008) and neurochemical (Liu and Reichelt 2001) as well as animal behavioural studies (Oades 2007) indicated serotonin's (5-HT) involvement in the pathophysiology of ADHD. For example, exposing rodents to MPH during preadolescence resulted in increased depression-like behaviours in adulthood manifested in enhanced vulnerability to stressful environments and anxiety-eliciting situations, as well as reduced reactivity to natural rewards (Bolanos et al. 2003; Carlezon et al. 2003). These behavioural deficits could be reversed by subsequent treatment with a selective 5-HT reuptake inhibitor (Bolanos et al. 2008). Nevertheless, the long-term influence of MPH on 5-HT metabolism including the 5-HT transporter (5-HTT) remains unclear.

In view of the close interactions between the dopaminergic and 5-HT system in the striatum and the dorsal raphe–striatal 5-HT projection system (Soghomonian et al. 1987) the present study

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sought to determine the striatal 5-HTT development in SHR and normal (Wistar Kyoto; WKY) rats as well as the long-term influence of MPH treatment on their striatal 5-HTT density.

## Materials and methods

### *Experimental animals*

Animal experiments were performed in accordance with German laws for the care and use of experimental animals (as approved by the Bezirksregierung Braunschweig, License AZ: 509.42502/01-08.00). SHR and WKY rats were obtained from a commercial breeder and used for further breeding in our own environmentally conditioned animal facility under standardized conditions. After mating, the dams were housed in single cages with free access to food and water. After weaning, the young rats (only male rats were used) were placed in separate cages, two per cage.

For studying the development of striatal 5-HTT density we took groups of six rats each and decapitated them at the age of 25 (preadolescence), 50 (late adolescence/young adulthood) and 90 days (adulthood).

In the second experiment, a 2-week MPH treatment was started in two groups of rats (WKY, $N=6$ and SHR, $N=6$) at day 25, in two other groups (WKY, $N=6$ and SHR, $N=6$) at day 50. MPH was administered via the freely accessible drinking water for a period of 2 weeks. The dose of MPH was adjusted to 2 mg/kg per day based on daily monitoring of amounts consumed by the two rats per cage and their body weight. These amounts were about 10% of body weight and remained unaffected by the addition of MPH. Littermates (WKY, $N=6$; SHR, $N=6$) served as controls and received normal tap water. All rats were decapitated at day 90.

### *Sample preparation and ligand binding assays*

For detailed information see Bock et al. (2005). Briefly, all rats were killed by decapitation at noon, brains were quickly frozen and stored at $-80^{\circ}$C. The frozen striatae (100–150 mg) were homogenized for 10 s in 5 vol of ice-cold PBS (potassium phosphate 10 mM plus 0.9% NaCl, pH 7.4, containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.02% thimerosal). The pellets were resuspended in 30 vol of ice-cold buffer (50 mM Tris–HCl, pH 7.4, containing 120 mM NaCl and 5 mM KCl). After low speed centrifugation ($1000 \times g$, 20 min, 4°C), the supernatants were centrifuged again at $40{,}000 \times g$ for 10 min at 4°C. The pellets were washed twice in the same buffer and the final pellets were resuspended in buffer to give a final concentration of 60 mg wet weight/ml.

For measurements of [$^3$H]paroxetine binding (as a measure of 5-HTT density), aliquots of the stored membrane suspensions were further diluted to 20 mg wet weight/ml in Tris-buffer (0.2–0.3 mg protein/ml) containing 120 mM NaCl and 5 mM KCl, and incubated for 2 h at room temperature with [$^3$H]paroxetine (specifity activity: 20.2 Ci/mmol) in the absence or presence of 225 μM 5-HT for the estimation of specific binding. The reaction mixture consisted of 100 ml membrane suspension (20–30 mg protein), 100 ml of [$^3$H]paroxetine containing buffer (six concentrations covering a range of final ligand concentrations in the assay system from 0.05 to 1.00 nM), and 100 ml buffer or 5-HT solution in buffer.

After incubation, the reaction mixtures were readily filtered through Wathman GF/B filters using a 12-channel cell harvester. The radioactivity trapped by the filters was determined by liquid scintillation spectroscopy.

### *Data analysis and statistics*

Nonparametric statistics were used due to the small sample size. The statistical significance of differences between the means of [$^3$H]paroxetine-binding within each rat strain were tested by Kruskal–Wallis one-way analyses of variance by ranks. Additionally, post-hoc pairwise Mann–Whitney *U*-tests (two-tailed) were computed. The level of significance was set at $P$ values $<0.05$. All analyses were carried out using the Statistical Package for the Social Sciences (SPSS version 11.5, 2004).

## Results

For the development of striatal [$^3$H]paroxetine-binding we found a significant decrease from day 25 to day 50 followed by an increase until day 90 in both rat strains (Figure 1). While there was no difference between both strains comparing day 25, we found a significant higher binding of [$^3$H]paroxetine in SHR compared to WKY rats at days 50 and 90 (Table I).

In terms of treatment with MPH no significant changes in striatal [$^3$H]paroxetine-binding could be observed (Table II).

## Discussion

To our knowledge, this is the first study addressing the development of striatal 5-HTT density in SHR as well as the influence of two-week MPH treatment. In untreated SHR and WKY control rats striatal

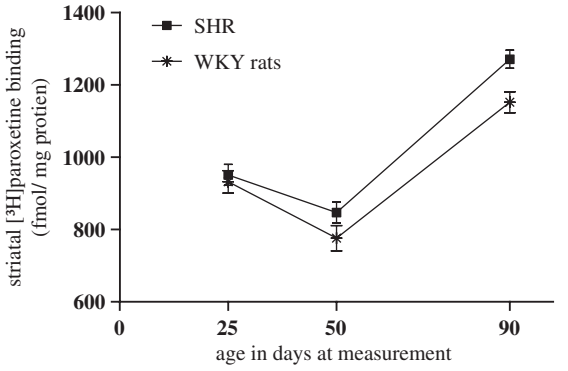


Figure 1. Development of [$^3$H]paroxetine binding (as a measure of 5-HT transporter density) in the striatum of non-medicated Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) measured at days 25, 50 and 90. For statistics see Table I.

5-HTT density slightly decreased from day 25 (accordingly preadolescence) to 50 (accordingly late adolescence) followed by an increase until day 90 (accordingly adulthood) exceeding the density at day 25. While at day 25 no significant difference was observed between both strains, the striatal 5-HTT density was higher in SHR than in WKY rats at day 50 and 90.

In accordance with our results, Moll et al. (2000) found only a trend for an age-associated increase of striatal 5-HTT density in WKY rats. Their observed moderate increase of 5.4% from day 25 to day 90 was less pronounced than that in the present study (12% day 25 to day 90). This slight difference could be ascribed possibly to differences within one strain as seen, e.g., also in tissue levels of 5-HT in the ventral striatum of WKY rats identical in strain, age and gender (Schwarting et al. 1998). Furthermore, in another rat model of ADHD, i.e. 6-OHDA-lesioned rats, increased striatal 5-HTT density compared to control rats as well as an increase with age in both strains was found (Zhang et al. 2002). This age-dependent increase of striatal 5-HTT has been linked to hyperactivity as a core symptom of ADHD. In SHR (van den Bergh et al. 2006) as well as 6-OHDA-lesioned rats (Zhang et al. 2002) hyperactivity was present only during early development (day 30 and day 24), and not in adulthood (day 59 and day 58). Similarly, the higher striatal 5-HTT in older SHR compared to WKY rats has been linked to impulsivity as another core symptom of ADHD, e.g., impulsivity measured by an intertemporal choice procedure was not different between SHR and WKY rats of young age (40 days old, Adriani et al. 2003) but elevated in adult SHR (8 months old, Fox et al. 2008).

However, these behavioural findings have not been consistent due to methodological differences and the fact that hyperactivity and impulsivity are fractionated in terms of behavioural output, neural basis and neurochemical modulation (Oades et al. 2002; Winstanley et al. 2006). In addition, relating the found age-dependent increase of striatal 5-HTT to behavioural abnormalities is highly speculative because the 5-HT system is very complex (e.g., numerous types of 5-HT receptors have been identified) and still poorly understood (Oades 2007).

Nevertheless, the found developmental course of striatal 5-HTT density in SHR and WKY rats might explain why numerous experiments in rodents demonstrated that stimulants improve impulse control in juvenile animals but failed to decrease, and in most cases increased, impulsive-like behaviour in adult ones (Bizot et al. 2007). This speculation implies a stronger serotonergic component of the impulsive-like behaviour in adult than in infantile and adolescent rodents. In summary, the found developmental course of striatal 5-HTT emphasizes once again that age should be considered when interpreting rat studies on behaviour and neurotransmitter systems in ADHD, because the age or stage of development of the different neurotransmitter components may confound the interpretation of their relationship (Oades 2007).

In both rat strains MPH treatment at different developmental stages had no influence on striatal 5-HTT density measured in adulthood (day 90). To our knowledge there are no comparable neurobiological data in literature, but the present finding of unchanged striatal 5-HTT density indicates that other factors might underlie the increased depression-like behaviours in rodents exposed to MPH during preadolescence reducible by treatment with a selective 5-HT reuptake inhibitor (Bolanos et al. 2008).

Although the sample size of the present study is in the common range of comparable animal studies, the fact that difference in striatal 5-HTT density between the rat strains missed the level of significance in both MPH treatment conditions might possibly be due to the high biological variation and should be regarded as a limitation of the present data.

In conclusion, there seem to be age-dependent changes in striatal 5-HTT density in both SHR and WKY rats resulting in increased striatal 5-HTT density in older ages. The higher striatal 5-HTT density in older SHR compared to WKY rats might indicate ADHD specific changes in the 5-HT system. In addition, this pilot study indicates that MPH has no long-term effect on striatal 5-HTT density in both healthy (WKY) and "ADHD" rats (SHR). But these preliminary findings need further investigation not only in animals.

Table I. Development of [$^3$H]paroxetine binding (as a measure of 5-HT transporter density) in the striatum of unmedicated WKY and SHR measured at age of 25, 50 and 90 days.

| | | | | | Pairwise Mann–Whitney *U*-tests (two-tailed) | | |
|---|---|---|---|---|---|---|---|
| [$^3$H]Paroxetine binding (fmol/mg protein) in the striatum of | Day 25 M (SD) | Day 50 M (SD) | Day 90 M (SD) | K-W rang-VA[a] *P* | Day 25/day 50 *P* | Day 25/day 90 *P* | Day 50/day 90 *P* |
| WKY rats | 932 (31) | 776 (35) | 1152 (29) | 0.001*** | 0.004** | 0.004** | 0.004** |
| SHR rats | 951 (29) | 847 (29) | 1271 (25) | 0.001*** | 0.004** | 0.004** | 0.004** |
| WKY vs. SHR[b] | 0.521 | 0.006** | 0.004** | | | | |

*** $P \leq 0.001$; ** $P \leq 0.01$.
[a]K-W rang-VA, Kruskal–Wallis one-way analysis of variance by ranks.
[b]Pairwise Mann–Whitney *U*-tests (two-tailed).

Table II. [$^3$H] paroxetine binding (as a measure of 5-HT transporter density) in the striatum of WKY and SHR with 2-week MPH treatment started at day 25 or day 50 measured at day 90.

| | | | | | Pairwise Mann–Whitney *U*-tests (two-tailed) | | |
|---|---|---|---|---|---|---|---|
| [$^3$H]Paroxetine binding (fmol/mg protein) in the striatum of | MPH treatment at day 25 M (SD) | MPH treatment at day 50 M (SD) | Unmedicated controls M (SD) | K-W rang-VA *P*[a] | MPH at day 25/MPH at day 50 *P* | MPH at day 25/unmed. controls *P* | MPH at day 50/unmed. controls *P* |
| WKY rats | 1066 (121) | 1122 (96) | 1106 (28) | 0.641 | 0.423 | 0.423 | 0.873 |
| SHR rats | 1163 (92) | 1143 (31) | 1213 (83) | 0.236 | 0.522 | 0.262 | 0.109 |
| WKY vs. SHR[b] | 0.200 | 0.749 | 0.037* | | | | |

* $P \leq 0.05$.
[a]K-W rang-VA, Kruskal–Wallis one-way analysis of variance by ranks.
[b]Pairwise Mann–Whitney *U*-tests (two-tailed).

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## Statement of interest

The authors have not declared any conflicts of interest.

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**BRIEF REPORT**

# The impact of one session of HF-rTMS on salivary cortisol in healthy female subjects

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**Abstract**

Previous studies in healthy volunteers reported a possible impact of high frequency repetitive transcranial magnetic stimulation (HF-rTMS) on stress hormones, like cortisol. In this sham-controlled, "single blind", crossover study, we examined whether HF-rTMS had an effect on the hypothalamic-pituitary-adrenal (HPA) axis, by analysing salivary cortisol levels. Two studies were conducted. First, HF-rTMS on the left dorsolateral prefrontal cortex (DLPFC) was performed in 28 young healthy female volunteers. Second, in a comparable, but different group of 26 healthy females, HF-rTMS was performed on the right DLPFC. Salivary cortisol levels were assessed before, immediately after and 30 min after real and sham HF-rTMS. We found no support for the hypothesis that one single session of HF-rTMS on the left or the right DLPFC has an immediate or delayed impact on the HPA–axis, as measured by salivary cortisol. Although we controlled for several methodological problems in HF-rTMS research, the hypothesis that one single session of HF-rTMS on the left or on the right DLPFC can influence the HPA-axis in healthy volunteers was not supported.

**Key words:** *HF-rTMS, salivary cortisol, healthy volunteers*

## Introduction

HF-rTMS on the DLPFC is currently used to treat affective disorders or as an experimental tool to investigate neurocognitive processes in healthy volunteers (Burt et al. 2002). An important aspect of the physiology of HF-rTMS could be related to the endocrinological response of the hypothalamic-pituitary-adrenal (HPA)-axis, such as cortisol secretion (Evers et al. 2001). Indeed, animal models suggest that rTMS on frontal brain regions attenuates the HPA system (Ji et al. 1998; Keck 2001; Hedges et al. 2003). A hypothesis on the underlying mechanism for its efficacy of the treatment of affective disorders could be that HF-rTMS induces endocrinological changes through its influence on cortico-subcortical neuronal connectivity (Post and Keck 2001).

Few studies examined the endocrinological HF-rTMS response on the HPA system in healthy volunteers. George et al. (1996) found in their open study that serum cortisol mean levels increased slightly 30 min after right and left prefrontal stimulation and then declined. Evers et al. (2001) observed only serum cortisol effects using infrathreshold HF-rTMS on the left DLPFC. The same volunteers (both men and women) were stimulated three times a day, so possibly sex differences and successive stimulation sessions could have biased their results (Otte et al. 2005). Also in healthy subjects, rTMS effects on thyroid function were only observed in the suprathreshold condition and recent data in the treatment of affective disorders indicates that suprathreshold rTMS has superior outcome results (Cohrs et al. 1998; Gershon et al. 2003).

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This sham-controlled study tried to evaluate the lateralized effects of one single session of suprathreshold HF-rTMS on the HPA-axis, measured with easy to obtain salivary cortisol samples. Salivary cortisol correlates highly with serum levels and represents the free, biologically active fraction of the hormone (Vining and McGinley 1986). Salivary cortisol responses can be observed 5–20 min after stress induction, with peak levels after 10–30 min (Kudielka and Kirschbaum 2005). It might be more indicative than serum total cortisol, because salivary cortisol, largely unbound, is independent of cortisol binding globulin (CBG) variations (Lac 2001). We applied HF-rTMS on both the right and left DLPFC in two different but comparable homogeneous samples of healthy female volunteers, stimulating just one cortical region in each participant. Importantly, as proposed by Herwig et al. (2001), taking into account anatomical brain differences, we determined the left and right DLPFC under MRI guidance. We hypothesized that compared to sham and regardless of hemisphere, one single session of HF-rTMS would be related to an increase of salivary cortisol.

## Materials and subjects

### *Subjects*

The choice of heterogeneous subject sampling could be an important methodological issue in TMS research (Martin et al. 2003). We chose to use uniform groups of young female subjects because age and gender might induce too much variability in our data. Both groups consisted of healthy, right-handed female volunteers recruited among medical and psychology students, receiving HF-rTMS on the left DLPFC ($n = 28$, mean age $24.7 \pm 5.9$ years) or HF-rTMS on the right DLPFC ($n = 26$, mean age $25.11 \pm 4.79$ years).

Psychiatric disorders were excluded using the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al. 1998). Safety guidelines of the International Society of Transcranial Magnetic Stimulation (ISTS) were followed (Anand and Hotson 2002). No drugs were allowed, except birth-control pills. The ethics committee of the Academic Hospital of the Free University of Brussels approved the study and all subjects gave written informed consent. Subjects were financially compensated.

### *Salivary cortisol sampling*

Saliva samples were collected using a Salivette (Sarstedt, Germany), with an insert containing a sterile polyester swab for collecting saliva, yielding a clear and particle-free sample. The salivettes were used according to the instructions provided by the manufacturer. Salivettes containing saliva were centrifuged at $2000 \times g$ for 10 min, and the filtrates were stored frozen ($-20^{\circ}$C). Before analysis, the samples were thawed and mixed. Saliva cortisol levels were measured by RIA (Diasorin, Italy), using a modification of an unextracted RIA method for serum cortisol. Briefly, 200 μl saliva were pipetted into the coated tube and incubated with [$^{125}$I]cortisol for 45 min at 37°C. The modified cortisol assay had a measuring range from 0.5–30 μg/l and within- and between-run coefficients of variation of $<5$ and $<10\%$, respectively.

### *Study protocol*

We used a Magstim high-speed magnetic stimulator (Magstim Company Limited, Wales, UK), connected to a specially designed figure-eight-shaped coil. Before each application, the motor threshold (MT) of the right abductor pollicis brevis muscle of each individual was determined. In order to accurately target the left and right DLPFC (Brodmann area 9/46), the precise stimulation site and position of the coil was determined using MRI non-stereotactic guidance. Perpendicular to this point the precise stimulation site on the skull was marked and stimulated. In each high-frequency (10 Hz) stimulation session, at stimulation intensity of 110% of the subject's MT, subjects received 40 trains of 3.9 s duration, separated by an intertrain interval of 26.1 s. Each session lasted 20 min (1560 pulses per session). For the sham condition, the coil was held at an angle of 90°, only resting on the scalp with one edge. The stimulation order was counterbalanced across subjects. To avoid possible interactions between the two sessions, there was a one week time lag between the real HF-rTMS and the sham procedure. During stimulation, all subjects wore earplugs and were blindfolded.

To obtain a baseline salivary cortisol level each subject delivered a salivette immediately before HF-rTMS stimulation (baseline $T_1$). All subjects then received sham or real rTMS targeted at the left or right DLPFC. All subjects were stimulated in the time interval between 10:00 and 12:00 h. Immediately after stimulation ($T_2$), participants delivered a second salivette and once more after 30 min ($T_3$).

### *Statistical analysis*

All results were analysed using SAS version 8.2 (SAS Institute, Cary, NC). The effects of real and sham HF-rTMS on the HPA-axis were analyzed separately using the non-parametric approach for the analyses

Table I. Mean ratings and standard deviations for salivary cortisol ($T_1$ = just before HF-rTMS, $T_2$ = just after HF-rTMS, $T_3$ = 30 min after HF-rTMS) measures for left and right HF-rTMS (real or sham condition) expressed in μg/l.

| | Real | Sham |
|---|---|---|
| Time | Mean (SD) | Mean (SD) |
| Left HF-rTMS effects on salivary cortisol ($n=28$) | | |
| $T_1$ | 6.35 (3.04) | 6.05 (2.87) |
| $T_2$ | 5.84 (2.95) | 5.41 (2.39) |
| $T_3$ | 5.21(3.04) | 4.72 (1.88) |
| Right HF- rTMS effects on salivary cortisol ($n=26$) | | |
| $T_1$ | 5.47 (1.80) | 6.89 (3.40) |
| $T_2$ | 4.64 (1.48) | 5.52 (1.78) |
| $T_3$ | 4.52 (1.63) | 5.48 (2.68) |

of a balanced two-period crossover design described by Hills and Armitage (1979).

## Results

Salivary cortisol data are presented as arithmetic mean and simple standard deviation in μg/l (Table I). Analyses on the salivary cortisol data were performed on the change during HF-rTMS ($T_2$–$T_1$), as well as the change observed 30 min after the end of each HF-rTMS session ($T_3$–$T_1$).

### *HF-rTMS on the left DLPFC*

The results of the Hills and Armitage non-parametric approach showed no evidence of a stimulation (treatment)-period or sequence effect. The mean difference in salivary cortisol concentrations between $T_1$ and $T_2$ after real and sham HF-rTMS was $0.12 \pm 2.26$ μg/l ($P = 0.872$, NS). Mean salivary cortisol levels at $T_1$ were for real HF-rTMS $6.35 \pm 3.04$ μg/l and for sham HF-rTMS $6.05 \pm 2.87$ μg/l. Salivary cortisol measurements declined were $5.84 \pm 2.95$ μg/l for real and $5.41 \pm 2.39$ μg/l for sham HF-rTMS at $T_2$. When considering the salivary cortisol change between $T_1$ and $T_3$, measures further declined in both HF-rTMS conditions (Mean salivary cortisol levels at $T_3$ were $5.21 \pm 3.04$ μg/l for real and $4.72 \pm 1.88$ μg/l for sham HF-rTMS) and no significant difference was observed ($P = 0.696$, NS). See also Table II.

### *HF-rTMS on the right DLPFC*

Due to two missing values, two volunteers were not included in the statistical analyses. At one of the fixed time points they did not produce enough saliva, needed to be analyzed. The Hills and Armitage approach non-parametric tests also did not demonstrate a stimulation (treatment)-period or sequence effect after one stimulation session on the right DLPFC. Mean difference in salivary cortisol levels immediately after stimulation ($T_2$–$T_1$) was $0.73 \pm 2.74$ μg/l ($P = 0.505$, NS). In analogy with the other group of female volunteers, immediately after stimulating the right DLPFC, salivary cortisol levels diminished in both conditions (from $5.47 \pm 1.80$ μg/l for real and from $6.89 \pm 3.40$ μg/l for sham stimulation at $T_1$ to $4.64 \pm 1.48$ μg/l and $5.52 \pm 1.78$ μg/l at $T_2$, respectively). Thirty minutes after stimulation ($T_3$–$T_1$), salivary cortisol values further diminished ($4.52 \pm 1.63$ μg/l for real and $5.48 \pm 2.68$ μg/l for sham stimulation at $T_3$), and no significant difference between real and sham HF-rTMS was demonstrated ($P = 0.271$, NS). For details see Table II.

Table II. Data are means ± SD. Analyses on the salivary cortisol data were performed on the change during HF-rTMS ($T_2$–$T_1$), as well as the change observed 30 min after the end of each HF-rTMS session ($T_3$–$T_1$). A negative value implies a lowering of that value. *P* values were assessed using methods described by Hills and Armitage ($P < 0.05$). For salivary cortisol mean ratings and standard deviations expressed in μg/L for $T_1$, $T_2$ and $T_3$ see Table I.

| HF-rTMS | Real | Sham | Difference (mean ± SD) | *P* value |
|---|---|---|---|---|
| Left ($n=28$) | Change on salivary cortisol immediately after one session ($T_2$–$T_1$) | Change on salivary cortisol immediately after one session ($T_2$–$T_1$) | | |
| | $-0.52 \pm 1.71$ | $-0.64 \pm 1.38$ | $0.12 \pm 2.26$ | 0.872 |
| | Change on salivary cortisol 30 min after one session ($T_3$–$T_1$) | Change on salivary cortisol 30 min after one session ($T_3$–$T_1$) | | |
| | $-1.15 \pm 2.56$ | $-1.34 \pm 1.78$ | $0.18 \pm 3.07$ | 0.696 |
| Right ($n=26$) | Change on salivary cortisol immediately after one session (T2-T1) | Change on salivary cortisol immediately after one session ($T_2$–$T_1$) | | |
| | $-0.70 \pm 0.74$ | $-1.43 \pm 2.42$ | $0.73 \pm 2.74$ | 0.505 |
| | Change on salivary cortisol 30 min after one session ($T_3$–$T_1$) | Change on salivary cortisol 30 min after one session ($T_3$–$T_1$) | | |
| | $-0.98 \pm 1.17$ | $-1.25 \pm 2.53$ | $0.27 \pm 2.84$ | 0.271 |

$T_1$ = just before HF-rTMS, $T_2$ = just after HF-rTMS, $T_3$ = 30 min after HF-rTMS.

## Discussion

Even though corrections for individual differences in brain anatomy were anticipated, our results indicate that one single session of HF-rTMS on the left and/or right DLPFC may not be able to influence the HPA –axis in healthy female volunteers. The general decrease in cortisol concentrations under real and sham condition most likely represents the decline during the daytime as part of the circadian variation of cortisol levels (Hanson et al. 2000). Our results are in agreement with former controlled suprathreshold HF-rTMS studies in normal volunteers, using serum cortisol (Evers et al. 2001).

There are some possible explanations for our negative findings. First, HF-rTMS effects on the HPA-axes in unipolar depressed patients are generally found two weeks or more after successful daily HF-rTMS treatment (Zwanzger at al. 2003; Loo and Mitchell 2005). So, one stimulation session may be too short to influence the HPA-axis and the duration of stimulation may be more important than the intensity, as also stated by Evers et al. (2001). Second, another important variable is gender (Kudielka et al. 2004). It is possible that oral contraceptives (OC) and the menstrual cycle phase could have had an impact on salivary measurements: in a study by Kirschbaum et al. (1999), women using OC showed blunted free cortisol responses to psychological stress compared to medication-free women. Almost all of our female volunteers used OC, which might partially explain the lack of salivary cortisol changes due to the higher free cortisol binding. Third, despite the fact that salivary cortisol sampling is not invasive, salivary cortisol measurement produces more variability than does serum cortisol (Reynolds et al. 1998). Fourth, although sham stimulation in our study was performed at a 90° angle, ensuring minimal stimulation of the DLPFC, it is possible that in our study a partially active placebo was used (Loo et al. 2000).

To avoid hormonal bias further HF-rTMS research on the HPA axis would need to be carried out in male volunteers or menstrual cycles should be taken into account in larger samples of healthy females. Besides salivary cortisol both total and free serum cortisol following strict time schedules could be sampled, as put forward by other researchers (Reynolds et al. 1998; Van Duinen et al. 2005). Because cortisol may be affected differently in healthy individuals and depressed patients (Erickson et al. 2003), it remains also of interest to assess the impact of one single session HF-rTMS on the HPA-axis in depressed patients.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**BRIEF REPORT**

# Verbal working memory and functional outcome in patients with unipolar major depressive disorder

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**Abstract**
In this naturalistic cross-sectional study, the author tested the hypothesis that verbal working memory (WM) in major depressive disorder (MDD) would predict functional outcome. The subjects consisted of 54 clinic adult out-patients. The author found that, in the patients with current episode of MDD, functional outcome was significantly correlated with depressive scores, but not with Digit Sequencing Task scores. Meanwhile, in a sample of full remitted or partial remitted (mildly depressed) patients, functional outcome was significantly correlated with both Digit Sequencing Task scores and depressive scores. Moreover, in a sample of full remitted or partial remitted (mildly depressed) patients, the Digit Sequencing Task score significantly contributed to the prediction of the functional outcome, but the depressive score did not. The findings in this study suggested that enhancement of verbal WM function may be useful to achieve normalization of functioning as an important component of remission in addition to symptomatic remission.

**Key words:** *Cross-sectional study, functional outcome, major depressive disorder, verbal working memory*

## Introduction

Major depressive disorder (MDD) is a significant health problem with economic implications, and estimates of the economic burden of depression range from $52 billion in 1990 to $83 billion in 2000 (Malone 2007). Among several factors, employment is considered to have a great impact on the societal costs of depression, due to lost income, lost productivity, and disability income payments.

In a previous report (Kaneda et al. in press), the author demonstrated that neurocognitive performance, particularly verbal working memory (WM), was more important than clinical symptoms to predict employment status in patients with schizophrenia. Patients with major depressive disorder also have been reported to perform less well in neurocognitive tests than normal controls, even after their depression is successfully treated with modern antidepressants (Gualtieri et al. 2006; Reppermund et al. 2008). In a recent report, Gualtieri and Morgan (2008) reported that substantial numbers of patients with depression are cognitively impaired, and the author also demonstrated that a depression-associated deficit in verbal WM existed even after remission (Kaneda in press). However, little emphasis has been placed on relation between neurocognitive function and psychosocial or functional outcome in studies of depression to date. The purpose of this study was to test the hypothesis that a specific type of cognitive impairment, namely verbal WM, in patients with MDD would predict functional outcome.

## Experimental procedures

### *Sample*

The subjects for this naturalistic cross-sectional study consisted of 54 clinic adult out-patients (aged 21–59 years): 22 patients who met DSM-IV (American Psychiatric Association 1994) criteria for current episode of unipolar MDD (nonpsychotic) and 32 patients who were in full remission or partial remission (mild depression). Patients had no comorbid psychiatric disorders and no medical, neurological or developmental conditions that might affect cognition. The investigation was carried out in accordance with the Declaration of Helsinki and the informed consent was obtained from all subjects.

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Thirty-five (65%) were women; the patients had a mean age of 41.0 (SD = 10.6), and a mean age at onset of 37.7 (11.7) years. Seven out of 22 (32%) patients with current episode of MDD were on antidepressants, and six (27%) of them were on benzodiazepines. Twenty-eight out of 32 (88%) remitted patients were on the antidepressants, and 16 (50%) of them were on benzodiazepines.

### *Clinical assessments*

The assessments were performed using the seven-item Hamilton Rating Scale for Depression (HAM-D7; McIntyre et al. 2002) for severity of depression and remission: full remission defined as an HAM-D7 of 3 or less, and partial remission (mild depression) as a score of 10 or less, and Brief Assessment of Cognition in Schizophrenia (BACS; Keefe et al. 2004) Digit Sequencing Task (patients are presented with clusters of numbers in random order of increasing length, and they are asked to tell the experimenter the numbers in order, from lowest to highest) for verbal WM. The BACS Digit Sequencing Task has been validated in normal controls (Keefe et al. 2004). Digit Sequencing Task scores for each depression group were normalized against their respective age-matched control group (data available upon request). Functional outcome (productivity), such as working, doing household chores, or going to school was assessed by the author based on the interviews with patients and their partners/parents/children, and was defined as follows: 0=non-impaired, 1=mildly impaired, 2=moderately impaired, 3=severely impaired). Demographic data are presented in Table I.

### *Data analysis*

JMI (Version 5.1.2) for Macintosh was used to perform the analysis. For numerical variables, the *t*-tests procedures for independent group comparison were used to compare the differences in variables between two groups, and the differences between three groups were compared by the analysis of variance (ANOVA), followed by *post hoc* comparisons. Pearson's correlation was used to examine the relationships between two numerical variables. A logistic regression model with forward selection criteria was used to predict the functional outcome using the demographic variables, depressive and verbal WM scores.

## Results

First, as reported elsewhere (Kaneda in press), Digit Sequencing Task scores were not significantly different between the two groups of patients with

Table I. Demographic data.

| | *N* (F/M) | Age (years) | Education (years) | Age at onset (years) | Duration of the illness (years) | Dose of antidepressants (mg/day)[1] | Dose of benzodiazepines (mg/day)[2] | HAM-D7 (Total) | BACS Digit Sequencing Task score (raw) | BACS Digit Sequencing Task score (*z* score[3]) |
|---|---|---|---|---|---|---|---|---|---|---|
| Total patients | 54 (35/19) | 41.0 (10.6) | 12.0 (2.2) | 37.7 (11.7) | 3.0 (3.9) | 18.7 (22.4) | 9.1 (14.0) | 10.4 (4.8) | 17.1 (4.4) | −0.75 (1.3) |
| Patients in acute depression | 22 (13/9) | 37.1 (9.0) | 11.9 (2.0) | 32.2 (11.2) | 4.4 (5.3) | 13.2 (22.8) | 9.8 (16.0) | 15.2 (1.9) | 17.2 (4.3) | −0.97 (1.5) |
| Patients in remission | 32 (22/10) | 43.7 (10.9) | 12.2 (2.3) | 41.1 (10.8) | 2.1 (2.2) | 22.5 (21.7) | 8.7 (12.7) | 6.9 (2.9) | 17.0 (4.5) | −0.59 (1.2) |

[1]Paroxetine equivalent data are given as mean (SD).
[2]Diazepam equivalent data are given as mean (SD).
[3]*z* scores were calculated using the age-matched control group means and standard deviations.
BACS, Brief Assessment of Cognition in Schizophrenia; HAM-D7, seven-item Hamilton Rating Scale for Depression.

current episode of MDD and in full remission or partial remission (mild depression), even after controlling for the education level.

Second, examination of the relationships between functional outcome and Digit Sequencing Task and depressive scores in the two groups, patients with current episode of MDD and in full remission or partial remission (mild depression), the results were different between the two: in the patients with current episode of MDD, functional outcome was significantly correlated with depressive scores ($r=0.45$, $df=21$, $P<0.05$), but not with Digit Sequencing Task scores ($r=-0.14$, $df=21$, $P=0.54$). Meanwhile, in a sample of full remitted or partial remitted (mildly depressed) patients, functional outcome was significantly correlated with both Digit Sequencing Task scores ($r=-0.43$, $df=31$, $P<0.05$) and depressive scores ($r=0.38$, $df=31$, $P<0.05$).

Third, in a multiple regression analysis with a forward stepwise procedure, the depressive score in the group of patients with current episode of MDD ($F=5.1$, $df=1$, $P<0.05$) significantly contributed to the prediction of the functional outcome, but the Digit Sequencing Task score did not. Meanwhile in a sample of full remitted or partial remitted (mildly depressed) patients, the Digit Sequencing Task score ($F=4.5$, $df=1$, $P<0.05$) significantly contributed to the prediction of the functional outcome, but the depressive score did not.

Fourth, examination of the relationships between Digit Sequencing Task and depressive scores revealed that Digit Sequencing Task scores were not significantly correlated with depressive scores in either patients with current episode of MDD ($r=-0.03$, $df=21$, $P=0.89$) or in full remission or partial remission (mild depression) ($r=-0.32$, $df=31$, $P=0.08$). In addition, there were no significant correlations between Digit Sequencing Task scores and the dose of antidepressants ($r=-0.13$, $df=31$, $P=0.49$) or benzodiazepines ($r=-0.30$, $df=31$, $P=0.09$) in a sample of full remitted or partial remitted (mildly depressed) patients.

## Discussion

These findings in this study suggested relations between MDD-associated deficit in verbal WM and functional outcome in a sample of full remitted or partial remitted (mildly depressed) patients. The findings seems to be inconsistent with those of Kennedy et al. (2007), who reported, in their review, that residual symptomatology after remission from depression may lead to enduring psychosocial impairment, as may subtle neurocognitive deficits. Nonetheless, the findings in this study do not underscore the importance of clinical remission from depression, which is defined objective outcome indicated by a quantifiable score with a depressive symptom measurement tool. On the contrary, symptomatic full remission should be always achieved as the primary goal of treatment, since it is the optimal outcome in depression (McIntyre et al. 2005; Möller 2008). Meanwhile, the findings in this study suggested that enhancement of verbal WM function by, e.g., cognitive rehabilitation may be useful to achieve normalization of functioning as an important component of remission (Zimmerman et al. 2006) when symptomatic full remission is failed to achieve. Besides, the possibility of influence of medications, particularly benzodiazepines (Stewart 2005), on the verbal WM dysfunction cannot completely be ruled out. Another limitation in this study was that patients in full remission and partial remission (mild depression) were combined for statistical analyses, mainly because there were few patients in full remission. Therefore, a further longitudinal study using patients without benzodiazepines might be necessary to confirm the results of the present study.

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## Statement of interest

None.

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**BRIEF REPORT**

# Case–control study of association between the functional candidate gene *ERBB3* and schizophrenia in Caucasian population*

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**Abstract**

Schizophrenia is a common psychiatric disorder with a complex genetic aetiology. Evidence shows that the oligodendrocyte and myelin-related genes including *ERBB3* are closely related to schizophrenia. Two recent studies (Kanazawa et al. (Am J Med Genet B Neuropsychiatr Genet 2007;144:113)) and Watanabe et al. (Neurosci Res 2007;57:574) reported there was no association between *ERBB3* and schizophrenia in Japanese population. We investigated the *ERBB3* gene given the putative functional nature of the gene and population heterogeneity between Asian and Caucasian. Scottish case and control samples were sequenced with four SNPs (rs705708 at intron 15, rs2271189, rs773123, rs2271188 at exon 27). We detected rs773123, which is a nonsynonymous Ser/Cys polymorphism located seven bases downstream of rs2271189, with $P=0.034$. The subgroups of male patients and patients with age at onset $<45$ showed evidence of significant association with $P$ values of 0.0046 and 0.0055, respectively. To our knowledge, this is the first association study between *ERBB3* and schizophrenia in the Caucasian population. Further investigation with large sample size should be helpful to clarify the nature of the gene.

**Key words:** *association, case-control, ERBB3, myelin, schizophrenia*

## Introduction

Schizophrenia is a severe, often chronic and common complex debilitating mental illness with a large genetic component (Faraone et al. 2002; Rujescu 2008). The oligodendrocyte and myelin-related genes including *ERBB3* (*HER3*) have been found to be highly related to schizophrenia (Hakak et al. 2001; Tkachev et al. 2003). The *ERBB3* gene maps to human chromosome 12q13 covering 22,702 base pairs. The region 12q13 has been reported to be involved in schizophrenia (Deb-Rinker et al. 2002). *ERBB3* expresses a 6.2-kb transcript in a variety of normal tissues of epithelial origin (Kraus et al. 1989). The neuregulin receptor ERBB3 is a member of the epidermal growth factor receptor family, which plays fundamental roles in the regulation of cell survival, proliferation, differentiation, and cellular transformation in response to various specific growth factors (Holbro et al. 2003; Walters et al. 2003).

In a microarray study, Tkachev et al. (2003) found that the amount of the *ERBB3* mRNA was reduced by 2.29-fold in patients with schizophrenia. Moreover, quantitative polymerase chain reaction (PCR) analysis on brains have confirmed significant down-regulation of *ERBB3* by 1.97 ($P=0.0005$) and 2.62 ($P=0.001$) in schizophrenia and bipolar disorder, respectively (Tkachev et al. 2003). DNA microarray has also shown the ERBB3 receptor to be down-regulated in schizophrenics (Hakak et al. 2001). The high degree of correlation between the expression changes in schizophrenia and bipolar disorder provide compelling evidence for common pathophysiological pathways that may govern the disease phenotypes of schizophrenia (Hakak et al. 2001; Deb-Rinker et al. 2002; Tkachev et al. 2003). Moreover, it has been found that loss of *ERBB3*

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results in embryonic lethality in mice with brain defects (Cho and Leahy 2002; Niendorf et al. 2007). In addition, the neuregulin 1 gene (*NRG1*), which codes for the neuron-derived ligand for the ERBB3 receptor, has been identified as a promising susceptibility gene for schizophrenia by our group (Tang et al. 2004; Zhao et al. 2004; Li et al. 2006). There was evidence showing co-expression and functional inter-activity between *ERBB3* and *NRG1* (Britsch 2007; Parlapani et al. 2008), such as the entry of *NRG1* beta1 to the parenchyma of the brain and spinal cord (Kastin et al. 2004). Two recent studies (Kanazawa et al. 2007; Watanabe et al. 2007) reported there was no association between *ERBB3* and schizophrenia in the Japanese population. We studied the Scottish population by sequencing four single nucleotide polymorphisms (SNPs) (rs705708 at intron 15, rs2271189, rs773123, rs2271188 at exon 27).

## Methods

One hundred and ninety cases and 196 controls of Caucasian origin were genotyped using sequencing analysis. The cases contained 82 (71.9%) male and 32 (28.1%) female with a mean age at onset of 26.39 years, SD = 11.29 and the controls comprised 105 (59.3%) male and 72 (40.7%) female with a mean age at onset of 38.52 years, SD = 11.07 (based on the samples genotyped successfully). All the patients were interviewed and diagnosed by two independent psychiatrists according to American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders – IV (DSM-IV). A standard informed consent for the genetic analysis, which was reviewed and approved by the local psychiatry research ethical committee in Scotland, was given by all subjects after the nature of study had been fully explained. Peripheral blood samples were taken from the subjects, and genomic DNA was then extracted from the whole blood using the modified phenol-chloroform method (Gao et al. 2001).

The SNPs were genotyped using the Nested PCR-based sequencing analysis. The primers were shown in supplementary Table I. The PCR products were purified, and then subjected to heat inactivation. The products were then sequenced using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3100 DNA sequencer (Applied Biosystems). The protocols used were as described in one of our previous study (Liu et al. 2005).

The chi-square test was used to check if genotype distributions of the samples were in Hardy–Weinberg equilibrium. The statistical significance of the differences in the allele frequency distributions was estimated using the program Clump 2.2 (Sham and Curtis 1995). The *P* values were corrected as they were assessed using the Monte Carlo approach rather than the $\times 2$ distribution (Sham and Curtis 1995). Each computation was performed with at least 100,000 simulations. The odds ratio and relative risk (95% CI) were calculated using EPI (Ver. 5). The frequencies of multiple marker haplotypes were estimated using both the EHPLUS program (Zhao et al. 2000) and PHASE 2.02 (Stephens et al. 2001). The standardized measure of linkage disequilibrium (LD) for each pair of markers, denoted as D′, was estimated by 2LD software (Zapata et al. 2001). Linkage disequilibrium (LD) plots were generated by Haploview (Barrett et al. 2005). All tests were two tailed and significance was accepted at $P < 0.05$. We also combined our data with the data from Kanazawa et al. (2007) and Watanabe et al. (2007) using meta-analysis. The method was described in our previous studies (Li et al. 2006). The SNP rs2271188, which was investigated in Kanazawa's study, was excluded from the analysis because of low heterozygosity in our samples.

## Results

Genotype distributions of the SNPs were in Hardy–Weinberg equilibrium except rs2271189 in cases ($P = 0.0006$) (Supplementary Table II). The results showed that there was no evidence of significant association for rs705708 and rs2271189. However, we found rs773123 with low heterozygosity of 83% "TT" genotype and 17% "AT" genotype. It is located seven bases downstream of the synonymous SNP (rs2271189, Arg) in the 27th exon. Kanazawa et al. 2007 reported rs773123 was not associated with schizophrenia in Japanese population. However, there was a big difference on allele frequency at rs773123 between Asian and Caucasian populations (such as the T [Cys] allele is 69% in Kanazawa et al.'s study and 92% in our study). We detected evidence of statistically significant difference in the allele frequency distributions between patients and controls with *P* value 0.034 after Monte Carlo simulation (OR 0.41, 95% C.I. 0.18–0.96) (Table I). The subgroups of male patients and patients with age at onset $< 45$ showed evidence of significant association with schizophrenia with *P* values of 0.0046 and 0.0055, respectively (Table II). The *P* values from genotypic analysis were even slightly smaller than the *P* values from allelic analysis (Tables I and II). This nonsynonymous SNP can lead to the change of coding protein residue from AGC[Ser] to TGC[Cys]. Strong linkage disequilibrium was observed between rs2271189 and two other SNPs

Table I. Results of case-control analyses for each SNP.

| | | | | Patients | | Controls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SNPs[a] | Alleles | Distance[b] | Function | Allele1[c] | Allele2[d] | Allele1[c] | Allele2[d] | *P* value | Odds ratio (95% CI) | Relative risk (95% CI) |
| rs705708 | A/G | 0 | intron | 74 (48.68%) | 78 (51.32%) | 132 (42.86%) | 176 (57.14%) | 0.24 | 1.26 <0.84–1.90 > | 1.14 <0.92–1.40 > |
| rs2271189 | C/T | 6.078 | synonymous [Arg] | 119 (61.98%) | 73 (38.02%) | 208 (62.65%) | 124 (37.35%) | 0.88 | 0.97 <0.66–1.43 > | 0.99 <0.86–1.14 > |
| rs773123 | A/T | 6.085 | nonsynonymous, [Ser] to [Cys] | 7 (3.65%) | 185 (96.35%) | 28 (8.43%) | 304 (91.57%) | **0.034** | 0.41 <0.18–0.96 > | 0.43 <0.19–0.97 > |
| | | | | | | | | **0.028**[e] | | |

[a]NCBI SNP Cluster ID.
[b]Positions of SNPs are shown as distances (kb) from rs705708.
[c]Number of the first polymorphism alleles in alphabetical order for each SNP.
[d]Number of the second polymorphism alleles in alphabetical order for each SNP.
[e]*P* value from the genotypic analysis of AT vs. TT (OR = 0.39 <0.16, 0.93 >). *P* value <0.05 is in boldface.
The experimental work and statistical analyses were carried out during 2003–2004. Eight SNPs were selected for genotyping on a small set of samples, and only the SNPs with evidence of association were replicated in the total Caucasian subjects.

Table II. Results of different sex and age at onset groups.

| SNPs/Sex/Age at onset | *P* values | Odds ratio (95% CI) |
|---|---|---|
| rs705708 | | |
| Male | 0.46 | 1.19 (0.74,1.93) |
| Female | 0.47 | 1.33 (0.61,2.93) |
| Early age at onset | 0.62 | 1.12 (0.72,1.73) |
| Late age at onset | 0.43 | 1.76 (0.41,7.51) |
| rs2271189 | | |
| Male | 0.56 | 0.87 (0.54,1.41) |
| Female | 0.92 | 0.97 (0.49,1.91) |
| Early age at onset | 0.87 | 0.97 (0.63,1.48) |
| Late age at onset | 0.93 | 1.06 (0.26,4.39) |
| rs773123 | | |
| Male | **0.0046** | 0.09 (0.01,0.71) |
| | **0.0034**[a] | |
| Female | 0.44 | 0.55 (0.12,2.59) |
| Early age at onset | **0.0055** | 0.21 (0.06,0.7) |
| | **0.0039**[a] | |
| Late age at onset | 0.41 | 1.5 (0.16,13.75) |

[a]*P* value from the genotypic analysis of AT vs. TT. Early age at onset group was defined as individuals with age at onset <45.

($D' > 0.97$); however, LD between rs705708 and rs773123 was very weak (Supplementary Table III and Supplementary Figure). Meta-analysis of each SNP showed no evidence of significance (Supplementary Table IV). There was no evidence of significance for either overall or individual haplotypic analysis (overall $P = 0.068$) for the haplotypes with frequencies >5% in both cases and controls. However, the haplotype (GCT) showed a *P* value of 0.047 with frequency of 0.01 in cases and frequency of 0.054 in controls (Table III).

## Conclusion

To conclude, our study showed evidence of association between the nonsynonymous SNP (rs773123) with schizophrenia in the Caucasian population, in particular in male patients and patients of early age

Table III. Results of multi-marker haplotypes of the case-control study in the Caucasian population.

| Haplotype[a] | Frequency in cases | Frequency in controls | *P* value[b] |
|---|---|---|---|
| 111 | 0.0801 | 0.0401 | 0.10 |
| 121 | 0.4298 | 0.3609 | 0.19 |
| 211 | 0.455 | 0.5122 | 0.30 |
| 212 | 0.01 | 0.0536 | **0.047** |
| Overall *P* value, on 3 degrees of freedom = 0.068 (chi-square = 7.1291) | | | |

[a]For each haplotype, alleles are concatenated according to the order rs705708, rs2271189 and rs773123. 1 = the first polymorphism allele; 2 = the second polymorphism allele, as shown in Table I.
[b]*P* values <0.05 are in boldface italic.
The haplotypes with frequencies <0.05 in both cases and control were not shown.

at onset. To our knowledge, this is the first association study between *ERBB3* and schizophrenia in Caucasian population. Further study with large sample size should be carried out. The involvement of the epidermal growth factor receptor family genes including *ERBB3* in the pathogenesis of schizophrenia deserves further investigation.

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## Statement of interest

None.

## Electronic-database information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ for *ERBB3*;

Online dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ for *ERBB3*;

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ for *ERBB3*.

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**CASE REPORT**

# Obsessive-compulsive disorder followed by psychotic episode in long-term ecstasy misuse

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**Abstract**

*Aim.* We report the case of two young subjects who developed an obsessive-compulsive disorder (OCD) during a heavy use of ecstasy. After several months of discontinuation of the drug, major depression with psychotic features developed in one subject and a psychotic disorder in the other individual. No mental disorder preceded the use of ecstasy in any subject. *Findings.* A familial and personality vulnerability for mental disorder was revealed in one subject, but not in the other, and all physical, laboratory and cerebral NMR evaluations showed normal results in both patients. Remission of OCD and depressive episode or psychotic disorder was achieved after treatment with a serotoninergic medication associated with an antipsychotic. *Conclusions.* The heavy long-term use of ecstasy may induce an alteration in the brain balance between serotonin and dopamine, which might constitute a pathophysiological mechanism underlying the onset of obsessive-compulsive, depressive and psychotic symptoms. The heavy use of ecstasy probably interacted with a vulnerability to psychiatric disorder in one subject, whereas we cannot exclude that an "ecstasy disorder" ex novo affected the other individual.

**Key words:** *Ecstasy, obsessive-compulsive disorder, major depression, psychosis, serotonin*

## Introduction

The use of ecstasy (MDMA) can induce acute psychological effects including: (1) generally, euphoria and reduction of negative thoughts; (2) sometimes, hyperactivity, flight of ideas, insomnia, hallucinations, depersonalisation, anxiety, agitation and bizarre behaviour; (3) occasionally, panic attacks, delirium, or brief psychotic episodes (Green 2003).

In addition, MDMA shows long-term effects, which outlast the actual drug experience by months or years, such as cognitive impairment, greater impulsivity, panic attacks, recurrent paranoia, hallucinations and severe depression (Kalant 2001; Green 2003).

An increase in serotonin (5-HT) and dopamine (DA) release is the major mechanism underlying the acute mental effects of ecstasy (Kalant 2001), whereas the long-term effects have been suggested to depend on a decrease of serotoninergic function.

This damage has been clearly demonstrated in animal experiments (Green 2003; Gouzoulis-Mayfrank and Daumann 2006). In humans, one postmortem study (Kish et al. 2000) reported severe depletion (50–80%) of striatal 5-HT and 5-HIAA in the brain of a 26-year-old male subject who had regularly taken MDMA for 9 years. Moreover, several studies found a reduction of 5-HIAA in the cerebrospinal fluid of ectasy users, and brain imaging studies suggested a brain damage and glial proliferation in heavy MDMA users (Gouzoulis-Mayfrank and Daumann 2006). Therefore, some evidence suggests that a long-term effect of MDMA might induce brain alterations, particularly involving the serotoninergic system. However, some methodological problems suggest caution to infer a causal relationship between MDMA use, cerebral alterations and the onset of psychopathological conditions (Curran 2000).

We present the clinical history of two patients who showed the onset of obsessive-compulsive disorder (OCD) after a long period of use of ecstasy alone. Moreover, many months after discontinuation of MDMA, a depressive episode with psychotic features developed in one patient and a psychotic disorder in the other.

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### Case one

A 16-year-old girl used ecstasy for 1 year (four to five tablets per week; total lifetime intake of about 200 tablets). After the first 2–3 months of MDMA consumption, the patient began to spend most of her time performing complex rituals before leaving or after returning home. The compulsive behaviour was associated with obsessive thoughts: obsessive phobias, numerical, symmetry and order obsessions, over-crowding of thoughts accompanied by poor insight and resistance. The disorder significantly interfered with her daily life: even automatic activity such as writing or walking became influenced by exact, compulsive rules. The great interference of compulsive behaviour led the patient to abandon the school. She discontinued MDMA use because she considered ecstasy responsible for the OCD. After discontinuation, the OCD improved but did not disappear. Three months later, the patient was hospitalised for the onset of a severe depressive episode with psychotic features and worsening of the OCD. Anamnestic information did not report the existence of mental disorders before the ecstasy use. Moreover, clinical evaluation showed the presence of major depression and delusional disorder in relatives. Finally, the patient was diagnosed, using the Structured Interview for DSM-IV personality disorders (Pfohl et al. 1997), as affected by a borderline personality disorder. All physical, laboratory and cerebral NMR evaluations failed to show abnormal results. After 2 months of treatment with an antidepressant (clomipramine) and an antipsychotic (olanzapine), remission of depressive episode and OCD was achieved.

### Case two

A 23-year-old man started to use MDMA at 20 years of age and stopped the drug use after more than 2 years of consumption (one to two tablets per week for a total lifetime intake of about 150 tablets) because of the sudden onset of obsessions of contamination. The obsessions were associated with a compulsive potomanic behaviour, justified by the patient's need for "inner" purification. The severity of symptoms induced a water intoxication and the patient was hospitalised in a medical ward. After discharge, the obsessions of contamination worsened and were associated with different and complex compulsive acts of purification which caused great interference in his daily life; particularly, he was unable to work and to attend his daily activities. Eight months after MDMA discontinuation, a psychotic disorder developed and the patient was hospitalized in a psychiatric clinic. All physical, laboratory and cerebral NMR evaluations failed to show abnormal results. Anamnestic information and clinical evaluations did not support the existence of a mental or a personality disorder before the use of ecstasy. Moreover, no mental disorder was found in any relative. He was treated with an antipsychotic (risperidone) and a serotoninergic antidepressant (sertraline). A complete remission of both psychotic and obsessive-compulsive symptoms was achieved only after 1 year of treatment.

## Discussion

These case reports raise the question about the causal relationship between ecstasy misuse and onset of mental disorders. It is not easy to answer the question of whether MDMA induced psychopathological conditions in these subjects. However, we must take into account several findings.

No mental disorder affected the two patients before the use of ecstasy. Therefore, we can exclude that they used ecstasy as a self-medication for a preexisting depressive or anxiety disorder, as suggested by previous studies (Curran 2000; Lieb et al. 2002; Huizinik et al. 2006).

During the abuse of ecstasy, the two patients did not use any other drugs. Almost only poly-drug abusers were evaluated in previous studies investigating psychopathological effects of ecstasy. Concomitant opiate and alcohol addiction was found to be a risk factor for developing mental disorders in ecstasy users (Schifano et al. 2000).

The two patients first developed OCD after long-term use of ecstasy. In agreement with this finding, in a previous study Parrott et al. (2001) observed higher scores in OC subscale of SCL-90 in heavy ecstasy poly-drug users than in control subjects; more recently (Lieb et al. 2002), the prevalence of OCD was found to be twice as frequent in MDMA users than in non-users. However, the onset of OCD followed the drug abuse in only a minority (16%) of ecstasy users.

A dysfunction of the serotoninergic system has been hypothesized to underline the development of OCD in non-addicted subjects (Aouizerate et al. 2004; Chamberlain et al. 2005). In fact, the specific treatment response to serotoninergic medications and the transient exacerbation of symptoms after a pharmacological challenge with specific 5HT agonists suggest that an imbalance of 5HT is involved in the pathogenesis of OCD (Zohar et al. 2000; Micallef and Blin 2001).

A psychotic depressive episode or a psychotic disorder developed in the patients some months after MDMA discontinuation. Previous studies reported a high prevalence (nearly 30%) of depression

in MDMA users (Schifano et al. 1998; Topp et al. 1999; Lieb et al. 2002). Interestingly, in a high percentage (40%) of the ecstasy users major depression developed after drug abuse (Lieb et al. 2002). Also psychosis is a well-documented psychopathological condition associated with MDMA use (McGuire et al. 1994; Schifano et al. 1998; Topp et al. 1999; Curran et al. 2004). However, psychosis induced by ecstasy use is generally a short-lasting disorder (Topp et al. 1999; Curran et al. 2004), whereas in our patient the psychotic symptoms remitted after 1 year of treatment.

The association between OCD and major depression or psychotic disorder is a well-documented clinical condition in non-addicted patients (Aouizerate et al. 2004; Chamberlain et al. 2005**)**. Recently, this association has been investigated in neurophysiological, neuropsychological and neuroimaging studies (Aouizerate et al. 2004; Poyurovsky et al. 2004; Chamberlain et al. 2005; Bottas et al. 2005). The 5HT/DA dysfunction in cortical-striatal-thalamic-cortical pathways has been suggested as a possible pathophysiological mechanism underlying the association between obsessive-compulsive, depressive and psychotic symptoms in non-addicted patients.

Acutely, MDMA is known to increase 5HT and DA activity, whereas long-term use decreases 5HT function (this effect was observed after using ecstasy 25 times) (McCann et al. 1998). The long-term effect on the DA system appears to be more controversial (Green et al. 2003; Goñi-Allo et al. 2006). Therefore, the MDMA effect on the serotoninergic and probably on the dopaminergic system might be involved in the onset of psychopathological conditions, such as major depression, anxiety and psychotic disorders, in ecstasy users. In accord with this hypothesis, a higher incidence of these disorders was found in subjects with pre-existing MDMA misuse (Lieb et al. 2002), particularly in heavy users (Schifano et al. 1998).

Mental disorders do not develop in all ecstasy users. Therefore, a pre-disposition to psychiatric illnesses may need to exist for psychopathological symptoms to develop in some ecstasy users.

In view of these observations, a specific causal role as a primary risk factor for the onset of mental disorders in our patients cannot be attributed to ecstasy with certainty, even though heavy use of the drug lasted several months and was followed by mental disorders. However, in one of our patients ecstasy misuse probably interacted with a vulnerability to psychiatric disorders. In fact, the girl was affected by a borderline personality disorder which predisposes people to suffer from several mental disorders, such as substance abuse, depression, anxiety and eating disorders (Skodol et al. 2002; Lieb et al. 2004). Moreover, this patient also showed a familial vulnerability to major depression and psychotic disorder (Shih et al. 2004). In contrast, the young man did not show any personality or familial predisposition to mental disorders. However, we cannot exclude that this patient might have been vulnerable to psychotic disorders, because his psychotic disorder lasted much longer than the usual ecstasy-induced psychosis (Curran et al. 2004). Therefore, the onset of the mental disorder in the girl was probably induced by the interaction between ecstasy misuse and personality and familial vulnerability rather than representing an ex novo "ecstasy disorder". In contrast, it is difficult to say whether an ex novo "ecstasy disorder" affected the young man.

## Conclusion

Our case reports suggest that the assessment of personality and psychiatric family history in ecstasy users might be a useful clinical tool: (1) to identify a predisposition for the development of mental disorders; (2) to verify the association between ecstasy abuse and the onset of a specific mental disorder in vulnerable individuals; and (3) to clarify whether ecstasy may induce mental disorders in absence of other risk factors.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**CASE REPORT**

# Bispectral index monitoring during dissociative pseudo-seizure

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**Abstract**

Severe forms of dissociation or conversion can lead to events clinically often described as pseudo-seizures. Borderline personality disorder is a clinical condition which is often accompanied by a high susceptibility for dissociation and dissociative states are characterized by memory disturbance and perceptual alterations. We report a case of a patient with a complete anaesthesia, paralysis and amnesia for about 1 h. Within this time period we measured a bispectrum EEG index called BIS. Deepest recorded BIS value was 47. The described pattern of short but deep BIS reductions is completely discongruent to the findings during physiological sleep, during general anaesthesia, but very similar (and even more pronounced) to those during self-hypnosis. This makes former assumptions plausible that hypnosis and severe forms of dissociation (or conversion) may share common aetiologies.

**Key words:** *Bispectral index, dissociation, hypnosis, pseudo-seizure, EEG*

## Introduction

Severe forms of dissociation or conversion can lead to events clinically often described as pseudo-seizures. Borderline personality disorder is a clinical condition which is often accompanied by a high susceptibility for dissociation and dissociative states are characterized by memory disturbance and perceptual alterations (Ludascher et al. 2007). As an extreme variant, pseudo-seizures often appear as events with complete anaesthesia, paralysis and amnesia. In such cases patients have a high risk of iatrogenic complications resulting from a treatment of an assumed status epilepticus (Pakalnis et al. 1991).

Here, we report a case of a patient with a complete anaesthesia, paralysis and amnesia for about 1 h. Within this time period we measured a bispectrum EEG index called BIS, which is usually recorded during general anaesthesia to gain information about the 'depth of anaesthesia' and to prevent awareness. In short, bispectrum analyses are used to search for nonlinear interactions. The well-known Fourier transform of the second-order cumulant is the traditional power spectrum. To gain additional information about phases or coherences a third-order cumulant-generating Fourier transform can be performed which is called bispectrum. Bispectral index is a squared normalized version of the bispectrum yielding dimensionless numbers between 0 (indicating the absence of brain electrical activity) and 100 (indicating an awake patient) (Sigl et al. 1994).

## Case report

We report a female 32-year-old patient with a diagnosis of borderline personality disorder according to DSM-IV criteria without somatic comorbidity, who was in inpatient treatment at our institution. She had been suffering from pseudoepileptic seizures for about 2 years, neurological causes had been carefully ruled out. Body weight was 88 kg, height 163 cm. In initial clinical examination (12/2006) she presented without pathological findings. No pathological alterations were detected in routine laboratory investigations. Drug screening and pregnancy test were also negative. She received a stable medication with valproate 1200 mg/day, citalopram 20 mg/day and quetiapine 150 mg/day.

The patient initially hyperventilated for about 5 min and then presented with a pseudo-seizure

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with complete paralysis, anaesthesia, and unconsciousness for about 60 min. Glasgow Coma Scale (GCS) was 3 for the whole time period. She had a complete amnesia for this event. Neurological status was completely normal after returning to full vigilance.

BIS was recorded for about 12 min (see Figure 1) from 7 to 19 min after pseudo-seizure initiation. Deepest recorded BIS value was 47. The signal quality index (SQI) was at maximum during the whole recording and the BIS number was validated by the device (solid form displayed) throughout the measurement. No one touched the patient or attempted to arouse the patient during the time of the recording.

## Discussion

To the best of our knowledge this is the second report quantifying BIS during a dissociative pseudo-seizure with complete paralysis, analgesia and amnesia. There is just one case report of a patient suffering from a pseudo-seizure and a subsequent BIS recording. In the case of Donnelly et al. (2006) the patient had received hypnotic medication (benzodiazepines and phenytoin) before monitoring and time course was not graphically demonstrated. They described that immediate readings gave a BIS number in the mid-80s stabilizing between 92 and 95. Then, presumably after a short time period recording was stopped.

Physiological sleep and general anaesthesia share about the same levels of total BIS reduction: Slow wave sleep BIS levels are usually between 40 and 60, REM sleep levels between 50 and 60 (Sleigh et al. 1999; Nieuwenhuijs et al. 2002). During general anaesthesia (and, e.g., during anaesthesia for electroconvulsive therapy) BIS values drop to values between 40 and 60 (Johansen 2006; Sartorius et al. 2006). The main point with respect to this report is the slow and uniformly continuous manner of BIS reductions in sleep and anaesthesia. In our case the described pattern of short but deep BIS reductions (Figure 1) is completely discongruent to the findings during physiological sleep or during general anaesthesia, but very similar (and even more pronounced) to those during self-hypnosis (Burkle et al. 2005). Burkle et al. showed a graphical time course with three abrupt BIS droppings below 80 during the central 50 min of a surgical procedure (a mastectomy and a sentinel node biopsy in a female patient suffering from an invasive ductal breast carcinoma). This makes former assumptions plausible that hypnosis and severe forms of dissociation (or conversion) may share common aetiologies. Such associations have already been claimed for hysterical and hypnotic blindness (Sackeim et al. 1979) or hysterical conversion and hypnosis in general (Babinski 1914; Vuilleumier 2005).

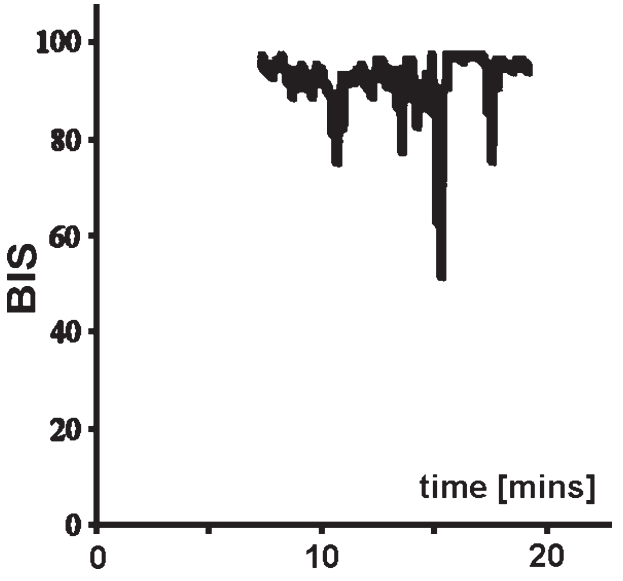


Figure 1. BIS-values during case of pseudo-seizure.

The time-pattern of multiple, short-term, discontinuous and striking BIS-'droppings' in our case and in the case of self-hypnosis (Burkle et al. 2005) is surprisingly different from the stable pattern of analgesia, paralysis, and unconsciousness, which were continuous and without abrupt fluctuations.

From an anaesthesiological point of view 'anaesthesia' can be split up into a triangle consisting of 'hypnosis', analgesia and areflexia. BIS reflects mainly 'hypnosis' and not analgesia or areflexia. The anaesthesiological term 'hypnosis' can be further split up into awareness, consciousness and loss of response or recall (amnesia) (Rosow et al. 2001). Therefore, a continuous BIS below a certain level seems not to be necessary (but sufficient) to reach 'hypnosis'.

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## Statement of interest

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**CASE REPORT**

# Myocarditis after overdose of conventional antipsychotics

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**Abstract**
Many reports are available regarding myocarditis induced by clozapine; however, it is an uncommon adverse effect with conventional antipsychotics. We are presenting a case who developed myocarditis after overdose of haloperidol and recovered in a few days after stopping the drug.

**Key words:** *Myocarditis, haloperidol*

## Introduction

Myocarditis is not very common after conventional antipsychotics use, though many case reports describe its emergence after clozapine treatment (Kilian et al. 1999; Coulter et al. 2001; Grenade et al. 2001). Diagnosis of myocarditis is based upon electrocardiographic changes (transient ST-T wave abnormalities), elevated cardiac enzymes and sometimes signs of heart failure (Kirpekar et al. 2001; Wynne and Braunwald, 2005). We describe a patient who developed myocarditis after overdose of conventional antipsychotic drugs.

A 65-year-old female presented to the emergency room with complaints of unconsciousness and difficulty in breathing. Her relatives reported that she had taken an overdose of antipsychotics which were prescribed to her for the treatment of schizophrenia (haloperidol 15 mg/day, chlorpromazine 100 mg/day and trihexyphenidyl 6 mg/day) since the age of 37 years. According to her family members she had few exacerbations in the initial 20 years of contracting the illness, but was doing well on the medications as described above and did not suffer any exacerbation over the past 8 years. Although she was upset for the past few days as she had to take the pills everyday, she never disclosed any wish to harm herself or symptoms suggestive of depression. She was performing all her chores and was under regular follow-up of a psychiatrist. Past history and other medical records (reports of routine laboratory investigations, ECGs that were repeated annually) did not have evidence of any other chronic medical disorder. When empty strips of drugs were examined it appeared that she had consumed 1200 mg of chlorpromazine, 80 mg of haloperidol along with 10 mg of trihexyphenidyl in a single day, just before she was found unconscious. On examination her pulse rate was 92/min and it was irregular; right brachial systolic blood pressure was 70 mmHg, while diastolic blood pressure was not recordable; she was afebrile and respiration was laboured and shallow. She appeared pale and was responding only to deep painful stimuli. Chest examination revealed bilateral coarse crepitations and neurological examination showed sluggish deep tendon reflexes with planter reflex equivocal bilaterally. A venous blood sample was drawn for investigations.

Due to her condition, 100% oxygen was started after securing airway at a rate of 2–3 l/min, intravenous dopamine infusion was started at a rate of 5 μg/kg per min. Immediate EKG was ordered and showed sinus rhythm, generalized ST depression along with T wave inversion in V3–V6 leads along with multiple ventricular ectopic beats. Arterial blood gas analysis gave evidence of metabolic acidosis that was treated with intravenous Sodabicarb. She was shifted to the intensive care unit, where rate of dopamine was increased to 8 μg/kg per min with Ringer Lactate infusion.

Her blood sample was sent for routine haematological and biochemical examination with electrolyte

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assessment and serum CPK. Results reveled that her hemoglobin was 11.6 g% (12–16 g%); TLC was 10,870/$mm^3$ (4000–11,000/$mm^3$); DLC: polymorphs 60% (55–65%), lymphocytes 38% (30–40%), eosinophils 2%; blood urea was 65 mg% (20–40 mg%); serum $Na^+$ was 134 mEq/l (130–145 mEq/l), serum $K^+$ was 4.0 mEq/l (3.5–5 mEq/l); random blood sugar was 145 mg%, and serum CPK was 1873 U/l (50–150 U/l). Lipid profile was also within normal limits. A diagnosis of drug-induced myocarditis was made and treatment continued with the addition of platelet aggregation inhibitors.

By the second day she improved; BP escalated to 114/74 mmHg, her chest cleared, and level of consciousness improved. She was started with nasal tube feeding, and on the third day respiratory support was withdrawn and dopamine stopped. Her vitals improved further; ECG and CPK level came down to normal after 4 days (1168 U/l on the second day; 698 U/l on the third day and 110 U/l on the fourth day). She was discharged after 6 days with risperidone 2 mg/day after disappearance of all symptoms.

## Discussion

In this case, diagnosis of myocarditis was made upon the clinical conditions of shock, ECG changes and cardiac enzymes. Other causes were excluded as the patient did not have any history of fever, substance use or exposure to any drug other than these psychotropic drugs that could induce myocarditis (Wynne and Braunwald, 2005), and the symptoms resolved after stopping the drug (Wynne and Braunwald, 2005). Myocarditis has been frequently reported after clozapine therapy (Kilian et al. 1999) and less commonly with other atypical antipsychotics e.g., quetiapine (Roesch-Ely et al. 2002); however, only 11 cases could be found for haloperidol and 14 for chlorpromazine (Coulter et al. 2001). It may occur due to type 1 hypersensitivity if it occurs early in the treatment (Kilian et al. 1999). Supporting reports are available that describe drug-induced myocarditis associated with peripheral eosinophilia and leukocytic infiltration in the myocardium (Kilian et al. 1999; Kirpekar et al. 2001; Ansari et al. 2003). However, we did not find peripheral eosinophilia and a histopathological report of myocardium was not available, we opine that direct myocardial damage, as hypothesized by Kilian et al. (1999), occurred in this case. This possibility is further supported by the elevated serum CPK level. In these circumstances, serum CPK elevation could occur in the presence of neuroleptic malignant syndrome, after skeletal muscle trauma, cardioversion, convulsions, etc. However, we did not find any evidence of skeletal muscle trauma, or convulsion, and patient did not have any feature suggestive of neuroleptic malignant syndrome, e.g., rigidity, fever, labile blood pressure, etc., during hospital stay. It was made sure from the records that she had not received any intramuscular injection. Since she was brought in in a state of shock, an immediate intravenous line was established and all drugs were injected only via this route. Moreover, the pattern of normalization of serum CPK is very typical to that seen after myocardial damage (Antman and Braunwald 2005). Since the patient had been on the same drugs for a fairly long period without any sign of myocardial damage in the past, it is possible that the higher doses of the drugs induced the toxic reaction in the myocardium precipitating the clinical syndrome.

There remains a possibility that the patient had overdosed to deliberately harm herself. As many as 10% of schizophrenics commit deliberate self harm, but it is more common in subjects with frequent exacerbations, poor drug responders, being young, male, recent rejection, functional impairment, dependence on treatment, hopelessness, etc. (Norquist and Narrow 2000). Few of these factors were found in this patient; however, it is still difficult to comment whether it was such an attempt, as the patient did not disclose it on interview. However, it should be remembered that this patient was under our treatment only for a short duration and had a comorbid serious medical illness, and this situation might have prohibited the development of a therapeutic alliance.

In conclusion, we feel that even those drugs that are considered non-lethal should be prescribed with caution and should be kept away from patients, especially where a risk factor of overdosing is present.

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**CASE REPORT**

# A case of non-SIADH-induced hyponatremia in depression after treatment with reboxetine

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**Abstract**

Hyponatremia is a well-known side effect of antidepressant treatment with serotonin reuptake inhibitors (SSRI) or combined serotonin and noradrenaline reuptake inhibitors (SNRI), and is linked to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) in most cases. In contrast, only very few data are available on hyponatremia following treatment with selective noradrenalin reuptake inhibitors (NaRI). In this report, we describe the case of a patient who developed severe hyponatremia after treatment with reboxetine. However, extensive laboratory testing did not reveal inappropriate secretion of ADH, suggesting that SIADH did not account for hyponatremia in our case. Proposing further examination of the underlying pathomechanism of hyponatremia as a side effect of NaRIs, we discuss the importance of careful monitoring of serum sodium levels in patients treated with NaRIs.

**Key words:** *Hyponatremia, SIADH, antidepressants, depression, reboxetine*

## Introduction

Sufficient evidence has been provided showing that recent antidepressants such as selective serotonin reuptake inhibitors (SSRIs), selective serotonin/noradrenaline reuptake inhibitors (SNRIs) or noradrenaline reuptake inhibitors (NaRIs) are safe and well tolerated and therefore particularly suitable for treatment of depression in the elderly. However, treatment with SSRIs and SNRIs (e.g., Kirby and Ames 2001; Romero et al. 2007; Singh 2007) is sometimes accompanied by hyponatremia, which is considered a potential side effect of antidepressants and linked to the syndrome of inadequate antidiuretic hormone secretion (SIADH) in the majority of cases ( Spigset and Hedenmalm 1996; Bouman et al. 1998; Leung et al. 1998; Strachan and Shepherd 1998; Kirby and Ames 2001), leading to a retention of solute-free water. Hyponatremia is a phenomenon often observed in the elderly, with drug side effects and polydipsia (Kirby and Ames 2001) being possible causes. Drugs may trigger the inappropriate secretion of the antidiuretic hormone, also known as vasopressin, and may thus lead to severe side effects, e.g., seizures and coma (e.g., Siegel et al. 1998). Compared to SSRIs and SNRIs there are only few data available on hyponatremia following treatment with NaRIs (Ranieri et al. 2000; Sand et al. 2002; Abdelrahman et al. 2003), some of them provided by reports to the manufacturing company (Schwartz and Veith 2000). These cases comprise observations of SIADH-induced hyponatremia in reboxetine-medicated elderly patients.

In this report, we present the case of another patient, who developed hyponatremia after treatment with reboxetine, however without any signs of inadequate ADH secretion. Remarkably, this patient had developed hyponatremia in a previous episode after treatment with an SNRI.

## Case report

Mrs K. was a 73-year-old patient who had been suffering from recurrent major depression for many years. When she was admitted to the Department of Psychiatry for the first time, antidepressant

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treatment with venlafaxine had led to severe hyponatremia with serum sodium levels of 115 mmol/l (reference range: 132–145 mmol/l). At that time, the patient had symptoms such as fatigue, disorientation, cognitive deficits, and delusions. Although antidepressant treatment with venlafaxine was discontinued and changed to reboxetine up to 8 mg/day, hyponatremia and hyponatremia-associated symptoms persisted until reboxetine dosage was reduced to 4 mg/day. During the following 2-year interval, the patient kept stable with regard to depression. Serum sodium levels during reboxetine treatment with a daily dose of 4 mg were within normal range. At this time, venlafaxine was considered to be the most likely cause for hyponatremia in this patient.

Two years later, depression recurred despite continuation of antidepressant treatment and the patient was again admitted to our hospital. At the time of admission her serum sodium levels were within a normal range. Antidepressant medication was changed to mirtazapine up to 60 mg and later to clomipramine up to 150 mg/day. At all times laboratory monitoring showed normal serum sodium levels. After a temporary improvement of depressive symptoms, antidepressant treatment again had to be changed because of another relapse. Since the patient had been stable during reboxetine treatment for 2 years and had a history of hyponatremia associated with SSRI/SNRI treatment, another course of reboxetine was initiated. However, after re-administration of reboxetine at a daily dose of 4 mg, serum sodium dropped to 123 mmol/l within 7 days. Severe hyponatremia-associated symptoms such as psychotic symptoms and aggressive behaviour recurred. After discontinuation of antidepressant treatment and substitution of sodium chloride the symptoms disappeared within a few days and the serum sodium returned to a low-normal level. Administration of haloperidol did not influence the patient's symptomatology.

Since hyponatremia is one of the most common electrolyte disorders in elderly patients and can occur due to a number of different reasons, extensive clinical and laboratory examinations were carried out during this hospitalisation period to rule out any other medical reason or contributing factors, e.g., tumours, respiratory diseases, acute nervous system or metabolic diseases potentially influencing volume and sodium level regulation (Ranieri et al. 2000). Apart from arterial hypertension the patient's medical history was unremarkable. For the last 5 years her blood pressure had been treated with metoprolol 95 mg and hydrochlorothiazide 12.5 mg, and was stable. Diagnostic screening for infections, tumours or neurological diseases was negative. The patient's EEG was intermittently slowed in the left occipital brain region, while MRI of the brain was normal with respect to the patient's age. Mini Mental State Examination at the time of admission was 28 points. There was no evidence of psychogenic polydipsia (Kirby and Ames 2001), volume depletion or polyuria. Ultrasonography of the kidney revealed only age-related changes. The patient suffered from mild hyperglycemia (HbA1c, 5.1%; reference range, 3.4–4.7%). The patient had no history of alcohol abuse, $\gamma$-GT (25 U/l, reference range, $<39$) and lipase (52 U/l, reference range, 13–60 U/l) were within the normal range. TSH (2.79 U/l, reference range, 0.35–4.5 U/l) was also within normal limits.

Moreover, laboratory blood and urine tests were carried out to screen for SIADH. However, serum levels of antidiuretic hormone (ADH) (1.3 and 1.7 pg/ml, reference range, 1.1–4.5 pg/ml), serum-osmolality (291 and 297 mOsm/kg, reference range, 280–320 mOsm/kg), urine specific gravity (1.010 g/ml, reference range, 1.010–1.025 g/ml), urine osmolality (351 mOsm/kg, reference range, 50–1200 mOsm/kg) and urine sodium (63 mmol/l, reference range, 54–150 mmol/l) were within the normal range. Therefore, SIADH was ruled out as a potential reason for hyponatremia in our patient.

## Discussion

In the present case, antidepressant treatment with reboxetine was followed by severe hyponatremia accompanied by typical hyponatremia-associated symptoms such as disorientation, delusions and agitation. This constellation is in accordance with four previous reports also showing the occurrence of hyponatremia during reboxetine treatment (Ranieri et al. 2000; Schwartz and Veith 2000; Sand et al. 2002; Abdelrahman et al. 2003). In line with earlier studies, symptoms improved after the administration of sodium chloride, while no response to antipsychotic medication was observed (Brown et al. 1983; Verghese et al. 1998; Kawai et al. 2002). This underlines the notion that observed neuropsychological symptoms seem to be hyponatremia-related. However, further clinical and laboratory parameters failed to detect any signs of inadequate ADH secretion. Thus, in the present case hyponatremia has unlikely been induced by SIADH. Apart from that other medical reasons for hyponatremia were ruled out by means of extensive laboratory and clinical examinations. It is remarkable that initially our patient had developed hyponatremia during treatment with an SNRI, suggesting a particular vulnerability to this side effect. It could also be speculated that subsequent treatment with reboxetine might have maintained hyponatremia and the

associated symptoms. Taking into account the improvement of symptoms after dose reduction, hyponatremia under reboxetine seems to be dose-dependent.

In conclusion, this is the first report on hyponatremia induced by reboxetine that is not associated with inadequate ADH secretion. The previous development of hyponatremia after treatment with SNRIs suggests an increased vulnerability to this kind of side effects in some patients, which might not be limited to antidepressant subgroups. The case underlines the necessity of close laboratory monitoring of serum sodium levels during antidepressant treatment both with SSRIs and NaRIs in elderly people – even though these compounds are usually safe and well tolerated. Therefore, further examination of the pathomechanism of hyponatremia as a side effect after NaRI treatment should be aimed at.

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**CASE REPORT**

# Misdiagnosis of bipolar disorder as borderline personality disorder: clinical and economic consequences

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**Abstract**

We report the case of a 26-year-old patient with bipolar spectrum disorder who was misdiagnosed with borderline personality disorder. In spite of trials of various psychotropic drugs and frequent, prolonged hospitalizations, the patient had remained chronically symptomatic. Following a detailed examination of the longitudinal illness course and confirmation of the diagnosis of bipolar spectrum disorder, antidepressants were discontinued and the patient was treated with lamotrigine and quetiapine. This treatment resulted in sustained euthymia and cessation of deliberate self-harm in addition to a significant reduction in utilization of health resources.

**Key words:** *Bipolar disorder, borderline personality disorder, misdiagnosis, deliberate self-harm, economic burden*

## Introduction

Borderline personality disorder occurs in 1–2% of the general population and is arguably the most common personality disorder encountered in clinical settings (Torgersen et al. 2001) affecting about 10% of psychiatric outpatients and up to 20% of inpatients (Skokol et al. 2002).

The relationship between borderline personality disorder and bipolar disorders remains controversial (Benazzi 2006; Gunderson et al. 2006; Paris et al. 2007). The overlapping symptoms between the two disorders such as affective instability, unstable interpersonal relationships, and impulsivity contribute to the diagnostic confusion. Among young people the presence of mixed episodes makes it difficult to distinguish between borderline personality disorder and an emerging bipolar disorder (Akiskal et al. 1997). Subsyndromal symptoms that occur interepisodically in patients with bipolar disorder may resemble borderline psychopathology (Akiskal et al. 1997).

The consequences of misdiagnosis of bipolar disorder as borderline personality disorder can be serious, as patients may not receive optimal treatment. We present the case of a patient with bipolar spectrum disorder who was misdiagnosed as having borderline personality disorder and discuss the clinical and economic consequences of misdiagnosis and mismanagement.

## Case history

Mrs D is a 26-year-old unemployed, happily married lady who developed symptoms of anorexia nervosa at the age of 15. The onset of symptoms coincided with her sexual abuse by a former boyfriend. Her first contact with psychiatry was at the age of 17 when she was hospitalized for anorexia nervosa. After discharge from hospital, the patient continued to struggle with symptoms of the eating disorder; however, she coped quite well over the next 3 years. The start of a new relationship with her current husband at the age of 21 appeared to trigger thoughts of her previous abuse and she developed symptoms of depression including sad mood, anhedonia, social withdrawal, disrupted sleep, decreased appetite, lack of energy, poor concentration, and thoughts of suicide. She denied having any symptoms of (hypo)mania or psychosis and there was no history of substance use disorders. Family history

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revealed presence of depression on the paternal side and anxiety disorders on the maternal side. Over a 4-year period, she was tried on multiple psychotropic medications including neuroleptics, and benzodiazepines, and antidepressants, but never received mood stabilizers. Of the antidepressants tried, five were considered adequate trials, five were discontinued due to side effects and four were not used at a therapeutic range for an adequate treatment duration. Adequacy was defined as one trial of an average daily dose of a minimum 20 mg fluoxetine equivalent for a period of 90 days (Weilburg et al. 2003). There was an initial response to some of the antidepressants but the improvement was not sustained. Of the neuroleptics used, quetiapine was reported as beneficial in the reduction of anxiety and improvement of sleep only. The patient did not report any benefit with the use of benzodiazepines. Compliance to medications did not play a role in lack of response. In addition to a pharmacological approach she also participated in emotion-focused psychotherapy with a community psychologist on a weekly basis.

Soon after the introduction of antidepressants she began to engage in self-harm initially on a weekly basis and gradually the self-mutilation in the form of cutting became a daily occurrence. There had been episodes of self-harm on a couple of occasions as a teenager but never on a consistent basis. She had made 63 visits to the emergency department over a 4-year period including five visits for drug overdoses, 16 for suicidal ideation, and 42 for self inflicted lacerations. She had required 11 admissions to the inpatient psychiatry unit, one admission to the mood disorders unit and there were at least 65 outpatient hospital visits, which did not include weekly psychotherapy sessions. She was given multiple diagnoses including post-traumatic stress disorder, anorexia nervosa-restricting type, major depressive disorder, and dependant personality disorder but borderline personality disorder remained the main diagnosis. Interestingly, a formal personality assessment using the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID II) (First et al. 1997) completed by a community psychologist showed no evidence of Axis II psychopathology.

### *Admission to the Mood Disorders Program*

Mrs D was referred to the Mood Disorders Program with a history of refractory depression and chronic suicidal ideation. She presented with symptoms of low mood, irritability, anger, racing thoughts, low energy, poor concentration, reduced interest and sleep disturbance. Her appetite was low but her weight had been stable. The Structured Clinical Interview for DSM IV (First et al. 1994) confirmed the current diagnosis of major depressive disorder and lifetime diagnoses of anorexia nervosa-restricting type, and post-traumatic stress disorder. According to the SCID-I there was no evidence of a bipolar disorder; however, she did meet the diagnostic criteria for bipolar spectrum disorder as proposed by Ghaemi et al. (2002). Using the Clinical Global Impressions Scale for use in bipolar illness (CGI-BP) (Spearing et al. 1997) for both depression and overall bipolar illness, the patient scored 6 (severely ill) on the severity of illness scale.

The patient met criteria A and B as well as six D criteria including hyperthymic personality, recurrent major depressive episodes, brief major depressive episodes, early age of onset of major depressive episodes, antidepressant wear-off and lack of response to $>3$ antidepressant treatment trials.

Her medications at the time of admission included nortriptyline 50 mg, quetiapine 100 mg, and trazodone 200 mg/day. She was weaned off the antidepressants due to lack of effectiveness. Moreover, there was evidence that antidepressants had lead to induction of mixed episodes in that after their initiation the patient reported a further decrease in mood, increased anger, irritability and racing thoughts. The quetiapine dose was optimized to 150 mg and she was started on lamotrigine which was gradually increased to 200 mg a day. During the 6 months following her discharge, she had three emergency department visits but there were no admissions to hospital. During this period she had reduced the lamotrigine dose to 150 mg due to

Table I. A proposed definition of bipolar spectrum disorder.

A. At least one major depressive episode
B. No spontaneous hypomanic or manic episodes
C. Either of the following, plus at least two items from criterion D, or both of the following plus one item from criterion D:
   1. A family history of bipolar disorder in a first-degree relative
   2. Antidepressant-induced mania or hypomania
D. If no items from criterion C are present, six of the following nine criteria are needed:
   1. Hyperthymic personality (at baseline, non-depressive state)
   2. Recurrent major depressive episodes ( $>3$)
   3. Brief major depressive episodes (on average, $<3$ months)
   4. Atypical depressive symptoms (DSM-IV criteria)
   5. Psychotic major depressive episodes
   6. Early age of onset of major depressive episode ( $<$age 25)
   7. Postpartum depression
   8. Antidepressant "wear-off" (acute but not prophylactic response)
   9. Lack of response to $>3$ antidepressant treatment trials

From: Ghaemi et al. (2002) Can J Psychiatry 47:125–134 (reproduced with permission).

financial concerns about her ability to pay for the drug costs. Once drug coverage was obtained the lamotrigine dose was increased to 200 mg and quetiapine to 300 mg/day. Over the past 2 years she has not had any visits to the emergency department and she has not engaged in self-harm behavior. Once the medications were adjusted she was classified as much improved on the CGI-BP, compared with the preceding and worst phase of illness. The patient did not report any medication side effects that interfered with her function or comfort.

### *Economic burden*

We calculated the economic burden associated with emergency room visits and hospital admissions. Over a 51-month period prior to her admission to the Mood Disorders Program, the total cost was calculated at $102,968 with an average yearly cost of $24,228. Since being diagnosed with bipolar spectrum disorder and managed with lamotrigine and quetiapine, she has had three visits to the emergency department and no admissions to hospital. The total cost incurred over this 12-month period was $627.

## Discussion

The patient's primary diagnosis prior to admission to the Mood Disorders Program was borderline personality disorder. However, a closer examination of the longitudinal course suggested a diagnosis of bipolar spectrum disorder as proposed by Ghaemi et al. (2002). Additionally the diagnosis of mixed depression should be considered (Benazzi et al. 2004). Mixed depression is based on the coexistence of a major depressive episode and at least two of three excitatory symptoms including inner tension, psychomotor agitation, and racing thoughts. This clinical picture emerged after the patient received antidepressant medications. Antidepressants are known to cause treatment refractoriness in patients with a bipolar diathesis (Sharma 2001; Sharma et al. 2005) and may contribute to suicidality by inducing mixed episodes (Balazs et al. 2006). Increased risk of suicidality in mixed depression may be mediated by irritability and psychomotor agitation (Balazs et al. 2006). When there is suspicion of a bipolar diathesis, antidepressants should be used with caution in monotherapy or in combination with mood stabilizers and/or neuroleptics. Despite trials of several antidepressants used alone or in combination with neuroleptics, she remained highly symptomatic and engaged in self-abusive behaviour on a daily basis. It is likely that both the discontinuation of antidepressants as well as the addition of lamotrigine to quetiapine contributed to the patient's ability to achieve and maintain euthymia.

Differentiating borderline personality disorder from bipolar disorder is challenging in the clinical setting particularly in regards to the overlap of affective instability and impulsivity. To further complicate the diagnostic challenge, studies demonstrate a high co-occurrence between these two disorders (Magill 2004; Paris et al. 2007). A detailed longitudinal history can help differentiate if the symptoms represent discrete episodes or a long-standing pattern of functioning. With the overlap of affective lability and impulsivity between bipolar disorder and borderline personality disorder one would expect a similar response to mood stabilizers and atypical neuroleptics. Mood stabilizers and atypical neuroleptics are effective in controlling mood fluctuations in bipolar disorder (Evins 2003; Moller and Nasrallah 2003; Hadjipavlou et al. 2004; Perlis et al. 2006); however, in borderline personality disorder these medications primarily effect impulsivity (Pinto and Akiskal 1998; Hollander et al. 2001, 2005; Zanarini and Frankenburg 2001; Frankenburg and Zanarini 2002; Hilger et al. 2003; Bogenschutz and Nurnberg 2004; Preston et al. 2004; Zanarini et al. 2004). More research will need to be done to make direct comparisons of response to medications specifically in patients without comorbid illness (Binks et al. 2006).

The financial burden associated with the misdiagnosis of bipolar disorder can be staggering, but the emotional pain and suffering that the patients and their families endure is of prime concern. Once the patient is labelled with a personality disorder, health care professionals believe that the patient is unlikely to improve, will not benefit from medication management and any treatment compliance will be poor (Lewis and Appleby 1988). Patients may feel that they are not being listened to or taken seriously. This can further exacerbate their symptoms as they become desperate to do impulsive things to be heard. Often times they give up on themselves and believe that there is no chance of improvement. This was the case with Mrs D, who felt that once diagnosed with borderline personality disorder her symptoms were not taken seriously.

Unfortunately, once the patient has been diagnosed as having a personality disorder, psychiatrists often see these patients as problematic and undeserving of medical care (Lewis and Appleby 1988; Pfohl et al. 1999; Krawitz 2004). Without the proper diagnosis and management they continue to place a large burden on the mental health system. This case emphasizes the need to be cautious about diagnosing individuals with a personality disorder based solely on a cross-sectional assessment. Importance must be placed on obtaining a detailed longitudinal history to

avoid the negative consequences that can be deleterious for both the patient and the mental health care system.

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**CASE REPORT**

# Limbic encephalitis presenting with anxiety and depression: A comprehensive neuropsychological formulation

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**Abstract**

Limbic encephalitis (LE) is a paraneoplastic neurological disorder in which, typically, the neurological symptoms occur before the cancer is diagnosed. We report on a 52-year-old male with LE who has depressive and anxiety symptoms. Cranial MRI revealed increased hippocampal signal intensities in both temporal lobes. Extensive range of symptoms concerning emotion, personality and social functioning was assessed with a comprehensive neuropsychological formulation. The neuropsychological test battery showed dysfunction of hippocampus, medial temporal lobes, limbic system and frontal diencephalic structures. The current literature about the neurological mechanisms underlying the neuropsychological findings of LE is also briefly reviewed in this report.

**Key words:** *Limbic encephalitis, anxiety, depression*

## Introduction

Limbic encephalitis is a paraneoplastic neurological disorder in which, typically, the neurological symptoms occur before the cancer is diagnosed. In a recent study, the diagnostic criteria were defined as: (i) a compatible clinical picture; (ii) an interval of less than 4 years between the development of neurological symptoms and tumour diagnosis; (iii) exclusion of other neurooncological complications; and (iv) at least one of the following: CSF with inflammatory changes but negative cytology, MRI demonstrating temporal lobe abnormalities, EEG showing epileptic activity in the temporal lobes (Gultekin et al. 2000).

Neuropsychiatric symptoms include short-term memory disturbance, epileptic seizures, confusion of acute onset, changes in personality, hallucinations, depression and cognitive disturbances. Here we report on a case of limbic encephalitis presenting with depressive and anxiety symptoms, and review the neurological substrates of psychiatric findings. We believe the case to be particularly important because of the neurospychological battery involved.

## Case report

A 52-year-old, divorced male patient with a primary level of education admitted to our hospital with complaints of fear (i.e. of staying alone at home and of strangers looking at him in the street), trembling, unsteady and choking, feeling sad, hopeless, anhedonia, suicidal thoughts and forgetfulness in December 2005. His complaints started, with forgetfulness and decreased concentration, 9 months previously after being posted to another city from his hometown. Fears, akathisia, feelings of trembling, unsteadiness and choking, along with feeling sad, anhedonia and suicidal thoughts appeared later. However, neither he nor his family described hallucinations, aggressive behaviours or increase in appetite. The patient applied three times to the psychiatry department of a state hospital and was followed briefly with the diagnoses of bipolar disorder, depression and paranoid disorder, and was treated with olanzapine, valproate, amisulpiride, quetiapine and risperidone but to no avail. Past medical history was positive for alcohol abuse of 25 years' duration, cigarette smoking (20 packet-years)

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and an attack of myocardial infarction 5 years previously. He was also diagnosed with melanoma 10 years previously. The patient quit drinking alcohol after the diagnosis of melanoma. He had no history of any substance abuse. Family history was positive for maternal loss at the age of 12 years due to homicide where the offender was the father.

Physical and neurological examination at the time of admission revealed no pathology. At the initial mental status examination, the patient was found to be a well-groomed male with limited communication. Orientation, spontaneous and voluntary attention, concentration and memory were normal. Judgement, reality testing, abstract thought and intelligence were normal. Speech was hypophonic and poor in content. Thought content was notable for anxiety and hopelessness. Mood was judged to be depressive, affect dysphoric. Sleep was decreased; libido and appetite were found to be normal. There was a significant psychomotor retardation.

While the aetiology was investigated, sertraline (titrated up to 100 mg/day) and alprazolam (1.5 mg/day) were introduced to alleviate his anxiety, fears and insomnia. Upon failure, sertraline was changed to paroxetine 20 mg/day, and olanzapine 5 mg/day was added to treat the paranoid symptoms. Serum biochemistry, liver and renal function tests, lipid screening, vitamin B12 and folate, complete blood count, thyroid function tests and viral serology were all within normal limits.

A chest X-ray and 16-channel EEG were found to be normal. Cranial MRI in January 2006 revealed increased hippocampal signal intensities in both right and left temporal lobes, which were more pronounced in the latter (Figure 1). This was thought to be due to limbic encephalitis of probable viral origin.

A neurology consultation was initiated due to the neuro-radiological findings and the patient was referred to the inpatient unit of the neurology department to evaluate the aetiology. Lumbar puncture, viral and tumour markers were all found to be normal. To rule out a paraneoplastic syndrome due to a primary focus, abdominal USG, and CT of the thorax, upper and lower abdomen were performed. These were found to be normal, except for simple cortical cysts in both kidneys. An initial blood smear and a re-evaluation were done to rule out lymphoma. A few atypical lymphocytes were noted. However, other markers were all normal. During the patient's stay in neurology, increasing levels of anxiety and disorganized behaviour were noted. There were also brief periods of amnesia with confabulation and spells of hyperventilation. He also complained of disorientation, especially in the morning when awakening. The patient was therefore readmitted to the psychiatry inpatient unit.

Wechsler Memory Scale-Revised (WMS-R), Digit Span Learning, Tower of London (ToL), Wisconsin Card Sorting Test (WCST), Face Recognition, Judgment of Line Orientation, Verbal Memory Processes, Verbal Fluency, Wechsler Adult Intelligence Scale-Revised (WAISR) similarities subtest and Stroop Color Word Test (SCWT) were applied during neuropsychological evaluation (Lezak 1983; Oner 1997; Karakas 2004; Mesulam 2004; Atalay 2005).

In the WMS-R, the patient recalled personal and actual information only with phonemic cues, simple attention was narrowed, and there were problems in maintaining attention. The performances in visual and verbal paired associates, visual reproduction and figural memory subtests were decreased. In the subtests of WMS-R, searching and formation of memories was distorted. This pattern was thought to display dysfunction of hippocampus, medial temporal lobes, limbic system and some diencephalic structures. Verbal fluency was decreased and there were problems in planning. During the WCST session, he had problems in formation of categories,

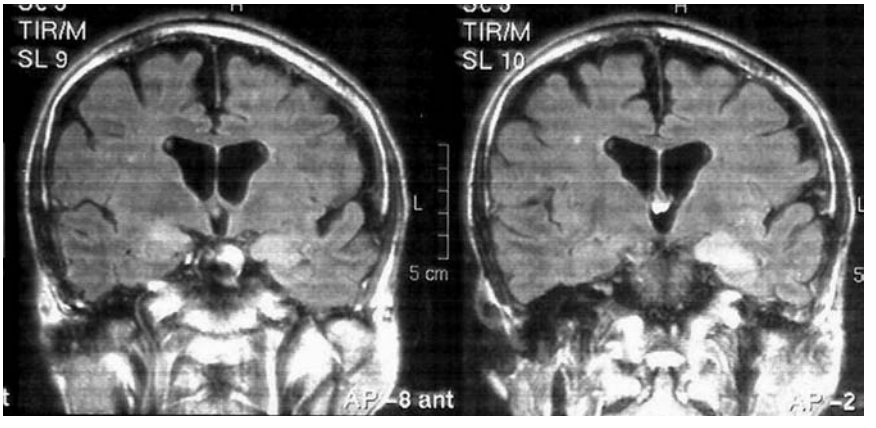


Figure 1. Coronal FLAIR sequences of brain MRI revealed increased signal on bilateral limbic areas: 153 × 73 mm (150 × 150 DPI).

resisting interferences and maintaining formed categories. Consequently he showed perseverative errors and was unable to form a category. This executive dysfunction (problems in adapting to varied stimuli, maintaining this adaptation, making choices, abstracting, and frontal complex attention) corresponded to early levels of frontal dementia.

SCWT displayed problems in resisting interference and inhibiting unwanted responses. This pattern was congruent with frontal dysfunction and seemed to be due to problems in cognitive flexibility, maintaining goal-directed behaviour and speed of cognitive processing. Verbal Memory Processes displayed a frontal type of degeneration with severely distorted free recall; however, being capable of making a choice among presented data. He evidenced an inefficient capacity to search long-term memory for information. Visio-spatial processes were also dysfunctional. The dismal performance displayed in the tests of Judgment of Line Orientation and Face Recognition may be evidence of a dorsal degeneration, possibly involving the parietal association cortex.

The performance in the similarities subtest of WAIS-R was also congruent with a dementia process in the early stages, with problems in daily logic and a preference for concrete answers. He couldn't succeed in even one of the 12 attempted trials in the Digit Span Learning test, which may reveal problem with encoding, storage and recall of information which may in turn be a marker for damage to medial temporal and hippocampal structures. He also displayed problems in the ToL test which evaluates executive functions, such as problem solving, planning, behavioural inhibition, cognitive flexibility, judgement and rule-governed behaviour.

The patient was sent for whole body positron emission tomography imaging in June 2006 to search for the origin of the possible malignancy, to the Istanbul University, Cerrahpasa Medical Faculty Hospital. This revealed a hypermetabolic area (an increased enhancement of [$^{18}$F]fluorodexyglucose; maximal standard update value was 7.8) in the suprahilar zone of the right lung, highly suspected for malignancy. Bronchoalveolar lavage did not show atypical cells. However, the location of the suspected lesion was not suitable for an endobronchial or transthoracic needle biopsy. Thoracotomy was offered, but the patient and his family did not accept it.

A flexible endoscopy of the larynx was undertaken during the second period of hospitalization to further rule out a malignancy because of the presence of melanoma in the past medical history. According to the autoimmune hypothesis for limbic encephalitis, Anti-Hu and Anti-Yo antibodies are frequently observed. However, our case was negative for the aforementioned antibodies in serum. The depressive and anxiety symptoms of the patient decreased on treatment with olanzapine 5 mg/day and alprazolam 1 mg/day, and he was discharged.

## Discussion

Because the panic, anxiety, depressive and paranoid symptoms of our patient were acute, of late-onset and he had no pre-morbid symptoms, an organic aetiology was primarily suspected. Nevertheless, because the psychiatric symptoms were predominant and disabling, psychotropic medication was started and which was beneficial for the patient. To clarify the organic aetiology, diagnostic tests (neuro-imaging, biochemical, neuropsychological tests, etc.) were performed. According to the diagnostic criteria used by Gultekin et al. (2000), MRI findings, clinical symptoms, neuropsychological and laboratory findings and exclusion of other aetiologies for encephalitis (i.e. herpes virus, neurosyphilis), the case was diagnosed as limbic encephalitis.

Neuropsychological symptoms precede cancer diagnosis in 60% of patients with paraneoplastic limbic encephalitis (Gultekin et al. 2000). Limbic system involvement was shown with radiological (i.e. hippocampal lesions in MRI), functional (i.e. neuropsychological testing) and clinical findings in our case. Anti-Hu, anti-Ta and anti-Ma antibodies were negative, as in 40% of all patients with limbic encephalitis (Gultekin et al. 2000). In patients without these antibodies, tumour distribution was diverse, with cancer of the lung the most common (36%), and 57% had positive MRI. Although it could not be confirmed with further diagnostic procedures, the positron emission tomography results in our case also revealed a lesion in the right lung highly suspected of malignancy. Our patient had been diagnosed with melanoma 10 years previously. However, there is no temporal relationship between the diagnosis of melanoma and the neuropsychiatric findings in the patient.

On psychological examination, our patient was noted to have depressive symptoms and anxiety. The thought content of our patient was notable for anxiety and hopelessness. Mood was judged to be depressive, affect dysphoric. Sleep was decreased. As the patient and his family described an acute settling of the sustained symptoms (rather than fluctuations), and no hallucinations, aggressive behaviour or change in appetite or libido, frontotemporal dementia was excluded.

In our patient, the predominance of frontal dysfunction shown in neuropsychological tests may be explained by his childhood. Considering the importance of the hippocampus in both emotional

regulation and memory, one would not be surprised to learn that hippocampal activation patterns of humans differ according to attachment status. Indeed, Buchheim and colleagues (2006) showed that subjects with unresolved attachments, especially traumatic events, had increased activation of medial temporal regions, including the amygdala and hippocampus, in the course of a functional MRI procedure, compared to controls. They interpreted this finding as linking unresolved attachment to emotional dysregulation of the attachment system.

Recently, Benke et al. (2004) also reported a case with limbic and cerebellar paraneoplastic syndrome, which was associated with a squamous lung carcinoma, who had severe anterograde memory loss, frontal executive dysfunction and behavioural alterations. Brain MRI of this case revealed inflammatory changes followed by progressive atrophy affecting the cerebellum and both temporal lobes, and the authors suggested that the related atrophy may present as a chronic, progressive, multifocal encephalopathy, and that the associated cognitive impairments may include several cognitive domains in LE.

The dysfunction at the hippocampal level may also have acted on the structures upstream, causing them to dysfunction. Hippocampal dysfunction may hamper learning, memory formation and therefore frontal executive functions. Through its link with the amygdale, this may disrupt the matching of novel stimuli with long-term memory representation of its affective significance. Also, via afferents projecting from the amygdala to striatal circuits, it may disrupt emotional expressiveness (hence apathy, anhedonia), motor activity (hence the psychomotor retardation) and cognitive processes (hence problems in executive function, behavioural inhibition and paranoid ideations) (Habib 2000).

Although less frequent than depression, anxiety is also observed with focal brain lesions, usually involving the temporolimbic lobe. In mammals, the amygdala is a key structure in fear conditioning. And direct electrical stimulation of any limbic sector in humans may evoke a visceral sensation or an emotion, usually fear or anxiety (Bakchine 2000).

Studies of patients with focal lesions to temporolimbic structures provide an extensive range of symptoms related to emotion, personality and social functioning. A large network, including the hippocampus, amygdala and multiple cortical and subcortical circuits, appears to modulate affect and emotional behaviour (Bakchine 2000). The connections with the frontal and prefrontal orbital appear especially important. Most temporal symptoms are difficult to localize specifically to the temporal lobes, or to the left or right sides. This may be explained in terms of the extended temporolimbic system working as a parallel distributed processing network. The findings in our patient illustrate the importance of neuropsychological test battery involvement in evaluation of LE cases for better understanding the neurological mechanisms underlying the behavioural symptoms.

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## Statement of interest

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**CASE REPORT**

# A case with occurring adverse effects when cross-over titration from fluvoxamine to paroxetine associated with increasing the plasma fluvoxamine level in major depressive disorder

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**Abstract**

Selective serotonin reuptake inhibitors (SSRIs) are first line drugs for treating not only depressive disorder but also anxiety disorder. Fluvoxamine, a SSRI, is mainly metabolized by cytochrome P450 (CYP) 2D6 and 1A2. However, paroxetine, an another SSRI is potent inhibitor for CYP 2D6. We report a case with depression whose plasma fluvoxamine level rapidly increased after the addition of paroxetine while switching from fluvoxamine to paroxetine. The case indicates that emerging adverse effects via the pharmacokinetic interaction of these drugs when switching patients from fluvoxamine to paroxetine can occur.

**Key words:** *Fluvoxamine, paroxetine, plasma concentration, depression*

## Introduction

It is generally accepted that significant plasma concentration–response relationships exist for tricyclic antidepressants and non-tricyclic antidepressants, such as nortriptyline (Asberg et al. 1971), imipramine (Glassman et al. 1977), desipramine (Nelson et al. 1982), mianserin (Otani et al. 1991), and trazodone (Mihara et al. 2002). Fluvoxamine belongs to the class of selective serotonin reuptake inhibitors (SSRIs). It is effective for the treatment of depression, panic disorder, obsessive-compulsive disorder, and eating disorders (De Wilde and Doogan 1982; Palmer and Benfield 1994; Papakostas et al. 2007). Compared to classical tricyclic antidepressants, fluvoxamine has fewer anticholinergic side effects, such as dry mouth, abnormal accommodation, and urinary hesitation and seems to be devoid of cardiotoxic and proconvulsive effects (Benfield and Ward 1986). However, fluvoxamine sometimes induces nausea and somnolence (Palmer and Benfield 1994; Ueda et al. 2001). Gerstenberg et al. (2003) reported that there is a therapeutic threshold for the steady-state plasma concentrations (Css) of fluvoxamine, and that a Css of fluvoxamine plus fluvoxamino acid, a major metabolite in fluvoxamine, of above 180 ng/ml best predicts a good response to fluvoxamine in depressed patients. We have reported that the lowest level of plasma fluvoxamine for a response to occur was 98 ng/nl in bulimic patients (Ikenouchi-Sugita et al. 2007). Kasper et al. (1993) reported that higher concentrations of fluvoxamine were significantly associated with more side effects. We have also demonstrated that patients with emerging nausea induced by fluvoxamine had slightly higher plasma levels of fluvoxamine than those without emerging nausea (Ueda et al. 2001). These findings suggest that plasma fluvoxamine levels are associated with the drug's clinical response and adverse effects. Therefore, plasma drug monitoring of fluvoxamine might be useful in maximizing drug response and avoiding drug adverse effects. In the present study, we reported a case with depression, whose plasma fluvoxamine level was rapidly increased after adding paroxetine when cross-over titration from fluvoxamine to paroxetine, a switch which might have been associated with adverse effects seen.

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## Case report

A 44-year-old female patient was diagnosed with major depressive disorder according to the DSM-IV-TR. She complained of depressed mood, self-blame, anxiety, agitation, and insomnia and her score on the Hamilton Rating Scale for Depression (Ham-D) was 26. Her physical condition was not particular. Treatment with fluvoxamine was initiated with a dose of 50 mg/day, and the dose was increased to 200 mg/day and maintained for 6 weeks with the same dose. Her Css of fluvoxamine at 200 mg/day was 128 ng/ml. Since her depressive state was not improved (her score on the Ham-D was 20), she was switched from fluvoxamine to paroxetine. The dose of fluvoxamine was reduced to 150 mg/day, and 20 mg of paroxetine was started (added on fluvoxamine). Five days after paroxetine administration (her Css paroxetine levels was 22 ng/nl), she complained of somnolence, dizziness, nausea, and orthostatic hypotension. Her Css of fluvoxamine at 150 mg/day was increased to 224 ng/ml, despite the decrease of the dose. Thereafter, paroxetine was stopped, and only fluvoxamine (150 mg/day) was continued. The adverse effects disappeared, and her Css of fluvoxamine at 150 mg/day was 98 ng/day. She had not received any other medication besides fluvoxamine and paroxetine. Subsequently, milnacipran was administered and increased to 150 mg/day instead of fluvoxamine, but her depressive state persisted. Finally, paroxetine was restarted and increased to 40 mg/day (her Css paroxetine level was 56 ng/nl), and her depressive state remitted without any adverse effects occurring. We examined her genotype of cytochrome P450 (CYP) 2D6, and the result was a CYP 2D6 was *1/*10 (extensive metabolizer).

## Discussion

Approximately 50–60% of all treated depressed patients fail to show adequate response to their initially prescribed SSRIs. Patients with an insufficient response to one SSRI are often switched to another SSRI (Ruhe et al. 2006). Fluvoxamine is one of the candidate drugs for treating not only depressive disorder, but also anxiety disorder, obsessive-compulsive disorder, or eating disorders (De Wilde and Doogan 1982; Palmer and Benfield 1994). We have demonstrated that plasma fluvoxamine level was raised in parallel with the increase in its dosage (Ueda et al. in press). Fluvoxamine is mainly metabolized by CYP 2D6 and 1A2 (Brosen et al. 1993; Perucca et al. 1994; Carrillo et al. 1996; Brosen 1998; Vandel 2003). However, paroxetine, another SSRI, is a potent inhibitor for CYP 2D6. In the present study, we reported a case with emerging adverse effects whose plasma fluvoxamine level was acutely increased after adding paroxetine when switching from fluvoxamine to paroxetine. Paroxetine is a strong CYP2D6 inhibitor (von Moltke et al. 1995). Thus, the administration of paroxetine significantly impaired the activity of CYP2D6 to contribute to the metabolism of fluvoxamine, which led to a two-fold increase in the fluvoxamine plasma level, despite a modest decrease in the fluvoxamine dosage (from 200 to 150 mg/day). Previous studies demonstrated that higher plasma fluvoxamine levels were related to adverse effects (Kasper et al. 1993; Ueda et al. 2001). Therefore, it is probable that the adverse effects were associated with a high plasma fluvoxamine level. In addition, the most important finding in the present case was that the increase of the plasma fluvoxamine level occurred within only 5 days after paroxetine administration. This suggests that the inhibition of CYP2D6 by paroxetine emerges rapidly. This finding is in accordance with the previous reports that plasma levels of several antidepressants metabolized by CYP 2D6 were increased within 7–10 days after paroxetine addition (Sandson 2003). Administration of paroxetine alone (40 mg/day) improved her depressive symptoms without any adverse effects such as somnolence, dizziness, nausea, and orthostatic hypotension, suggesting that treatment with paroxetine was independent of the emergence of those adverse effects.

The result of patient's genotyping of CYP2D6 was *1/*10 (extensive metabolizer). The gene coding for CYP1A2 is highly polymorphic and DNA testing can identify common variations that result in dramatically differences in patient exposure that may lead to adverse reactions or lack of efficacy at normal therapeutic doses. Since CYP1A2 activity is the undisturbed subroute of fluvoxamine metabolisation, co-administration of the CYP2D6 inhibitor paroxetine should partly (although not completely) be balanced by CYP1A2 activity. Therefore, a genetically decreased activity of CYP1A2 could be expected in this case of drastically increased plasma fluvoxamine levels. However, we did not determine the patient's genotyping of CYP1A2 in the present case. In any case, to combine fluvoxamine and paroxetine is a high risk, since this leads to inhibition of CYP2D6 by paroxetine and of CYP1A2 and CYP2C19 by fluvoxamine. Generally, the combination should be avoided, and clinicians should be very cautious for occurring unexpected adverse effects when cross-over titration from fluvoxamine to paroxetine.

In conclusion, we reported a case of depression whose genotypes of CYP2D6 was *1/*10 (extensive metabolizer) with adverse effects occurring in parallel with the rapid increase of her plasma fluvoxamine

level after paroxetine addition. Caution should be exercised when cross-over titration from fluvoxamine to paroxetine via the inhibition of CYP2D6 by paroxetine. Initiating paroxetine in a smaller dose might be a safer course.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**CASE REPORT**

# Quetiapine-associated dysphagia

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**Abstract**
We report a case of quetiapine-induced dysphagia in a geriatric patient which improved with discontinuation of the antipsychotic. The patient had developed dysphagia while being treated with antipsychotics for bipolar disorder. The patient's dysphagia showed significant improvement when she was taken off quetiapine. We review the available literature on antipsychotic-related dysphagia and suggest that clinicans need to be aware of the potential for this syndrome even with lower potency antipsychotics.

**Key words:** *Antipsychotics, geriatric*

## Introduction

Antipsychotics, also known as neuroleptics or major tranquilizers, are often used for the treatment of psychoses such as schizophrenia (Sokoloff and Pavlakovic 1997). In the elderly, antipsychotics are often used to treat aggressive or disruptive behaviour in the context of dementia with psychosis (Jenike 1988). Antipsychotics as a class can exacerbate or even cause oro-pharyngeal dysphagia by causing extrapyramidal side effects (EPS) (Rainer et al. 2007).

Antipsychotics are divided into subgroups based on potency and adverse side effect profiles. Low potency antipsychotics (such as thioridazine or chlorpromazine), are less commonly associated with EPS and more likely to cause anticholinergic side effects such as sedation, orthostatic hypotension and dry mouth. High potency agents (such as haloperidol) are more likely to cause EPS and less likely to cause anticholinergic side effects. Moderate potency agents (such as loxapine) are thought to have an intermediate potential for all side effects (Maletta et al. 1991).

The mechanism of action of antipsychotics involves blockade of dopamine receptors in the basal ganglia. EPS including drug-induced parkinsonism occurs in 12–45% of patients on antipsychotics, with higher rates in the elderly. Symptoms include tremor at rest, rigidity, bradykinesia, and blunted affect with masked facies (Casey 1993). Atypical antipsychotics such as olanzapine and quetiapine are less likely than conventional antipsychotics such as haloperidol to cause EPS (Miller et al. 1998).

Drug-induced parkinsonism may have serious implications for swallowing (Stoschus and Allescher 1993). Up to 50% of patients with Parkinson's disease may have some degree of dysphagia, and treatment with levodopa or dopamine agonists may lead to improved swallowing in some affected patients (Weiden and Harrigan 1986). However, more recent studies have shown limited impact of levodopa on Parkinson's disease patients with dysphagia (Hunter et al. 1997). Oro-pharyngeal deficits in Parkinson's disease include defective tongue movements, reduced initiation of tongue movement, slowed oral transit time, delayed initiation of swallow, misdirected swallowing, irregular epiglottic movement, and silent aspiration (Buchholz 1994).

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In this article, we report a case of a geriatric patient with quetiapine-induced dysphagia who improved with cessation of use of antipsychotics.

## Case report

A 66-year-old female with a 30-year history of bipolar disorder was admitted to an inpatient psychiatric hospital unit for worsening depression. The patient also had prominent referential beliefs and paranoid delusions. Nine months earlier she was diagnosed with cerebellar dysfunction due to a possible stroke. She had a swallow evaluation at that time with a modified barium swallow (MBS) that showed delayed oral stage of swallowing and silent aspiration of thickened fluids. At that time, she had been on aripiprazole.

On admission at this time, she was on quetiapine at a dose of 200 mg/day as well as on lorazepam 1.5 mg/day and citalopram 20 mg/day. She had been on this regimen for several months with no change in medications or doses. She was noted to have difficulty swallowing and had an MBS which showed delayed transfer of puree and fluids with aspiration of thick fluids, with the recommendation of a dyphagia pureed diet with thick fluids as she failed trials of thin fluids. The patient did not demonstrate any other extrapyramidal side effects. On neurological exam, the patient had residual cerebellar symptoms of dysmetria, dysarthria and ataxia with no change from that observed 9 months earlier. A repeat magnetic resonance imaging of the brain showed no change from 9 months earlier. The patient was tapered off the antipsychotic medication and had a repeat modified barium swallow evaluation performed 1 month later by the same speech and swallow therapist at the speech and swallow laboratory, using the same tmethodology as previously. This study was done to determine whether the patient improved while off quetiapine. The repeat study recommended a pureed dysphagia diet but allowed for regular thin fluids. There were no other changes in her medication regimen although she did receive a course of electroconvulsive therapy (ECT).

## Discussion

The rate of oropharyngeal swallow dysfunction is about 6% in the general population (Groher and Bukatman 1986). Dysphagia in the elderly most commonly occurs due to neurological disease or structural changes in the oro-pharynx. Swallow problems including choking, aspiration pneumonia and asphyxia have been commonly found in the psychiatric population (Fioritti et al. 1997). The rigidity and bradykinesia caused by antipsychotics may be responsible for the impairment in the oral phase of the swallowing (Robbins et al. 1986). One study reported the prevalence of dysphagia in a population with mental health disorders to be 32%, although it did not report whether those patients were on antipsychotics. Dysphagia in patients with psychiatric illness may also be due to non-pharmacological causes such as behavioural changes, environmental changes and cognitive dysfunction (Regan et al. 2006). The rate of death from asphyxia in psychiatric patients in Ireland was reported to be 100 times that of the general population (Craig 1982).

One literature review of 11 case reports of EPS-related dysphagia showed an age range of 35–79. Medium to high potency antipsychotics were implicated in eight of the cases, while three were related to atypical antipsychotics. In four of the cases, dysphagia was the only prominent manifestation of EPS. The onset of symptoms from initiation of the antipsychotic is about 1 month, with a range of a few days to 3 months. There were case reports of dysphagia induced by risperidone and olanzapine, but not by quetiapine (Dziewas et al. 2007).

The case reported above involved a patient whose dysphagia improved when taken off quetiapine. We believe that the discontinuation of the atypical antipsychotic led to improvement in the dysphagia of the patient, even if it did not completely eliminate it. In this case, the patient's quality of life was improved as she was able to be switched to much more palatable regular fluids instead of thick fluids when off the antipsychotic. Furthermore, we believe that the case raises the possibility of dysphagia exacerbated or worsened even by lower potency atypicals such as quetiapine. In this case, it is unlikely that the dysphagia resolved spontaneously as it persisted at the same level for 9 months until quetiapine was stopped. While the patient's cerebellar symptoms contributed to the dysphagia initially, the dysphagia worsened on quetiapine and improved off it with no change in her neurological condition. There were two separate speech and swallow evaluations done in the same laboratory, by the same therapist, using the same technique. The speech and swallow therapist was blinded to the fact that quetiapine was discontinued.

From a clinical point of view, early diagnosis of bradykinetic EPS-related dysphagia presents a challenge. Diagnosis of EPS-related dysphagia can be made either by modified barium swallow (MBS) or by fiberoptic endoscopic evaluation of swallowing (FEES). The FEES may be a more useful test, as endoscopy has the potential to minimize the effect of behavioural issues on the results of the test. In

addition, the FEES does not expose patients to radiation (Langmore 1982).

For reducing dysphagia, several strategies can be used; non-pharmacological strategies can include education of patients about rate and volume of food bolus, staff monitoring and intervention, as well as speech and swallow screenings. Pharmacological strategies include discontinuing antipsychotic treatment, lowering the dose or changing to another drug which is less likely to cause EPS. Sometimes, even switching from one high-potency antipsychotic to another may lead to improved swallowing (Dziewas et al. 2007).

## Conclusion

We believe that our case raises the possibility of worsening or exacerbation of dysphagia, even with low potency atypical antispychotics such as quetiapine. We suggest that clinicians need to be aware of the potential for this syndrome, even with lower potency antipsychotics. The Naranjo algorithm can be used to assess the likelihood that a change in clinical status is the result of an adverse drug reaction (ADR) rather than the result of other factors, such as progression of disease (Berry et al. 1998). Using the Naranjo algorithm, this case had a score of 5, meaning that an ADR is probable.

EPS-related dysphagia is a dangerous but potentially reversible side effect in patients receiving antipsychotics. Having a high index of suspicion and clinical awareness may facilitate early diagnosis and appropriate treatment. In the future, further research including prospective observational studies are needed to learn more about the incidence and course of this manifestation and to evaluate systematically different treatment options.

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## Statement of interest

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**CASE REPORT**

# Beneficial effects of *N*-acetylcysteine in treatment resistant schizophrenia

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**Abstract**

Poor response to antipsychotics is still an important problem in the treatment of many schizophrenia patients. *N*-Acetylcysteine (NAC) is a compound that exerts anti-oxidant and scavenging actions against reactive oxygen species. This paper reports a case of poorly responsive schizophrenia patient who improved considerably with add-on NAC 600 mg/day. The NAC might work through activating cysteine-glutamate antiporters or reducing in nitric oxide (NO) metabolites, free radicals and cytokines or through both of these mechanisms.

**Key words:** *N-Acetylcysteine, schizophrenia, drug therapy, combination, treatment resistant*

## Introduction

Poor response to antipsychotics is still an important problem in the treatment of many schizophrenia patients. Augmentation strategies as suggested in schizophrenia treatment guidelines such as with lithium (Schulz et al. 1999) and anticonvulsants (Basan et al. 2004), fail to produce adequate benefits in significant number of patients. Therefore, it is necessary to look for alternative or additional drug treatment possibilities.

The growing evidence of increased oxidative stress and diminished enzymatic antioxidants may be relevant to the pathophysiology of schizophrenia. These findings may suggest some clues for the new treatment strategies with antioxidants in schizophrenia (Akyol et al. 2002; Zoroglu et al. 2002).

*N*-Acetylcysteine (NAC) has long been used clinically as a mucolytic agent for chronic bronchitis and for patients with acute lung injury or acute respiratory distress syndrome (ARDS). *N*-Acetylcysteine is a compound that exerts anti-oxidant and scavenging actions against reactive oxygen species. In patients with ARDS, NAC has been shown to reduce the symptoms and to shorten the duration of ARDS, presumably acting as an anti-oxidant and restoring the decrease in glutathione in cases of lung injury (Kao et al. 2006).

This paper reports a case of treatment-resistant schizophrenia patient who improved considerably with add-on NAC 600 mg/day.

## Case

Mrs H.D. was a 24-year-old single, unemployed woman living with her parents. She was brought into Psychotic Disorders Unit of Psychiatry Clinics at the Gaziantep University School of Medicine Hospital by her parents for excitation and worsening psychosis. She claimed that "her parents had wanted to poison her, and they were talking negatively and despised her". She was irritable, aggressive and guarded. She had depressed mood, decreased of sleep, poor appetite, auditory hallucinations, and persecutory delusions. She has been followed at our clinic for 7 years prior to this presentation. After psychiatric evaluation she was hospitalized for exacerbation of schizophrenia, paranoid type based on DSM-IV-TR criteria. Results of neurological and physical examinations and laboratory tests were within normal limits. She did not have any other medical illnesses. Her vital signs were normal.

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She did not have any history of substance abuse or dependence. There were no history of psychiatric disorderds in her relatives and extended family.

The symptoms had worsened 5 weeks earlier while she was on ziprasidone 160 mg/day and zuclopentixole decaonate 200 mg intramuscular (i.m.) every 15 days. A week after worsening of her symptoms she was admitted to psychiatric unit at another hospital. Ziprasidone and zuclopentixole decaonate had been discontinued and risperidone 8 mg/day had been initiated. Because of acute distonia biperiden HCl 4 mg/day and because of akathisia clonazepam 2 mg/day had been added to risperidone. She had been discharged after 4 weeks of inpatient psychiatric treatment and she was brought to our clinic ome day after her discharge from other hospital. Ther was no significant improvement in her psychiatric symptoms.

In history; the patient's age of illness onset was 17. Over 7 years she was continuously treated with several typical and atypical antipsychotics at full therapeutic doses for adequate periods. Overall she demonsrated slight clinical improvement. Her persecutory delusions, auditory hallucinations, social withdrawal and cognitive impairment contunied. This current episode was diagnosed as exacerbation of schizophrenia. Delirium, probable organic causes, and substance abuse were ruled out.

Admission to our unit was the tenth psychiatric hospitalization of the patient. She was diagnosed with treatment-resistant schizophrenia according to the NICE criteria (NICE 2002) as evidenced by a lack of satisfactory clinical improvement despite the sequential use of the recommended doses of at least two antipsychotic drugs for 6–8 weeks, at least one of which was a second-generation antipsychotic. At the day of administration initial Positive and Negative Syndrome Scale (PANNS) score was 143, Clinical Global Impression (CGI) severity-of-illness score was 6 and Calgary Depession Scale score was 11. Based on history, the patient benefited most from combination treatment with olanzapine and zuclopenthixole decaonate. Thus olanzapine 20 mg/day, zuclopenthixole decaonate 200 mg once every 15 day were started. Clonazepam 4 mg/day were continued and risperidone was discontinued. The patient developed oculogyric crisis, which was treated with biperiden HCl 4 mg/day successfully. After 22 days after this treatment regimen PANSS score decreased to 78, CGI score to 4 and Calgary Depression Scale score to 8. Around 37th day of admission her psychotic symptoms worsened. The PANSS score increased to 102 and CGI score increased to 6. Calgary Depression Scale score was still 8. The informed consent was obtained from her next of kin and NAC 600 mg p.o. daily was added on to the mentioned treatment regimen. Seven days after addition of NAC the PANSS score decreased to 59, CGI score decreased to 4. Calgary Depression Scale remained to 8. She was discharged on the 67th day of the hospitalization. At discharge, PANSS score was 56, CGI score was 4. Calgary depression scale score was 8. The patient and relatives reported marked improvement in spontaneity, social skills and family relations. She was doing routine housework such as cooking and cleaning spontaneously and her self-esteem was normal. One month after discharge from hospital her PANSS, CGI and Calgary depression scale scores were 56, 4 and 8, respectively.

## Discussion

A number of studies have indicated that free radical-mediated neuronal damage plays a role in the pathophysiology of psychiatric disorders such as depression, bipolar disorders, and schizophrenia. Oxidative damage may account for deteriorating course and poor outcome in schizophrenia (Akyol et al. 2002; Savas et al. 2002, 2006; Zoroglu et al. 2002; Yanik et al. 2004; Selek et al. 2007). Recently the role of free radicals and nitric oxide (NO) have been studied in schizophrenia (Zoroglu et al. 2002). Increased NO production by nitric oxide synthetases (NOSs) suggests a possible role of NO in the pathophysiology of schizophrenia (Akyol et al. 2002). The generation of NO following *N*-methyl-D-aspartate (NMDA) or norepinephrine receptor activation seems to be important in the context of central nervous system pathology (Moncada et al. 1991). NO is associated to both neurotoxic and neuroprotective effects. NO is responsible for the glutamate-induced activation of guanylate cyclase which is considered its physiological target, but is associated to glutamate neurotoxicity and dopamine-induced cell-death. Oxidative stress plays a role in the cognitive deficits of schizophrenia patients. This may involve reduced glutamatergic neurotransmission since the oxidation of the redox-sensitive site in the NMDA receptor reduces the activation of this receptor (Perez-Neri et al. 2006).

There is growing evidence that the glutamatergic system plays a role in the pathogenesis of schizophrenia (Olney et al. 1995; Heckers et al. 2002). Since glutamate and dopamine interact in a complex way (Farber et al. 1998) the glutamatergic hypothesis of schizophrenia could also integrate dopaminergic abnormalities (Olney et al. 1995; Farber 2003). In a previous study, increased Glu signals in patients with recurrent episodes of schizophrenia

treated with neuroleptics were reported (Tebartz van Elst et al. 2005). In a recent study, significant correlations were found between prefrontal Glu concentrations and rating scores for schizophreniform symptoms, but not with antipsychotic medication in first episode schizophrenia patients. Those Glu findings might reflect factors intimately associated with schizophrenia but not reflect chronic medication effects or factors involved in the progress of illness (Olbrich et al. 2007).

Application of phencyclidine and other glutamate receptor blockers can cause schizophreniform symptoms (Olney et al. 1995). In a previous study, activation of cysteine-glutamate antiporters by using the cysteine prodrug NAC reversed psychomimetic effects in rodent phencyclidine model of schizophrenia (Baker et al. 2007). Mahadik et al. (2006) reported that dietary supplementation of antioxidants and omega-3 fatty acids were found to improve symptoms of schizophrenia. However, in this study, the patients were treated by a combination of antipsychotics and these agents; therefore it is hard to estimate the effect due of antioxidants alone (Mahadik et al. 2006).

In our case we envisaged that the patient might benefit from supplementation of NAC as an antioxidant and a precursor of glutathione. The NAC might work through activating cysteine-glutamate antiporters or reducing in NO metabolites, free radicals and cytokines or through both of these these mechanisms.

Finally, with the hypothesized mechanism(s) of action, NAC treatment demonstrated beneficial effects in a treatment-resistant schizophrenia patient and it has been well tolerated. In some cases of schizophrenia, especially in treatment-resistant cases, addition of NAC to the treatment regimen might be a useful intervetion.

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## Statement of interest

None.

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**CASE REPORT**

# Unilateral rubral tremor following treatment with risperidone

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**Abstract**
Rubral tremor is a rare movement disorder that occurs typically with midbrain damage. The main features of this tremor are its low frequency, irregular rhythm, presence at rest, and acceleration during posture and active movement. Antipsychotic agent-induced tremors are usually bilateral parkinsonian tremors. We found no previous reports of unilateral rubral tremor in the literature. A 23-year-old man had unilateral rubral tremors as a result of a midbrain lesion plus risperidone exposure for treatment of manic symptoms. After we stopped the use of risperidone, the tremor became less apparent and then disappeared. This case highlights the importance of being aware of this rare complication in susceptible patients receiving risperidone treatment.

**Key words:** *rubral tremor, parkinsonian tremor, antipsychotic agents, risperidone*

## Introduction

Rubral tremor is a rare movement disorder that occurs typically with midbrain damage. Its frequency usually is 2–5 Hz and includes resting, postural and kinetic components. It becomes more pronounced with a fixed posture, and further increases in amplitude with intentional voluntary movement (Vidailhet et al. 1998). Some authorities believe that it is actually a cerebellar postural tremor plus a parkinsonian tremor (Vidailhet et al. 1998), and can be caused by multiple sclerosis (MS) (Poser and Brinar 2004), vascular insults (Tan et al. 2001), tumours (Leung et al. 1999), head trauma (Krack et al. 1994), neuroleptic exposure (Friedman 1992), and toxoplasma abscess (Pezzini et al. 2002).

Currently, numerous atypical antipsychotic agents such as risperidone, olanzapine, quetiepine and aripiprazole are approved for the treatment of acute manic episodes (Derry and Moore 2007). Patients on these dopamine receptor antagonists may experience all of the common motor symptoms of idiopathic parkinsonism, including tremor, rigidity and bradykinesia. Drug-induced parkinsonian tremor is usually bilateral and commonly occurs during the first 5–30 days of treatment. We found no reports of drug-induced unilateral rubral tremor in the literature. We report the first case of a manic patient with unilateral rubral tremor related to a midbrain lesion plus risperidone exposure.

## Case report

Mr L, a 23-year-old man, was admitted to the hospital because his relatives were worried about his "unusual behaviour". He was convinced that a particular woman he knew from long before was communicating with him to express her love. A psychiatric examination revealed racing thoughts, and speech that was circumstantial, partly incomprehensible, and not directed. His mood was elated and sometimes irritable. He was unusually self-confident, showed psychomotor agitation, and reported a decreased need of sleep. These symptoms had developed within a few days and had now lasted for about 2 weeks. Clearly, he was suffering from a manic episode with psychotic features.

During hospitalization, the patient was treated with mood stabilizers, such as valproate and clonazepam and behavioural therapy. The mood symptoms subsided gradually but disruptive behaviours were still present. We then added an atypical antipsychotic, risperidone, for further management. We titrated the daily dose of risperidone from 2 to

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4 mg in 3 days. On the fourth day of risperidone use, there was a unilateral resting tremor over the right extremities at a frequency of 3–4 Hz which was accelerated during sustained posture and guided movement. There was no tremor on the opposite limb.

We consulted a neurologist and the patient was evaluated under the impression of rubral tremor. Tremography of the right upper extremity revealed regular burst formation at a frequency of 3–4 Hz in either a synchronous or alternative pattern with a particular posture. Brain MRI revealed increased signal density over the left cerebral peduncle, left paramedian of the mid-brain (including left red nucleus) and dorsal pons on T2-weighted imaging. Small bright lesions in the bilateral corona radiata were also noted which may have been related to the midbrain abnormalities. The possible differential diagnosis included multiple sclerosis, encephalitis, vasculitis and other rare neurological illnesses. IgG synthesis of cerebrospinal fluid (CSF) ruled out multiple sclerosis. On agarose gel electrophoresis of the CSF, no multiple bands in the "slow" IgG region were found when compared with control CSF. Results of other laboratory tests including the erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, antistreptolysin-O, rapid plasma regain, and human immunodeficiency virus test ruled out encephalitis. In addition, CSF studies such as cell counts, proteins and glucose levels, aerobic and anaerobic cultures, polymerase chain reaction analysis of herpes simplex virus and varicella-zoster virus, India ink for *Cryptococcus* infection, acid–fast stain for tuberculosis infection, and fungus stain revealed no abnormal findings. Results of antinuclear antibody, complement levels, antineutrophil cytoplasmic antibody and angiograms of cerebral, mesenteri or renal arteries ruled out vasculitis. Other laboratory exams to rule out rare illnesses such as Wilson's disease, Hallervorden–Spatz syndrome and seizure disorders including plasma concentrations of copper and ceruloplasmin, blood analysis for acanthocytes and evoked potentials also revealed no abnormal findings.

We discontinued risperidone and the tremor became less apparent and then disappeared. Another mood stabilizer, carbamazepine, was added to control manic symptoms and disturbing behaviours. Treatment with the two mood stabilizers, valproate and carbamazepine, combined with behaviour therapy, resulted in gradual improvement of his manic symptoms.

## Discussion

To our knowledge, this is the first description of unilateral rubral tremor as a result of midbrain lesion plus risperidone exposure in treating a manic patient. It appears that a therapeutic dose of risperidone can lead to rubral tremor in susceptible patients. Taken together, our case and a previous report (Friedman 1992) of risperidone-induced rubral tremor in a patient with post-traumatic ataxia suggest that the therapeutic use of risperidone could lead to rubral tremor in certain patients who have underlying brain lesions. However, our patient had only right-side extremity rubral tremor, which is different from the previous report. The unilateral rubral tremor may be related to his left-side midbrain lesion plus treatment with risperidone.

Previous reports have indicated some risk factors for development of rubral tremor and extrapyramidal symptoms in patients receiving antipsychotic medication (Friedman 1992; Worrel et al. 2000). These factors include a past history of mood disorder or substance abuse, rapid dosage increase or a large total dose of the drug, advanced age, underlying brain problems and concurrent antidopaminergic medications. Our patient had a mood disorder, rapid dosage increase and underlying brain lesions that might have led to the development of unilateral rubral tremor after risperidone treatment.

The neural mechanisms underlying rubral tremor have been investigated in monkeys (Ohye et al. 1988). Three major neural elements in the ventromedial tegmental (VMT) areas, the parvocellular division of the red nucleus, the cerebellothalamic fibres passing through the red nucleus, and the nigrostriatal fibers have been found to have interactions in producing tremors. In an animal study, these three elements were destroyed stereotaxically from the VMT area either separately or in various combinations. When all three elements were destroyed, rubral tremors appeared (Ohye et al. 1988).

In our case, the development of unilateral rubral tremor could have been due to lesions which destroyed only the parvocellular division of the red nucleus and cerebellothalamic fibers passing through the red nucleus but spared the nigrostriatal fibers. The patient appeared normal when not taking an antipsychotic agent. An antipsychotic agent, such as risperidone might have some effects on the nigrostriatal fibers and cause dysfunction of all three elements. Rubral tremor developed in our patient similar to those in experiments in monkeys after the three elements were destroyed.

In conclusion, we present the first case of unilateral rubral tremor as a result of midbrain lesion plus risperidone exposure in treating mania. This case highlights the importance of being aware of rare complications in susceptible patients receiving risperidone treatment.

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## Statement of interest

The author has no conflict of interest with any commercial or other associations in connection with the submitted article.

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**CASE REPORT**

# Transcranial direct current stimulation in a patient with therapy-resistant major depression

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**Abstract**

Transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) has been reported to exert significant antidepressant effects in patients with major depression. Several recent studies found an improvement of depressive symptoms in drug-free patients. Here we report the case of a 66-year-old female patient suffering from recurrent major depressive episodes who underwent anodal tDCS of the left dorsolateral PFC over 4 weeks as an add-on treatment to a stable antidepressant medication. Only a modest improvement of depressive symptoms was observed after tDCS, i.e. reduction of the baseline scores in the Hamilton Depression Rating Scale from 23 to 19 and in the Beck Depression Inventory from 27 to 20. However, there was an increase from 52 to 90% in the Regensburg Verbal Fluency Test. In addition, EEG was used to assess the acute effects of tDCS. Low resolution brain electromagnetic tomography (LORETA) showed a left unilateral focal effect (25–40% reduced power) in the delta, theta and alpha frequency bands. The same effect appeared in the surface analysis of the EEG. The absolute, as well as the relative power decreased significantly in the delta, theta and alpha bands after a comparison of the spectral analysis. Though tDCS over 4 weeks did not exert clinically meaningful antidepressant effects in this case of therapy-resistant depression, the findings for cognitive measures and EEG suggest that beneficial effects may occur in depressed subjects and future studies need to further explore this approach also in therapy-resistant major depression.

**Key words:** *Major depressive disorder, transcranial direct current stimulation, tDCS*

## Introduction

The method of transcranial direct current stimulation (tDCS) is known since the 1960s and based on experimental research in animal models (Bindman 1962). In humans, anodal tDCS enhances working memory and reaction time due to excitability alteration in neurons (Nitsche et al. 2005). Several studies suggested that tDCS may be helpful in post-stroke rehabilitation (Hummel et al. 2005) and in the treatment of central pain in traumatic spinal cord injury (Fregni et al. 2006c). The reduction of depressive symptoms has been shown in consecutive studies by Fregni and co-workers. Fregni et al. (2006a) found a significant decrease in the Hamilton Depression Rating Scale and Beck Depression Inventory after 5 days of active stimulation in comparison to a group of patients with sham stimulation. In another study (Fregni et al. 2006b) including 18 antidepressant-free patients with recurrent major depressive episodes, a significant reduction in the Hamilton depression score was found after 5 days of active tDCS. Interestingly, mood improvement and cognitive improvement were not correlated suggesting that independent mechanisms are responsible for cognitive and mood changes. This finding has been replicated by Boggio et al. (2007a). In 40 antidepressant-free patients suffering from unipolar major depression, Boggio et al (2007b) found a significant reduction in the Hamilton and Beck depression scores after DLPFC tDCS over 2 weeks compared to occipital and sham tDCS. Despite the particular need for novel effective antidepressant interventions in therapy-resistant depression there are no published data on tDCS in this difficult-to-treat group to our knowledge.

Here, we report the case of a 66-year-old woman suffering from a drug-resistant recurrent major depressive episode who was treated with anodal

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tDCS of the left dorsolateral prefrontal cortex over 4 weeks.

## Case report

The 66-year-old female patient was suffering from a recurrent major depression (DSM-IV: 296.33) and had depressive episodes in 1986 and 1990. The current episode began in 2006 following a treatment of tinnitus with corticoids. The patient had undergone several failed antidepressant trials: the first combination of mirtazapine, venlafaxine and olanzapine had no obvious effect on the improvement of depressive symptoms. The second combination of paroxetine 40 mg, reboxetine 4 mg, mirtazapine 30 mg, risperidone 1 mg and lithium 800 mg brought a partial response of the depressive syndrome. When tDCS was considered the patient was still suffering from anhedonia, lack of energy and concentration. ECT was rejected by the patient. The treatment regime had been stable for 6 weeks and was carried on during tDCS treatment.

In accordance with the safety criteria of tDCS in humans, we used the protocol suggested by Nitsche et al. (2003a,b). We applied a weak direct current of 1 mA to the cortex. We used a CE-certified DC-stimulator (eldith DC-stimulator, neuroconn, Ilmenau, Germany). The anode was placed over the left dorsolateral prefrontal cortex (DLPFC – EEG F3/int. 10–20 system), the cathode was placed over the right supraorbital region. The sponges (35 $cm^2$) were soaked with physiological 144 mmol/l NaCl solution. After 10 stimulations we changed to water-soaked sponges. The duration of the each stimulation was 20 min with phases of 15 s ramp up and 15 s ramp down. Over all we performed 16 stimulations in a period of 27 days, initially daily, and afterwards two-daily when the patient changed to outpatient status.

In advance of the stimulation we performed several neuropsychological tests and ratings: Beck Depression Inventory (BDI), Hamilton Depression Rating Scale (HAMD) and Clinical Global Impression (CGI) for clinical assessment, the Regensburg Word Fluency Assessment (RWT) and the Verbal Learning and Memory Test A/B (VLMT in four parallel versions A–D) for neuropsychological assessment. After a cycle of five stimulations we measured again BDI, HAMD, CGI, RWT and VLMT-C. After the second cycle of five stimulations we rated BDI, HAMD, CGI, RWT, VLMT-D. The rater was not blinded to the treatment. After the 16th stimulation the patient refused to continue treatment and ratings for personal reasons.

tDCS was started at 1 mA over 20 min with a ramp up and a ramp down of each 15 s (in toto 1230 s). The measured impedance initially showed variations between 50 and 70 kΩ in accordance to the skin contact and the pressure applied to the sponges and dropped to 10–20 kΩ during ramp-up-phase. During the stimulation all parameters were supervised constantly by the Eldith DC Stimulator. An excess of limitations, e.g., impedance rise by drying up or chute of the electrodes the stimulation was automatically finished. The itching sensation under tDCS application with 144 mmol/l NaCl solution was reported as uncomfortable but not painful. The duration of the sensation was about 2 min. After changing to water-soaked sponges, the duration of the itching sensation diminished to ∼1 min. Stimulation with water was reported more comfortable due to less itching sensation. Skin lesions did not appear (Palm et al. in press).

Overall, clinical rating showed a modest improvement of depressive symptoms (Table I). We found a reduction of the baseline HAMD score from 25 points at baseline to 22 after five stimulations and to 19 points after 10 stimulations. Further ratings did not take place as the patient wanted to stop the treatment. The BDI showed a reduction from 27 to 20 or 21 points after five and 10 stimulations, respectively. In the Regensburg word fluency assessment (RWT) in different versions, we found a percentage of 90% after 10 stimulations in comparison to 52% at baseline. The VLMT was constant at 27 points. The CGI was constant at 4. Although CGI showed no difference from baseline to the end, there has been a mild subjective and objective cognitive improvement. The further antidepressant treatment was moderately changed after end of tDCS and consisted in lithium 800 mg, reboxetine 4 mg and paroxetine 40 mg, but there was no improvement of depressive symptoms in the follow-up examinations.

Table I. Scores in measures of psychopathology and cognition.

| | HAMD | BDI | CORE | PANAS | VLMT W-F | RWT | CGI | MMST |
|---|---|---|---|---|---|---|---|---|
| Baseline | 25 | 27 | 12 | 16/16 | A: 27 B: 42 | PR 52 | 4 | 28/30 |
| *5 tDCS stimulations* 1. rating | 22 | 20 | | 18/12 | C: 27 | | 4 | |
| *5 tDCS stimulations* 2. rating | 19 | 21 | 12 | 20/17 | D: 27 | PR 90 | 4 | |

Acute effects of tDCS on the EEG were assessed with a Neuroscan Synamps apparatus using an electrode cap with 33 electrodes (all referred to channel Cz). After EEG baseline recording, we measured after treatment with 20 min sham tDCS (Eldith sham device), the patient was blinded to this condition. Fifteen minutes after the first active tDCS treatment EEG recording was repeated, the time was needed for drying the hair to avoid malfunction. Electrode skin impedance was always less than 5 kΩ. We used an electrooculogram below the left eye. The electrodes were placed according to the International 10/20 system (Jasper 1958) with the additional electrodes FC1, FC2, FC5, FC6, CP5, CP6, P09, P010. Fpz served as ground electrode. The patient was instructed to remain in an alert state with her eyes closed in a sound-attenuated room. The EEG was recorded for 10 min with a sampling rate of 1000 Hz and an analogous banpass filter (0.16–200 Hz). Offline we changed the sampling rate to 250 Hz and used a 70-Hz low-pass filter. Before analysis, artefact detection was performed visually with the exclusion of all EEG segments that contained obvious eye or muscle artefacts or a decrease in alertness. The scalp sites were grouped into three sagittal regions, frontal (Fp1, Fp2, F3, FC1, F4, FC2, FC5, F7, F8, FC6, Fz), central (T3, T4, CP5, CP6, C3, C4, Cz) and posterior (T5, T6, P3, P4, Pz, O1, O2) for the surface EEG analysis. After recomputation to the average reference, spectral analysis was performed for 27 electrodes (we excluded the electrodes T1, T2, P09 and P010 due to bad quality). The EEG was Fourier transformed for at least 2-s epochs using the Brain Vision Analyzer software Version 1.05. Epochs were reduced to 80 epochs (2 min 40 s). The EEG was analyzed in four frequency bands: delta (1–3 Hz), theta (4–7 Hz), alpha (8–12 Hz) and beta (13–25 Hz). A summary of real versus placebo treatment results are presented in Table II.

In addition we carried out a source analysis with LORETA (Pascual-Marqui et al. 1994). The LORETA changes showed up an asymmetric and focal effect (25–40% reduced activity) in the following frequency bands: delta (1.0–6.0 Hz), theta (6.5–8.0 Hz), alpha 1 (8.5–10.0 Hz) and alpha 2 (10.5–12.0 Hz) (see Figure 1).

## Discussion

In our case, tDCS had very limited effects on the course of a therapy-resistant depressive episode. The improvement in clinical symptoms was modest. Verbal fluency improved after ten tDCS sessions, but no effect was observed on verbal memory performance. A placebo effect on cognitive tasks may be possible, but the cognitive enhancement by tDCS has frequently been reported. Learning effects are not likely due to different versions of the tasks. Interestingly, the acute effects of a single session of tDCS on the EEG were rather pronounced. The differences between EEG effects after sham and after active tDCS suggest a specific efficacy of tDCS on neuromodulation and are probably more intensive after a couple of stimulations. Moreover, we observed similar somatosensory side effects as described by Dundas et al. (2007) in their trial of perception of comfort during tDCS. The use of water showed no other methodological and functional difficulties than the use of NaCl, in both cases the measured impedances depended on the skin contact of the sponges, and in both cases an erythema was found under the sponges after stimulation. In summary, tDCS was well tolerated and no adverse effects were reported.

Boggio et al. (2007b) found a decrease of depressive symptoms in the HAMD from 21 to 13 after 2 weeks of 2 mA treatment which means an average reduction by 8 points. Fregni et al. (2006b) found a decrease by 58% in the HAMD from the baseline score of 23.5 (stimulation with 1 mA over 5 days). In our case, the HAMD decreased from 25 to 19 (6 points) after 2 weeks of 1 mA treatment. In contrast to the studies of Boggio et al. (2007), Fregni et al. (2006a,b) we applied tDCS in a patient with therapy-resistant major depression and continuous psychopharmacological treatment. The moderate effects that we found suggest a lower efficacy of weak tDCS in this case of chronic and therapy-resistant depressive disorder. Another question is whether an antidepressant or

Table II. Summary of significant differences in the surface EEG after active tDCS compared to sham treatment.

| | Absolute power ($\mu V^2$) | | | Relative power (%) | | |
|---|---|---|---|---|---|---|
| Comparison tDCS EEG vs. baseline EEG | Delta | Theta | Alpha | Delta | Theta | Alpha |
| Frontal | ** ↓ | | *** ↓ | ** ↓ | * ↓ | *** ↓ |
| Central | | | ** ↓ | | | ** ↓ |
| Posterior | | | | | | |

Paired *t*-test significance: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; ↓ = significantly decreased.

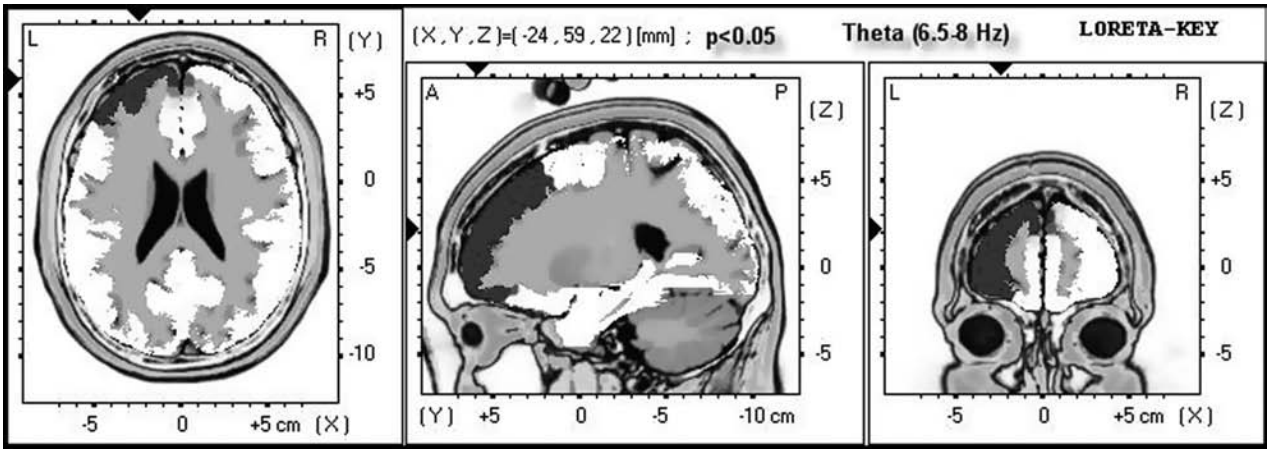


Figure 1. The acute effect of real tDCS on the current densities measured by LORETA. The signal measured about 15 min after the real prefrontal tDCS at the EEG Point F3 decreased the current density in the delta, theta and alpha bands. The figure shows the significant effect between real versus sham measurements for the theta frequency band. The effect was laterally in the superior frontal gyrus (*xyz* = −24, 59, 22; BA 9). The paired *t*-test was used with corrected *P* values: $P \leq 0.05$. *xyz* = maximum in the Talairach space. TF, converted *z*-value.

phase-prophylactic medication may alter the effect of tDCS on neuroplasticity and result in lower antidepressant effects.

However, tDCS has been proven to be a powerful tool for changing cortex excitability. The acute EEG changes following tDCS point in this direction and even more pronounced changes are probable when tDCS is repeated over a longer period. In contrast to former studies with antidepressant-free patients, the application of tDCS in this single case of a multi-drug-resistant and chronic depressive patient seems to have not the same quick and significant effects. The protocol we used was restricted to 1 mA for reasons of safety, and higher stimulation intensity and/or longer tDCS sessions may yield more robust results.

In conclusion, there is further need for studies evaluating tDCS as an add-on-treatment in therapy-resistant patients.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**CASE REPORT**

# Late-onset obsessive compulsive disorder associated with possible gliomatosis cerebri

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**Abstract**

Onset of obsessive-compulsive disorder (OCD) after the age of 50 years is rare, and should alert the physician to possible "organic" causes of OCD. These include infections, degenerative disorders, brain injury and cerebrovascular lesions, principally involving the frontal lobes and basal ganglia. The current patient had obsessive images, anxiety, auditory hallucinations and seizures following (possible) gliomatosis cerebri, with onset around 69 years of age. The atypical presentation, lesions involving the cortical–basal ganglia–thalamic–cortical circuit and the association with neurological signs/symptoms, was characteristic. However, late-onset OCD has not been commonly reported with diffuse lesions, and the association with gliomatosis cerebri is not known. This patient's case illustrates the need for careful screening of older patients with recently acquired OCD, and for further systematic study of OCD in the broad range of neuropsychiatric disorders affecting the elderly.

**Key words:** *Obsessive-compulsive, late-onset, gliomatosis cerebri*

## Introduction

Onset of obsessive-compulsive disorder (OCD) after the age of 50 years is rare. In one study only five such patients were found, out of an OCD patient population of over 1000, in whom symptoms of OCD first developed late in life (Weiss and Jenike 2000). Although thresholds for late-onset OCD differ across studies, most patients with onsets in the fifth decade, or later, appear to develop the illness secondary to a variety of neurological conditions. Accordingly, late-onset OCD has been reported subsequent to encephalitis, Parkinson's disease, Tourette's syndrome, Huntington's or Sydenham's chorea, cerebrovascular lesions, traumatic brain injury, brain tumours, etc. Localization of brain pathology also spans the anatomical spectrum from diffuse involvement to concentration in focal areas such as the frontal and parietal lobes, as well as the basal ganglia. The involvement of frontal lobes and basal ganglia seems particularly characteristic of late-onset OCD with almost all patients showing evidence of focal lesions in these areas (Philpot and Banerjee 1998; Chacko et al. 2000; Coetzer 2004).This concurs with what is known about the neuroanatomic basis of OCD. Although a primary pathological process underlying core OC symptoms has not been definitively identified, morphometric and functional imaging studies have consistently implicated the cortico–striato–pallidal–thalamic circuitry in "idiopathic" OCD (Gross-Isseroff et al. 2003; Desarkar et al. 2007).

## Case report

A 69-year-old retired accountant presented initially to neurology, from where he was referred to the psychiatric services. He had no past history of psychiatric problems, although his eldest daughter had suffered from depression. The patient himself had undergone bilateral cataract extraction; following intra-ocular implants his vision was normal. He had a thyroid nodule removed, subsequent to which he developed tetany, but recovered and was well on calcium and vitamin D supplements. He had also been diagnosed with grade II benign prostatic hyperplasia, but had been asymptomatic since being instituted on a 5α reductase inhibitor.

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His initial complaints included mild psychic anxiety, palpitations and a "sinking" sensation in the epigastrium. These would occur in stereotyped episodes, each lasting for about 15 min to 1 h, without any accompanying impairment of consciousness. He also complained of dizziness and had considerable difficulty falling asleep. Shortly after this, he started having obsessive images of nude females and external genitalia of his young grandson. Despite recognizing these as senseless, he was very distressed by such images and felt extremely guilty that he was "seeing" such things. He prayed a lot, but by doing so was able to control these images from recurring only for a short while. Over the next 2–3 months, he consulted several private psychiatrists, who initiated him on combinations of different SSRIs and benzodiazepines. However, he did not continue any of the SSRIs for more than 2 weeks, because of numbness of extremities and worsening of insomnia, which he attributed to them. A few weeks later he had three episodes of generalized tonic-clonic seizures, which led to an admission to the neurology inpatient unit. Neurological examination was unremarkable, but an MRI-brain showed bilateral (including corpus callosum), asymmetrical, multi-focal white and grey matter lesions, which were non-enhancing and had no mass effect (Figure 1).

Magnetic resonance spectroscopy ($^1$H-MRS) showed decrease in *N*-acetylaspartate levels (NAA) and increase in choline (Cho) levels, suggestive of mitotic lesions. Other physical causes for his anxiety symptoms, such as hypothyroidism or hypoparathyroidism were ruled out since thyroid functions, serum calcium, electrolytes, haemogram, renal function tests were all normal. CSF examination, CECT chest and abdomen and whole body PET-CT were also unremarkable. A 20-channel EEG with a recording time of about 25 min did not show any epileptiform discharges. A provisional diagnosis of gliomatosis cerebri (GC) was made based on the radiological findings; he was started on phenytoin (300 mg/day). The seizures were well controlled with phenytoin; hence the EEG was not repeated. Although he had no further seizures, within a week he started experiencing auditory hallucinations, both elementary sounds and voices. At this point he was referred to psychiatry. The patient and his family were reluctant to try SSRIs again. This coupled with the unusual response and non-compliance with these drugs in the past prompted a conservative approach to treatment. He was continued on phenytoin, to which low doses of clonazepam (0.5 mg/day) and haloperidol (up to 2.5 mg/day) were added. Hallucinations remitted completely with this treatment within about 10 days, anxiety symptoms were also helped, but his obsessive images persisted. The patient was taught the technique of thought stopping, but he was unable to do this on a regular basis.

A follow-up MRI scan after 3 months showed marked increase in size of the lesions, perifocal edema and mid-line shift (Figure 2).

The patient's health also began to deteriorate and he started developing episodes of altered sensorium, disorientation and agitation. The tentative diagnosis was disclosed. It was mentioned that a brain biopsy might be required for confirmation, but after a discussion of the benefits and risks the family did not want it to be done. Unfortunately, the patient's condition worsened further over the next few

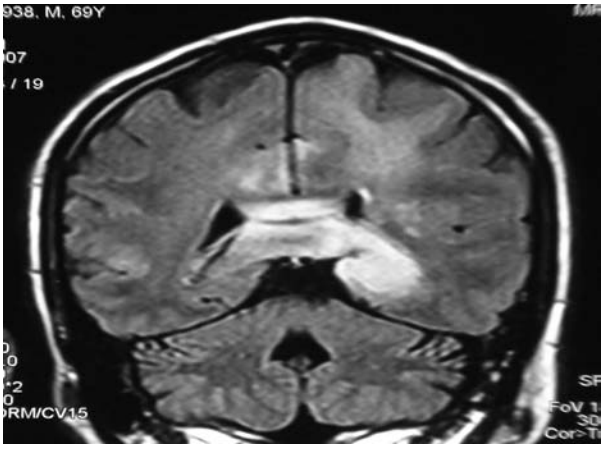


Figure 1. Baseline MRI. Coronal T2 FLAIR image showing hyperintense lesions in the left temporal lobe in the hippocampal region. Also evident are diffuse asymmetrical white and grey matter hyperintensities in the parietal lobe as well as corpus callosum.

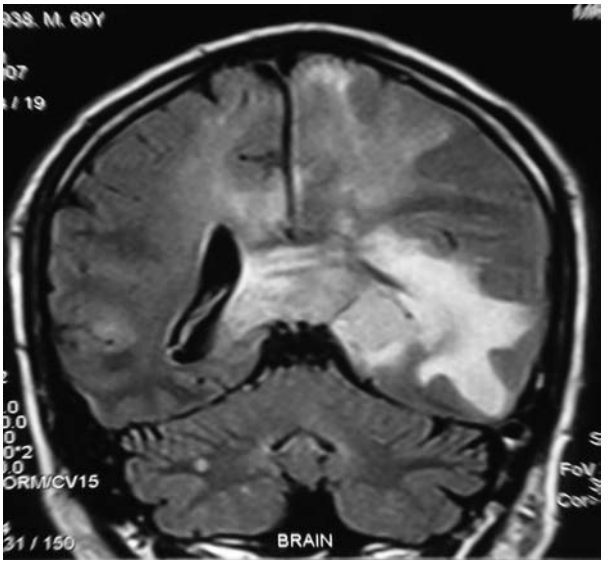


Figure 2. Follow-up MRI. Coronal MRI done 3 months later revealed increase in the size of lesion with mass effect in temporal lobe.

months as he developed right hemiparesis and aphasia. Despite best efforts, he did not survive for more than a month thereafter.

## Discussion

Epidemiological studies indicate that the mean age at onset of idiopathic OCD is usually in the second or third decade of life (Rasmussen and Eisen 1992). Onset of OCD after the age 50 should, therefore, alert the physician to possible "organic" causes of OCD.

In the index patient OC symptoms were most probably secondary to GC. Serial MRIs showed that the lesions were first visible in the left temporal lobe, following which they gradually extended to involve several regions of the brain. The final picture showed extensive involvement with multiple diffuse white and grey matter lesions in different brain areas, including the corpus callosum. Marked increase in size of the lesions, perifocal oedema and mid-line shift were also evident. A MRI showing diffuse, extensive, contiguous involvement (particularly of the corpus callosum), with preservation of the overall cerebral structure is often characteristic of GC (del Carpio-O'Donovan et al. 1996; Bendszus et al. 2000). However, changes on the MRI can be non-specific and other causes of non-enhancing lesions (listed in the table) need to be considered (Felsberg et al. 1994; del Carpio-O'Donovan et al. 1996; Bendszus et al. 2000) (see Table).

Additionally, decrease in NAA levels and increase in Cho levels were noted on MRS in this patient. The most common finding on MRS in GC is a decreased level of NAA; increased Cho, increased choline/creatinine and Cho/NAA ratios have also been reported (Bendszus et al. 2000; Guzmán-de-Villoria et al. 2007). The diagnosis was further supported by the absence of evidence of any primary lesion, a normal CSF examination and a normal metabolic profile. The final confirmation of GC is usually by histopathology (Guzmán-de-Villoria et al. 2007), which was not possible as a brain biopsy was not consented to. Therefore, the diagnosis of GC remained provisional.

GC is a rare primary brain tumor, commonly characterized by diffuse infiltration of the brain with neoplastic glial cells that affect various areas (Kim et al. 1998). Extensive hemispheric white matter and corpus callosum infiltration is characteristic, with lesser spread to subcortical and cortical grey matter. Surgery is often not practical considering the extent of the disease; standard chemotherapy has been unsuccessful, and although brain irradiation can stabilize or improve neurological function in some patients, its impact on survival has yet to be proven Consequently, the prognosis for GC is generally poor, with a median survival time of only 12 months (Vates et al. 2003). Considering these factors, the family of this patient preferred him to continue on symptomatic treatment, which unfortunately did not prolong his life for long.

Table I. Differential diagnosis of non-enhancing white matter lesions

| |
|---|
| Ischaemia |
| Gliomatosis cerebri |
| Multiple sclerosis |
| Adrenoleukodystrophy |
| Subacute sclerosing panencephalitis |
| Viral encephalitis |
| Metachromatic leukodystrophy |

Psychiatric symptoms have been previously reported among patients with GC including depression, dementia and personality changes (Duron et al. 2008). However, to the best of our knowledge the association of GC with late-onset OCD has not been reported in the past.

In this patient the initial involvement of the left temporal lobe could explain the auditory hallucinations. As the lesion spread, it involved other areas including the components of limbic system which might be responsible for the anxiety symptoms. Involvement of the frontal lobe and basal ganglia could account for the obsessive images. Presence of multiple diffuse lesions would explain the generalized tonic-clonic seizures.

Apart from the late-onset and presence of neurological illness, this patient had other features common with previous reports of late-onset OCD secondary to organic factors. These included the atypical presentation, lesions involving the cortical–basal ganglia–thalamic–cortical circuit, and the association with neurologic signs/symptoms such as seizures, dizziness, unilateral weakness and changes in behaviour/personality, which also happen to be common presenting features of GC (Kim et al. 1998). Then again, diffuse brain lesions causing OCD has not been commonly reported in earlier descriptions of late-onset OCD.

Response to treatment of late-onset OCD secondary to neurological disorders is highly variable. Some authors have indicated that the majority of these patients had a relatively poor outcome, with limited response to psychotropic medication (Weiss and Jenike 2000); others have concluded that the presumed organic aetiology in late-onset OCD does not preclude the possibility of successful treatment (Philpot and Banerjee 1998; Carmin et al. 2002). In this patient, however, the unusual response to SSRIs and the eventual poor outcome was in all probability related to the underlying condition, i.e. GC.

In conclusion, the unfortunate case of this elderly gentleman demonstrates that late-onset OCD is often associated with all manner of neurological conditions. A high index of suspicion for such conditions and careful work-up of older patients with recently acquired OCD is thus required. Furthermore, given the paucity of information regarding OCD later in life, a systematic study of OCD in the broad range of neuropsychiatric disorders affecting the elderly is now indicated.

## Acknowledgements

None.

## Statement of Interest

None.

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**CASE REPORT**

# Repeated intravenous ketamine therapy in a patient with treatment-resistant major depression

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**Abstract**

*Background:* The intravenous administration of ketamine, an *N*-methyl-D-aspartate receptor antagonist, results in a great improvement of depression symptoms, but it is not clear for how long. This single-case trial was conducted to explore the duration of improvement and the effects of a second administration on the clinical outcome. *Methods:* In an open label trial, a 55-year-old male patient with treatment-resistant major depression and a co-occurring alcohol and benzodiazepine dependence received two intravenous infusions of 0.5 mg/kg ketamine over the course of 6 weeks. Depression severity was assessed by means of a weekly clinical interview, the 21-item Hamilton Depression Rating Scale (HDRS), and the 21-item Beck Depression Inventory (BDI). *Results:* The first ketamine infusion lead to a pronounced improvement of symptoms, peaking on the second day post infusion (HDRS −56.6%, BDI −65.4%). Positive effects started fading by day 7, reaching baseline by day 35. The second infusion was less efficacious: HDRS and BDI were reduced by 43 and 35%, respectively, and returned to baseline by day 7. *Conclusion:* In this patient with a co-occurring substance use disorder, repeated administrations of ketamine produced positive results. Since the second application has been less efficacious, doses and schedule of administrations need to be further investigated.

**Key words:** *Treatment-resistant major depression, N-methyl-D-aspartate receptor antagonist, ketamine*

## Introduction

Although the number of antidepressant substances has grown considerably over the last decades, many patients suffering from depression continue to be symptomatic despite intensive treatment. It is widely accepted that at least 20% of all depressed patients do not respond adequately to several antidepressant drugs (Crown et al. 2002). While most investigations had revealed that monoaminergic system dysregulation contributed to depression (Delgado 2000; Hirschfeld 2000) it was not until recently that an involvement of the glutamatergic system was demonstrated (Krystal et al. 2002). Consequently, it was suggested that by directly targeting the NMDA receptor, it might be possible to modulate glutamate and GABA systems, thus creating a target for novel antidepressants (Krystal et al. 2002). The exact mechanisms through which NMDA receptor antagonists exert their rapid antidepressant effects are still being discussed, however there is some speculation if directly targeting the NMDA receptor might eliminate long neurotrophic signalling cascades, which could be the reason for the delayed effects of traditional antidepressants (Manji et al. 2003).

Several NMDA receptor antagonists are currently under investigation as antidepressants (e.g., memantine, riluzole and ketamine). Although early results are promising, sample sizes are still small and clinical results preliminary.

A recent study with i.v. ketamine resulted in a pronounced improvement of depression within hours of beginning the treatment (Zarate et al. 2006). In this trial, 71% of all otherwise treatment-resistant patients responded (>−50% on the HDRS) to a single ketamine application (Zarate et al. 2006).

Ketamine is a chiral compound and a dissociative anaesthetic. Because of vivid hallucination induced by ketamine in dosages necessary for anaesthesia

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(0.7–2 mg/kg bodyweight) its use in human medicine has decreased. For antidepressant effects a dosage of 0.5 mg/kg has been needed. In that dosage no anaesthesia is induced.

Known side effects include perceptual disturbances, confusion, elevations of blood pressure, euphoria, dizziness, and increased libido. These adverse effects cease about 80–110 min after an i.v. infusion.

So far, patients with a current co-occurring substance use disorder were excluded from studies on ketamine's antidepressant action. While such exclusion might have been necessary to establish effectiveness, it also constitutes a serious limitation to a wider applicability, since substance use disorders are very frequent in depressed patients (Kessler et al. 2005). Petrakis et al. (2004) have shown that subjects with a family history of alcohol dependence react slightly different to the infusion of ketamine than those without. However, they found no differences regarding mood states post infusion but a blunted response to the dissociative, intoxicant, and dysphoriant properties of ketamine, whereas negative symptoms were slightly more pronounced. In our eyes, these findings do not constitute an a priori reason for excluding patients with a current diagnosis of alcohol dependence from this procedure – rather the opposite. Furthermore, we found no reports on repeated applications of ketamine in the same subject. This led to questions about the sustainability of ketamine's effect on depression; and if effects indeed faded over time, whether a second infusion would result in improvements.

Therefore, our objectives were to replicate and further evaluate ketamine's antidepressant effects in a patient with a treatment-resistant depression and a co-occurring substance use disorder.

## Methods

A fifty-five year old male subject who met DSM-IV criteria for a treatment-resistant major depression and a co-occurring dependence on alcohol, benzodiazepines, and nicotine was included in this study. At the time of inclusion the subject was severely depressed as indicated by weekly clinical interviews, scores on the 21-item HDRS (Hamilton 1960) of 36, and a BDI (Beck 1974) score of 26. The Structured Clinical Interview for Axis I and II DSM-IV disorders (Spitzer et al. 1992) revealed no history of schizophrenia, schizoaffective, bipolar or personality disorders.

Over the course of 4 years the subject had been admitted seven times to inpatient psychiatric services with the following symptoms: restlessness, feelings of hopelessness, helplessness, feelings of guilt, sadness, anxiety, early morning awakening, blunted libido, loss of interest in formerly enjoyable activities, and suicidal ideation.

Past pharmacological treatments had included citalopram (40 mg/day), paroxetine (40 mg/day), mianserine (120 mg/day), mirtazapine (30 mg/day), venlafaxine (375 mg/day), trimipramine (350 mg/day), trazodone (900 mg/day), and escitalopram (20 mg/day). Augmentation therapy had comprised metylphenidate, valproic acid, lithium, olanzapine, quetiapine, buspirone, buprenorphine, and chlorprothixene. All antidepressant medications were given over an adequate time period. Additionally, the subject had received cognitive behavioural therapy. All interventions had been well tolerated, but had failed to achieve remission.

Consumption of nicotine and alcohol had been stable over the course of 6 months at three packs of cigarettes/day and 2.5 l beer/day, respectively, while consumption of benzodiazepines had risen over the same period from 5 to 15 mg lorazepam/day. The subject did not consume any other legal or illegal substances as indicated by urinalysis. A routine medical work up showed no irregularities, with the exception of a high blood pressure (RR 155/95 mmHg). Routine laboratory evaluation revealed the following abnormalities: cholesterol 8.3 mmol/l, and $\gamma$-GT 97 U/l. A cranial MRI, an EEG, an ECG, and chest radiography showed no pathological findings. BMI was 24.8.

Before the first ketamine infusion, anti-depressant medication with trimipramine was tapered to zero to avoid cardiac complications.

A first intravenous infusion of saline solution with 0.5 mg/kg of ketamine hydrochloride (Ketalar®, Pfizer) via an infusion pump over 50 min was followed by a clinical assessment of depression severity (on days 1 and 2, and thereafter on a weekly basis), by the HDRS, carried out by the same psychiatrist. The subject also completed the self rating questionnaire BDI two hours post infusion and on days 1, 2 and 7 after treatment.

Once the subjected reported a complete fading of effects and reached initial values on the HDRS and BDI, a second infusion of an unchanged ketamine dose was administered. This design was chosen first to test the sustainability of effects after the first infusion and second to allow for a comparison of effectiveness between the first and the second application.

During administration of ketamine, ECG, blood pressure, and oxygen saturation were continuously monitored.

Written informed consent from the subject was obtained after the procedures had been fully explained.

## Results

Regression of symptoms started within 25 min of the first ketamine infusion. The subject started to report feelings of dizziness, nausea and dissociative symptoms. He then depicted a sensation of being "lulled in cotton" and being "slightly agravic". However, these sensations vanished 2 h after the end of the infusion.

With the onset of the above effects the subject also began to describe a reduction in restlessness and tension. During the procedure the subject remained conscious and oriented. Thirty minutes after the end of treatment the patient felt entirely unimpaired and moved around freely. Immediately after the end of the infusion he reported feeling less depressed, exhibiting no more signs of sadness or anxiety. His mood continued to improve over the next several hours and days as indicated by declining scores on both the HDRS and the BDI.

At baseline, 2 h prior to the first infusion, depression scores on the HDRS had been 36 and 26 on the BDI, respectively. Two hours afterwards the BDI score had dropped by 9 points (or 35%) to 17 and continued to decrease to 11 (−58%) 1 day and to 9 (−65%) 2 days after the end of the first infusion. By day 7, BDI scores had risen back to 14. The HDRS score had sunk to 16 (−57%) 2 days after the intervention and to 14 (−61%) on day 7. Positive effects lasted for 14 days. By day 21 post infusion, the HDRS score had returned to 25, increasing to 27 on day 28 and to 35 on day 35. By this time, the BDI had increased to 25.

On day 35 the patient received a second infusion of ketamine (same procedure as infusion one). The subject rapidly experienced the same effects (e.g., dissociative symptoms, dizziness) as previously described. However, they were less pronounced compared to the first administration. Again, improvement of symptoms was almost instantly evident, as indicated by a 6-point decrease in BDI scores to 19 (−24%) 2 h and to 16 (−36%) 1 day post treatment. Two days after the second infusion at 10:00 h, HDRS score was at 20 (−43%); however, the same day at 17:00 h, BDI had returned to 25. Seven days after the second treatment HDRS score reached its initial value of 35. Overall, the subject reported effects as present, but less pronounced and faster fading in comparison to the first infusion. Monitoring remained uneventful following both ketamine applications and no cardiovascular complications or other side effects were observed.

## Discussion

To our knowledge, this is the first report on the effects of a treatment course with two subsequent intravenous infusions of 0.5 mg/kg ketamine.

The patient suffering from a treatment-resistant major depression lasting for 4 years, experienced a reduction of symptoms of −61% on the HDRS and of −65% on the BDI after the first infusion. On the self rating questionnaire BDI, even remission was indicated (<9). The positive effects started to fade 7 days following infusion, reaching initial values by day 35. This could indicate that a single infusion of ketamine might not lead to a sustained remission.

The second infusion of ketamine was accompanied by slightly less pronounced dissociative symptoms. The improvement, as indicated by a decrease of the HDRS score by 43%, was present, but was experienced as faster fading and less powerful by the patient. The exact reason for this remains unclear. The occurrence of tachyphylaxia following ketamine administration has not been reported in the anaesthesia literature and therefore seems unlikely. However, during the second infusion of the same ketamine dosage, feelings of "comfortable numbness" (Check 2006) were slightly less pronounced and heart rate was distinctively lower (average of 79 in comparison to 95 bpm during the first infusion), suggesting that the presence of a certain intensity of psychomimetic effects was necessary for the antidepressant effects to occur (Zarate et al. 2006).

In accordance with findings of Berman et al. (2000) and Zarate et al. (2006) we found ketamine to exert strong antidepressant effects despite the subject's current co-occurring abuse of benzodiazepines and alcohol. Therefore, treatment with ketamine might be a valuable option for a wider range of patients than previously reported. Interestingly patients with a life-time diagnosis of "any substance abuse/dependence" (ASAD) or with a life-time diagnosis of "alcohol abuse/dependence" (AAD) did not fare worse than those without (HDRS reduction: ASAD −63.3%; AAD −66.5%; without −47.3%, Zarate et al. 2006, table).

In comparison to trials with other NMDA receptor antagonists like riluzole (Zarate et al. 2004) in the same clinical context, it seems noteworthy that during both treatments, the onset of improvements started very rapidly within minutes after infusion. The use of NMDA receptor antagonists might open new ways of therapy but further studies with more

patients and different doses, intervals, and forms of application will be needed.

## Statement concerning human studies

We report about an open label trial of ketamine in a single case. The study procedures and possible side effects of the study medication had been carefully explained and patient's written informed consent was obtained. In this case of an off label clinical treatment and in agreement with Swiss law no authorization by a research ethical committee was required and therefore not obtained. This was confirmed by the president of the local ethics committee.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**CASE REPORT**

# Zuclopenthixol-induced neuroleptic malignant syndrome presenting as fever of unknown origin, hyperglycaemia and acute myocardial infarction in a 60-year-old man

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**Abstract**

Neuroleptic malignant syndrome (NMS) is a rare clinical condition and potentially life-threatening complication of antipsychotic medications. We report a patient with an atypical presentation of NMS. A 60-year-old man with schizophrenia was admitted to our hospital with disturbed consciousness, fever and marked extrapyramidal rigidity both in the upper and lower extremities. He had been given i.m. zuclopenthixol 200 mg/month but had not taken the last dose. Laboratory investigations showed that creatinine phosphokinase 428 IU/l (normal up to 130), lactate dehydrogenase 772 IU/l (normal up to 450), blood glucose 256 mg/dl (65–110). Urine analyses revealed ketonuria. White blood cell (WBC) count was 6100 cells/mm$^3$. Therefore, the patient was diagnosed as having NMS and antipsychotic medications were stopped. Adequate hydration was provided and bromocryptine 5 mg was started three times a day. Despite treatment, the patient died due to acute myocardial infarction after 3 days of hospitalization.

**Key words:** *Neuroleptic malignant syndrome (NMS), zuclopenthixol, hyperglycaemia, acute myocardial infarction, schizophrenia*

## Introduction

Neuroleptic malignant syndrome (NMS) is characterized by fever, signs of autonomic dysregulation (e.g., pallor, diaphoresis, blood pressure instability, tachycardia, pulmonary congestion and tachypnea), extrapyramidal rigidity and altered consciousness. NMS is usually induced by a reaction of antipsychotic medications and other drugs that affect the dopaminergic system on administration or withdrawal (Hasan and Buckley 1998). The frequency of NMS is approximately 0.07–2.2% of patients treated with neuroleptics. Risk factors are dehydration, use of multiple antipsychotic medications, agitation, previous episodes, rate and route of neuroleptic administration (Aruna and Murungi 2005). The rapidly fatal course is an uncommon event (Lenler-Petersen et al. 1990). The mortality rate of NMS in the literature is 10–20%. After the onset of symptoms and signs, death occurs within 3–30 days. The most common causes of death were respiratory or renal failure, cardiovascular collapse and arrhythmias (Caroff 1980; Levenson 1985; Shalev and Munitz 1986). We report a case of zuclopenthixol-induced NMS associated with hyperglycaemia and acute myocardial infarction in a 60 year-old man with schizophrenia.

## Case report

A 60-year-old man with schizophrenia was admitted to our hospital because of disturbed consciousness, fever and hypertonicity of both upper and lower extremities. The patient was diagnosed with schizophrenia 20 years previously. He was taking zuclopenthixol intramuscularly 200 mg once a month, but it was not known how long he had been taking this medication. He was not taking any other medications. No medication of any kind was prescribed of prior to zuclopenthixol. He received his last dose four days ago. No diabetes mellitus or cardiovascular

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disease were present in the patient's previous medical history, although a family history of cardiovascular disease was present.

On admission, his body temperature was 40°C and blood pressure was 100/60 mmHg. Laboratory investigations revealed: creatinine phosphokinase 428 IU/l (normal up to 130), lactate dehydrogenase 772 IU/l (normal up to 450). The level of plasma triglyceride (TG) 190 mg/dl, low density protein (LDL) 156 mg/dl, high density lipoprotein (HDL) 56 mg/dl.The patient's body mass index (BMI) was 24 kg/m$^2$. Central obesity and microalbuminuria were not observed. The level of blood glucose was elevated: 256 mg/dl at time of admission. In addition, ketonuria was detected on urine analysis. The patient was investigated by an endocrinologist and treatment was started immediately according to their suggestions. Two days after beginning therapy, level of blood glucose was decreased to 200 mg/dl, and ketonuria was observed at lower level in the urine. Nevertheless, 3 days after admission blood glucose rose to 298 mg/dl and ketonuria increased in the urine. White blood cells were 6100 cells/mm$^3$. Serum electrolytes showed: sodium 146 mEq/l, calcium 8.9 mg/dl, potassium 4.8 mEq/l, blood urea nitrogen (BUN) 25 mg/dl, serum creatinine concentration 0.8 mg/dl and myocardial creatinine phosphokinase (CKMB) 27 IU/l.

A computerized scan of the patient's head was within normal limits. Because the patient was admitted to hospital with fever of unknown origin, analysis of the cerebrospinal fluid (CSF) was performed but the results were unremarkable. No bacterial pathogens were identified in the blood, urine and CSF cultures. ECG examinations were normal initially. Consequently, the patient was diagnosed as NMS caused by use of zuclopenthixol.

At the beginning of therapy, antipsychotic medication was stopped and the patient was hydrated by 3500 cc/day isolayte solution. Bromocryptine 5 mg three times a day was administered. For agitation, diazepam 20 mg was started twice a day.

After 2 days of the therapy, the level of CK was decreased to 374 IU/l. Agitation and consciousness of the patient were slightly improved. The level of CK increased to 442 IU/l again; ST segment elevation was observed in the anterior derivation on the ECG examinations. The patient was examined by a cardiologist, and was diagnosed as having acute myocardial infarction and treatment was started at once. However, the patient died suddenly due to cardiogenic shock 3 days after admission.

## Discussion

NMS is an uncommon syndrome, which is usually induced by a reaction to antipsychotic therapy. In the NMS, there is a blockage of dopamine in the nigrostriatal tracts. According to Levenson (1985), diagnostic criteria for neuroleptic malignant syndrome include major and minor criteria. Fever, muscular rigidity and raised creatinine phosphokinase are the major criteria. Tachycardia, labile blood pressure, tachypnoea, altered consciousness, sweating, leucocytosis are minor criteria. Three or two major and four minor criteria are the diagnostic threshold. Rhabdomyolysis usually accompanies this syndrome (Hasan and Buckley 1998).

Early diagnosis and immediate treatment of NMS are very important because of its high potential lethality. In the literature, it is reported that death occurs within 3–30 days after the onset of symptoms and results from respiratory or renal failure, cardiovascular collapse and arrhythmias (Caroff 1980; Levenson 1985; Shalev et al. 1989). As soon as the patient was diagnosed with NMS, treatment was initiated at once; however, the he died on third day of therapy. Lenler-Petersen et al. reported that sudden death is very infrequent in the literature (1990).

The exact mechanisms responsible for NMS are still unclear. To authors' knowledge, NMS is believed to result from metabolic abnormalities of intracellular calcium. Therefore, treatment should include either bromocryptine or dantrolene. Dantrolene works by blocking the release of calcium from the sarcoplasmic reticulum, and bromocryptine is a direct dopamine agonist causing inhibition of calcium ion uptake in skeletal muscle (Mieno et al. 2003). Some authors suggested that the combination of these drugs is very effective for the treatment of NMS (Shalev et al. 1989; Caroff and Mann 1993), despite the fact that dantrolene is less recommended on its own.

In the lateral hypothalamus, inhibition of dopaminergic receptors may contribute to the fever (Fiebel and Schiffer 1981). In a previous report, the mortality rate of NMS was shown to be high, and the clinical condition is very serious in patients with fever (Hadad et al. 2003). Similarly, in our case body temperature was 40°C and very serious complications were present.

Zuclopenthixol decanoate is a long-acting form of zuclopenthixol that has been made more lipophilic by esterification of decanoic acid. The esters of zuclopenthixol are dissolved in fractionated coconut oil and, when injected i.m., diffuse slowly from the oil depot to the body water phase where they are rapidly hydrolysed to the active substance.

Maximum serum concentrations of zuclopenthixol are reached 3–7 days following i.m. injection (Szukalski et al. 1986). In our case, the last dose of zuclopenthixol was administrated 4 days before admission. Thus, NMS developed while zuclopenthixol was at maximum serum concentrations.

There is a little knowledge about cardiac abnormalities in NMS. To our knowledge, a few cases died from cardiac complications in NMS in the literature (Lenler-Petersen et al. 1990; Benaissa et al. 1998; Patrick et al. 2003). In one of these reports, cardiac and skeletal muscles showed similar changes, suggesting a common disease process on post mortem investigation (Patrick et al. 2003). In another report, elevated CK was an indicator of cardiac involvement in NMS in Parkinson's disease. In addition, high levels of serum myosine light chain I and CK–MB are useful indicators of lethality (Sato et al. 2005). Our case died due to acute myocardial infarction when serum CK level was elevated again.

Atypical antipsychotics are usually associated with a risk of metabolic syndrome (Goethe et al. 2007). The features of metabolic syndrome are impaired glucose regulation or diabetes, insulin resistance, raised arterial pressure $\geq$160/90 mmHg, raised plasma triglyceride levels, raised LDL cholesterol level, and/or low HDL cholesterol level, central obesity and/or BMI $>30$ kg/m$^2$ and microalbuminuria (Cihan and Pabinger 2007). Our patient was not diagnosed as having metabolic syndrome, because none of the features of metabolic syndrome were present, except for impaired glucose regulation and moderate hypercholesterolemia.

Here, we report a case of NMS in an elderly ma with hyperglycaemia. There were a few cases with hyperglycaemia in NMS in the literature. In one of them, Saeki et al. (1998) described a case of NMS associated with disseminated intravascular coagulation followed by hyperosmolar non-ketotic diabetic coma in an elderly patient with Parkinson's disease during l-dopa therapy. The symptoms recovered within several days on treatment. In another report, a patient with NMS, who developed diabetic ketoacidosis, was treated immediately but this patient died several days after admission (de Boer and Gaete 1992).

Certain antipsychotics may be associated with new-onset glucose intolerance, including acute diabetes and ketoacidosis. The pathophysiology of this effect is likely to be a complex mechanism (Bottai et al. 2005). Some authors report that hyperglycaemia can reduce dopaminergic transmission and increase postsynaptic dopaminergic supersensitivity, thus bringing about NMS (Saller and Kopin 1981; Chu et al. 1986). Some recent studies suggest that hyperglycaemia causes increased risk of myocardial infarction, peripheral vascular disease, stroke, renal damage, vision loss, cataract, etc. Also, hyperglycaemia induces oxidative stress and increases the production of proinflammatory molecules. These events eventually cause endothelial dysfunction and lead to tissue and target organ damage (Das Undurti 2003). Therefore, there may be a positive association among hyperglycaemia and cardiac abnormalities and mortality from NMS.

Since antipyschotics might induce hyperglycaemia, a physician must be alert to NMS in patients treated with antipsychotic medications, and especially to hyperglycaemia in order to prevent cardiac complications.

To the authors' knowledge, using bromocryptine for suppression of calcium in a few postpartum cases induced acute myocardial infarction (Hayashi et al. 2003). However, these authors did not report that using bromocryptine for NMS had worsened the cardiac complications. Sparrow et al. (2003) reported that postmortem histology of cardiac and skeletal muscle showed similar changes of focal cellular necrosis and vacuolation suggesting a common disease (Patrick 2003). Therefore, it is difficult to say that the cause of cardiac complications is the use of bromocryptine. Perhaps the cause of these complications is NMS itself. It is necessary that experimental and clinical investigations should be made to assess the link between bromocryptine and cardiac complications in NMS.

The mortality rate of NMS is high when associated with any unusual complications (de Boer and Gaete 1992). The early diagnosis and treatment of these complications are very important for the prognosis of NMS. However, the presence of these complications in NMS makes treatment difficult, particularly in elderly patients.

## Conclusion

We have demonstrated that NMS is a potentially life-threatening disease when accompanied by infrequent complications, and physicians should be alert to the presence of hyperglycaemia, because hyperglycaemia is a risk factor for cardiac complication in NMS in elderly patients treated with zuclopenthixol.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**CASE REPORT**

# Is anorexia nervosa a neuropsychiatric developmental disorder? An illustrative case report

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**Abstract**

We propose the concept that anorexia nervosa is a neuropsychiatric developmental disorder. In support of the concept we present a case report of a 12-year-old girl with high functioning autistic disorder who developed Tourette syndrome and obsessive-compulsive disorder. She subsequently experienced a distinct onset of partial anorexia nervosa characterized by fear of gaining weight, body image distortions, food preference idiosyncrasies including avoidance of fat, dietary restriction, a pursuit of thinness, episodic self-induced vomiting, the missing of her menstrual cycles, and a 10% decrement in expected weight for height. She fell short of the required 15% decrement in expected weight for height to qualify for the full syndrome. Our case presentation emphasizes the longitudinal commonalities and symptomatic overlap of her multiple comorbidities. We discuss treatment approaches typically used with individuals with neuropsychiatric developmental disorders which might benefit higher functioning individuals with eating disorders. We conclude with examples of a neuropsychiatric developmental approach to generate a research agenda for anorexia nervosa.

**Key words:** *Anorexia nervosa, Tourette syndrome, autism, obsessive-compulsive disorder, neuropsychiatric disorders*

Neuropsychiatric developmental disorders (NPDD) are defined by an onset during infancy, childhood, or the adolescent years (Kerbeshian and Burd 2007). There is a temporal unfolding and progression of the course of symptoms. As symptoms aggregate into definable syndromes there may be concurrent variation in syndromal comorbidities over time. This progression of dysfunction modifies and is conversely modified by the lines of normal and expectable development. This epigenetically programmed course is the product of a longitudinal transaction between genetic endowment and environmental experience. These progressive changes in syndromal phenomenology are captured in part by the concept of heterotypic continuity. As defined by Costello et al. (2003), there is continuity of the underlying vulnerability to a disorder that exposes a child to different disorders as a function of age, or there is an underlying disorder with differing manifestations at different ages.

The relationship between NPDD and its comorbidities is refracted through the developmental lens of age-dependent influences. Our working definition of comorbidity is that of a greater than chance likelihood that if one condition is present, it will be accompanied by another. Commonly the concept of comorbidity is applied contemporaneously, that is, the comorbid conditions are present at the same point in time. We might call this cross-sectional or horizontal comorbidity. However, the concept of comorbidity may also be given a developmental dimension and applied over a span of time. Clarkin and Kendall have defined this vertical or longitudinal comorbidity as the greater than chance likelihood that the occurrence of one condition will be followed by another (Clarkin and Kendall 1992; Kendall and Clarkin 1992). Such a perspective can add flexibility to the application of otherwise rigid hierarchical diagnostic schema. One is then enabled to distinguish second-order diagnostic patterns

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through the dimension of time, providing some clarity to what may appear to be indistinct overlapping diagnostic constructs. The subjective impression in some such cases might be that of one condition evolving into or being replaced by another, or in other words, heterotypic continuity.

Anorexia nervosa (AN) is an eating disorder characterized by refusal to maintain a minimal body weight, a fear of gaining weight, distortions in body image and, in postmenarchal females, amenorrhea (American Psychiatric Association 2000). In contrast to most NPDDs, AN is more prevalent in females and in affluent societies. Although family and twin studies point to biogenetic factors in the condition, the expression of AN is strongly mediated by ontogenetic and sociocultural variables (Nilsson et al. 1998; American Psychiatric Association 2006). AN frequently presents with multiple comorbidities including mood disorders; anxiety disorders, including obsessive-compulsive disorder; and substance use disorders (Wonderlich and Mitchell 1997). The DSM III and the DSM III-R classified AN with the disorders usually first evident in infancy, childhood or adolescence. The DSM-IV and DSM-IV-TR moved AN and other eating disorders to a separate eating disorders section as a number of them had a typical range of age of onset during later adolescence and early adulthood. Additionally it was noted that AN and other eating disorders are usually diagnosed, treated, and researched by specialists in the field of eating disorders (Shaffer et al. 1998).

The DSM-IV-TR definition of AN requires a weight of less than 85% of that expected for age and height. Although this could be seen as a severity of illness measure, which in most other DSM-IV-TR definitions has more of a quantifying than qualifying emphasis, it is nonetheless a necessary criterion for making a diagnosis of AN. By strict definition, an individual meeting all the criteria for AN with the exception of the weight criterion should be classified as having an eating disorder not otherwise specified. Crow et al. have convincingly argued that in AN there is unclear value in a diagnostic criterion that utilizes a dichotomous cutoff such as percentage of expected weight to establish syndromal severity (Crow et al. 2002). In their work, discriminant analysis failed to differentiate between full AN and partial AN (pAN).

In the DSM-IV-TR, obsessive-compulsive disorder (OCD) presents with obsessions and/or compulsions (American Psychiatric Association 2000). The symptoms are time-consuming, interfere with functioning, are not restricted in content to another disorder, such as AN, and are not attributable to substance abuse or a medical condition. Through imaging and neurochemical studies, the neurobiological substrate for the pharmacotherapy and the psychotherapy of OCD appears to be in the neural module defined by the orbitofrontal cortex and its reciprocal connections with striatal and thalamic nuclei (van den Heuvel et al. 2005). Serotonin reuptake inhibitor antidepressants and the cognitive-behavioural technique of exposure-response prevention appear to be about equally effective, while similarly affecting neuroanatomic substrates (American Academy of Child and Adolescent Psychiatry 1998).

Autistic disorder (AD) is the prototypic pervasive developmental disorder and a classic presentation of a NPDD (Volkmar et al. 1999). The current conceptualization of the condition includes an age of onset of before 3 years of age; the presence of impairment in social interaction, including the realm of peers, sharing and emotional reciprocity; impairment in communication, including delayed or deviant modes of communication; and restricted and stereotyped behaviours and interests (American Psychiatric Association 2000). Individuals with AD may exhibit pica, characterized by the eating of nonnutritive substances. AD frequently has multiple other feeding and eating components such as restricted food preferences or strong avoidance of some textures, colours, or odours of foods.

Tourette syndrome (TS) has been described as a model neuropsychiatric disorder of childhood (Cohen et al. 1997). Diagnostic criteria for Toilette's disorder include the presence of multiple motor tics and one or more vocal tics of greater than a year's duration, with an age of onset of prior to 18 years old. In individuals with TS, there may be a number of neurobehavioural correlates, which are not necessary in making the diagnosis and are variable in their expression (Freeman et al. 2000). Among these correlates is obsessive-compulsive disorder or obsessive-compulsive behaviour. Family and genetic studies point to the heritability of the condition.

We propose that, at least in some cases, AN may be conceptualized as a NPDD. As test of concept, we will present a complex case of pAN in a 12-year-old girl, who has also the comorbid syndromes of OCD, AD and TS. We make the assumption, bolstered by the work of Crow et al., that conclusions relevant to our case of pAN will also be germane to full AN (Crow et al. 2002). Where relevant, we will review data regarding comorbidity among the DSM-IV-TR disorders our patient exhibits and data regarding the influence of shared aetiological factors among these disorders. Our presentation of the shared phenomenologies among these conditions will infer that these symptoms may be reflective of common neurobiological substrates. We will attempt the challenging and complex exercise of simultaneously applying the concepts of

cross-sectional and longitudinal comorbidity to observed clinical phenomenon. Finally, we will give examples of the generation of testable hypotheses following from the application the neuropsychiatric developmental model to our case.

## Case study: Barbara U

We first evaluated Barbara U. as an 11-year-old fifth grader, referred for difficulties with an eating disorder, obsessions, compulsions, a developmental deviation, and a tic disorder. We utilize a pseudonym to protect the patient's confidentiality to meet the ethical requirements in conducting research. Barbara was her mother's first pregnancy. The pregnancy was complicated by hyperemesis gravidarum, resulting in a hospitalization at three months of gestation for treatment of dehydration. Mother did not use alcohol, tobacco, or illegal drugs during the pregnancy. The delivery was vaginal.

Barbara had significant difficulties with her development dating back to her birth. She was born at 7 months of gestation, weighing 2100 g. She required 2 months of neonatal intensive care for respiratory distress, required intubation, and mechanical ventilation. Barbara's third toes were longer than her large toes or second toes on both of her feet. Both of her fourth toes were short and incurving. Chromosomal studies and a paediatric genetic assessment were declined due to concerns about cost. One month after discharge, she was readmitted to the hospital for another month due to severe failure to thrive associated with poor suck. She was treated with intravenous and gavage feedings. She was walking by 13 months of age, with periodic toe-walking. At 18 months of age she had surgical correction of a ventriculoseptal defect. Around this time, the mother noted Barbara did not exhibit a reciprocal social smile, avoided eye contact and, as time went on, became actively and interpersonally avoidant. By age 2 years, these symptoms were quite prominent. She would lead her mother around by the hand and would mechanically point her in the direction in which she might want something done. She was uniformly indifferent to others but did have recognition of her mother and father. She sought contact with her parents, but without affection and only when she needed something or needed protection. She was toilet trained at 2 years of age.

As a preschooler, Barbara was quite agile and exhibited an exceptional sense of balance. Barbara had significant delays in her speech and language with the emergence of recognizable speech after age 3 years. She had normal brain stem auditory evoked potentials as an infant and again at age 4 years. At age 4 years, she exhibited a pattern of echolalia followed by conversations with herself consisting primarily of complex repetitions of things she had heard at other times. These interactive conversations would last late into the night. After pacing back and forth in her room, she would sleep for just 4 hours a night. During the day, she often exhibited body rocking, especially when excited, which was often accompanied with pulling out clumps of her own hair or hitting herself on the forehead. Barbara would become very distressed with even minor changes in her environment.

At age 4, after a workup with a normal EEG, brain CT scan, and thyroid function blood tests, she was diagnosed with autism. By 5 years of age, Barbara began to make significant improvements in her speech and language and interpersonal relatedness skills. She continued with abnormalities in the rhythm and prosody of speech, avoidance of eye contact, a persistent falsetto voice present in virtually all situations, and clumsiness and impaired use of metacommunicational motor movements. She also manifested odd accessory and other motor movements.

Early on, Barbara exhibited a number of precocious splinter skills. Before talking, she demonstrated excellent drawing skills, including the use of three-dimensional perspective. She lost this by 8 years of age. She was able to read prior to starting school, and by the first grade was reading several grade levels in advance of her classmates. While she was able to read sentences and passages fluently, she did not have even basic comprehension of the material. Until the fifth grade her comprehension abilities were far below her decoding skills. Barbara demonstrated excellent rote memory for dates and times, but had not mastered the savant calendar trick.

When Barbara was 3 years of age she would collect dryer antistatic sheets and sleep with them, apparently being stimulated by the smell. Between 6 and 9 years of age, she exhibited a marked hypersensitivity to tastes which to others were fairly bland. At 9 years of age, her parents built an addition to their house, and Barbara's bedroom was moved. She then began collecting her saliva in her mouth, being loathe to swallow it, complaining that it was dirty. As a consequence she would dribble her saliva and express concern that her lips were being dirtied. She began to constantly wash her hands and complained about fears of germs and contamination. She was quite selective in what she would touch. She developed specific concerns about contamination of her food. She would collect nonsense articles such as used erasers. There were no evening up or checking rituals. At night, while pacing on her toes in her room, she would talk about a number of thoughts

running through her mind, usually detailed and repetitive replays of the day's events.

Since 4 years of age, Barbara has had a pattern of waxing and waning motor and vocal tics. Her motor tics included bilateral facial grimacing, the spasmodic blinking of her left eye, a tensing of the left cheek and occasional shoulder shrugging. These movements have been fairly consistent and continual. By age 11 years, she developed a frequent spitting tic. Her echolalia continued and she used a high pitched falsetto voice in a breathy manner. She would also interrupt her speech with brief bursts of hyperpnoea.

Cognitive testing at 7 years 3 months with the Stanford-Binet found an IQ of 80, with a mental age of 6 years 2 months. At age 10 years, she was given a more complete neuropsychological evaluation. The full scale IQ on the Wechsler Intelligence Scale for Children was 84, Verbal of 82 and Performance of 90, and a Peabody Picture Vocabulary Test-Revised resulted in a standard score of 102. Additional speech and language testing indicated she had a good deal of difficulty with environmental cues, particularly as related to learning about other people or anticipating or predicting their actions (theory of mind). Her decoding skills in reading were at a Percentile Rank of 32.

On our initial contact, Barbara was 11 years old. The family history included two healthy younger brothers age 2 years. One of these brothers also had webbing between the second and third toes of both his feet. Her father had bouts of excessive alcohol usage. Maternal great-grandmother had a mental illness and the maternal grandmother had obsessions. Barbara's mother has had problems with cleanliness compulsions, occasional spasmodic eye blinking and tensing of the forehead with wide opening of the eyes. Mother walked on her toes until she was 18 years of age, and even as an adult has periods of toe walking. Barbara's mother worked in the home and her father was a student.

Barbara denied having auditory, visual, or olfactory hallucinations. She has not been seen as paranoid or overly suspicious. We had diagnosed her with the following: AD with hyperlexia, TS, and OCD. At age 11, we started Barbara on fluvoxamine, maintaining her on 12.5 mg/day, as higher doses were associated with nausea and urinary incontinence. Both her compulsions and tics would wax and wane and included touching compulsions. The fluvoxamine improved her spitting, but the facial tics and fears of contamination, especially of food, continued unchanged.

During our subsequent treatment of Barbara, we saw the evolution of an eating disorder. She developed an increasing preoccupation with her weight. Mother dates this preoccupation as following Barbara's seeing one of her classmates become sick and vomit after eating a meal in school. Barbara complained about feeling queasy. Following a miscommunication with mother, Barbara's fluvoxamine was stopped and she had increasing difficulties. Initially, she began to actively avoid food due to concern about contamination and germs. She subsequently became obsessively preoccupied with fear of becoming fat. She started weighing herself many times during the day and she lost weight (from 48.1 kg to 46.3 kg). She drank very few liquids and developed enuresis with a waxing and waning course. She would eat dry toast and especially avoided cheese or fatty foods. Teachers were concerned she would not drink milk in school and that her use of the bathroom diminished markedly. She then began to pull her clothes tight around her to make herself look thinner but her parents reported that her clothes appeared to be extremely loose. She then began to disrobe before her evening pacing routine. She was diagnosed with a nonspecific eating disorder. We referred her to a clinical psychologist who provided behavioural and cognitive-behavioural interventions and she was treated with clonidine and fluvoxamine.

Her paediatrician, who monitored her growth, indicated she was below the 10th percentile of her ideal body weight of 52.2 kg. Barbara had also begun to miss her menstrual periods. She met criteria for AN, with the exception of the 15% weight loss criterion. Her persistent weighing of herself continued. She became very upset if the scale showed even a minor weight increase.

Barbara's tics and obsessive-compulsive symptoms ultimately came under control with a combination of clonidine 0.025 mg qid and fluvoxamine 50 mg bid. This also improved her pattern of anxiety, especially the separation anxiety. She began to sleep well at night and her energy level was good. Her weight stabilized and she was able to maintain in the range of 47.2 kg. Mother also began to accommodate to Barbara's food preferences by avoiding fatty foods, but was able to get her to take in carbohydrates. Barbara continued to be preoccupied with food and with her weight. A year later following our first contact with Barbara, she had again lost weight down to 46.3 kg, and had begun episodically to vomit, usually after drinking large volumes of fluids. She would hide her vomitus about the house. She would tell her mother she felt skinny and bony, and that she was worried about gaining weight.

By the time of her entry into the sixth grade, Barbara was doing reasonably well, with the exception of her eating disorder. She was mainstreamed in most of her classes with resource room help. Her

reading comprehension scores were at average levels. She was pleased to report she had received 100's on some of her tests. She was enjoying school and appeared motivated. She slowly gained to 48.6 kg and was struggling hard not to vomit. She developed a capacity for verbal expression of her subjective experience of symptoms. For example, she reflected on why, in the past, she was compelled to avoid eye contact. She stated that when she looked another individual in the eye, she perceived a light emanating from the eye, causing her a sense of photophobia. This caused her to look away. If she wished to persist in maintaining eye contact, she would have to squint. Her description was congruent with a sensory phenomenon preceding the expression of a motor tic. Her falsetto diminished, there was a more natural prosody to her speech, she related more warmly and appeared less odd. She expressed an affect of sadness regarding some of her difficulties. We continued with her pharmacotherapy and psychotherapy. Barbara increasingly relied on postprandial vomiting to avoid gaining weight, rather than restriction of intake. By the end of her sixth grade year, which was cut short due to a natural disaster in the community, Barbara had dropped from 49.5 to 47.7 kg. At the end of that school year, the family moved to another community, and Barbara was lost to follow-up. Subsequently, we received a note from mother, indicating that Barbara's situation was again deteriorating. She asked for her medical records to be transferred to another clinic out of state.

Over a nearly 5-year period, Barbara's weight had fluctuated between 45.4 and 49.5 kg. We felt that she otherwise met full criteria for a diagnosis of AN, with the exception of the 15% weight loss criterion. This would place her in the grouping of patients described by Crow et al. (2002) as pAN. We propose that our findings regarding pAN in Barbara's case may have relevance for the full syndrome of AN

We will now review selected studies of the comorbidities that Barbara experienced, with the goal of emphasizing shared aetiological factors and common neurobiological substrates. There is a dearth of epidemiological studies exploring the prevalence of Barbara's other comorbidities and her eating disorder. In a group of 30 women with severe eating disorders, not all of whom had AN, Wentz et al. (2005) studied the prevalence of childhood onset neuropsychiatric disorders, a concept congruent with NPDD. They found 23% with autism spectrum disorders and 27% with a tic disorder, suggesting that having an eating disorder carries a greater risk for the expression of these other categories of conditions. The association between AN per se and TS is based primarily on case reports. There are reports of a subset of eating disorder patients with both TS and OCD (Guarda et al. 1999). AN and TS in a young man has been reported (Annibali et al. 1986). TS and AN was first reported in 1979 (Yaryura-Tobias 1979). The association between OCD and AN is more robust (McElroy et al. 1994; Lennkh et al. 1998; Wentz et al. 2001; Serpell et al. 2002; Halmi et al. 2003). The phenomenological similarities suggest that both AN and OCD may share common brain-behavioural pathways (Barbarich 2002). The relationship between AN and obsessive-compulsive personality may be an even stronger association (Anderluh et al. 2003). Dysregulation in the serotonin system has been implicated in both OCD and AN (Barbarich 2002; Kim 2003). Finally, the link between AN and OCD through the symptom pattern of body dysmorphic disorder as a disturbance of body image is interesting (McElroy et al. 1994; Rabe-Jablonska and Sobow 2000). A link between body dysmorphic disorder and TS is also noteworthy (Sverd et al. 1997). Associations between AN and AD have also been reported (Gillberg 1983). First-degree relatives of individuals with AN are more likely to exhibit symptoms of a pervasive developmental disorder. Multiple case studies of individuals with concurrent AN and AD have been published (Stiver and Dobbins, 1980; Fisman et al. 1996; Gillberg et al. 1996; Gillberg 1998; Gillberg and Billstedt 2000) The results of Wentz et al. (2001) notwithstanding, Bolte et al. (2002) note that in a subgroup of 29 significantly underweight autistic spectrum disorder patients none met criteria for AN. The risk of being underweight and of having an eating disorder is increased among Asperger syndrome patients (Sobanski et al. 1999). Serotonin abnormalities may connect some studies of AN and AD. Hyperserotonemia has long been an episodic finding in AD (Chandana et al. 2005). The related eating disorder of bulimia nervosa is associated with increased turnover of the serotonin metabolite, 5-HIAA. Serotonin selective reuptake inhibitors have been helpful in treating the repetitive behaviours of AD and the binge-eating/purging elements in anorexia nervosa (McDougle et al. 2000). If AN and the above-noted comorbidities in Barbara's case are related, one might expect common features of non-pharmacological treatment approaches. Cognitive-behavioural psychotherapy has long been utilized with benefit in treating AN and OCD (Mitchell et al. 2001). The related interventions of exposure with response prevention, habit reversal, and applied behavioural analysis have been shown to be helpful in the treatment of AN, AD, TS and OCD (Boutelle 1998; Grindle and Remington 2002; Kennedy et al.

1995; Lovaas 1993; Mavissakalian 1982; McEachin et al. 1993; Wilhelm et al. 2003).

In exploring the relationship among Barbara's comorbidities exclusive of her pAN we note the following. TS and AD co-occur at a greater than chance rate (Baron-Cohen et al. 1999). In individuals with AD, TS may serve as a positive prognostic indicator (Burd et al. 1987; Kerbeshian et al. 1990). Not only is there a greater likelihood of OCD and obsessive-compulsive symptoms occurring in individuals with TS, but about 10% of individuals with OCD will exhibit tics or TS. TS and OCD may be alternate expressions of a common diathesis (Comings and Comings 1991; Leckman 2002). The issue of concurrence between autism and OCD is somewhat controversial, due to overlapping phenomenology between the obsessive need for sameness and repetitive behaviour in individuals with AD, and the ritualistic and stereotyped patterns one sees in the symptoms of OCD (Baron-Cohen 1989; McDougle et al. 1995). However, there are convincing case reports supporting concurrence between AD traits and OCD (Bejerot et al. 2001). The association among TS, OCD, and AD is well accepted with TS serving as a bridging comorbidity between the latter two (Cath et al. 2001; Zappella 2002). Table I outlines the strength of associations among the combination of comorbidities noted in Barbara's case. These commonalities allow for the fitting of TS, OCD, and AD within the construct of NPDDs. The association among these disorders and pAN in Barbara's case is most interesting, given the commonly assumed greater salience of cultural, social, experiential, and psychological variables implicated in the genesis of AN, as compared to the others. AN typically has not been thought of as a NPDD. Challenging this perspective, Klump et al. (2007) conducted a study supporting early to middle adolescence as a developmental phase threshold for the expression of a genetic risk for disordered eating.

We will now discuss the microcosm of specific symptoms across Barbara's comorbidities that determine the macrocosm of her clinical syndromes. The DSM schema of mental disorders is based essentially on a syndromal classification strategy. Distinct syndromes are defined by patterns of symptomatic specificity conforming to a level of diagnostic validity. Validity is in part defined by the statistical separability of one syndrome from another. Specific symptoms, however, may be criteria in multiple syndromes, albeit often differently nuanced. One way of exploring the neuropsychiatric developmental roots of pAN in Barbara's case would be to list the permutations of the symptom-based criteria for her comorbidities across time and across diagnoses. We have previously used a similar mode of case analysis (Kerbeshian and Burd 2005). As an example, Barbara's persistent preoccupation with and intense fear of weight gain or of being fat in AN could as well be conceptualized as a preoccupation with stereotyped and restricted patterns of interest in a high functioning person with AD. The resultant behaviours could also be conceptualized as a pattern of adherence to nonfunctional routines or rituals. In the case of OCD, the persistent preoccupation with weight and associated behaviours may also be viewed as the recurrence of thoughts, impulses and images that define obsessions, and the driven repetitive behaviours or mental acts that define compulsions. In the case of TS, the more complex "mental tics" reported as premonitory urges to tic, or heightened, or distorted body sensory phenomenon are evocative of the body image distortion present in AN. In Table II, we summarize examples of shared phenomenology among Barbara's four comorbid diagnoses.

The sequence of emergence and overlap of NPDD comorbidities in Barbara's case are consistent with what one would expect, given the range of ages of onset of her conditions. By definition, the age of onset of AD is below 3 years of age. The median age of onset of TS is 6–7 years; the modal age of onset of OCD in females is 20–29 years, but childhood onset is fairly common; and the typical age of onset of AN is 14–18 years (American Psychiatric Association 2000).

Barbara experienced an evolution over time of specific symptoms across different syndromes, what we might term symptom ontogenesis. We will now track over time the transformation of component symptoms with the aim of inferring a microscopic

Table I. Strength of association among comorbidities.

| | | | | | | |
|---|---|---|---|---|---|---|
| Larger scale studies | | | | | X | X |
| Common diathesis | | | | | | X |
| Family history studies | | | | X | | X |
| Similar phenomenology | | | X | | | X |
| Common neurotransmitters | | X | | X | | |
| Common medication response | | X | X | X | X | |
| Case reports | X | X | X | X | X | X |
| Strength Of Association | AN&TS | AD&OCD | AN&OCD | AN&AD | TS&AD | TS&OCD |

Table II. The shared phenomenology of AD, TS, OCD, AN in the case of Barbara U.

| Criteria | AD | TS | OCD | AN |
|---|---|---|---|---|
| AD: Stereotyped and restricted patterns of interest | X | | X | X |
| AD: Adherence to nonfunctional routines or rituals | X | X | X | X |
| TS: Tics, (mental tics), premonitory urges or sensations | | X | X | X |
| OCD: Recurrent thoughts impulses or images | X | X | X | X |
| OCD: Driven repetitive behaviors or mental acts | X | X | X | X |
| OCD: Unrealistic prevention of distress or dreaded situation | | | X | X |
| AN: Illogical intense fear of gaining or becoming fat | | | X | X |
| AN: Distrubance in way body or shape experienced | | | X | X |
| AN: Restricting food intake | | | X | X |
| AN: Binge-eating/purging | X | X | | X |
| AN: impairment in perception of the self | X | | | X |
| | AD | TS | OCD | AN |

view of the progression of her longitudinal comorbidity. For example, let us trace the progression of her abnormal integration of food and feeding across her development. As a preterm infant and through the first month of her life, Barbara had a failure to thrive and poor suck requiring intravenous and gavage feedings. Although such a symptom pattern may be more attributable to the risks associated with preterm birth, we note that very preterm birth and birth trauma are risk factors for AN in girls (Cnattingius et al. 1999). At age three, she exhibited a repetitive seeking of olfactory stimulation from dryer antistatic sheets, symptomatic of her autism. By 6–9 years old, her olfactory sensitivity was replaced by a gustatory hypersensitivity, again most likely a symptomatic manifestation of her autism. By age 9, Barbara began to experience what was likely a precursor symptom to her OCD and her pAN, namely her being loathe to swallow her own saliva due to its being dirty, coupled with her concerns that her food was contaminated. At age 11, the spitting tic of her TS may in part have incorporated her sensations and concerns about swallowing her own saliva, and may have presaged the postprandial vomiting of her eating disorder through the intermediary of her vomiting also being a tic (van den Eynde, et al. 2007). By age 11, her feeling queasy after eating may have been a sustained echo phenomenon of her TS after seeing her classmate vomiting and/or a triggering element of her OCD symptom of fear of germs and contamination of her food. The fear of contamination of food was temporally connected with and ultimately replaced by her fear of becoming fat and her compensatory eating behaviours to avoid weight gain, leading to the symptoms of her pAN.

We believe we have made a plausible case for pAN being on a continuum with other NPDDs in Barbara's case. Her course of illness with comorbidities reflects one ontogenetic pathway to pAN, a pathway which is noticeably transparent due to the severity of her other NPDD comorbidities. It may be that analogous but less severe neurobiological, developmental, and psychosocial factors are present in other individuals who develop AN. These factors may not rise to the level of recognition of component or comorbid syndromal diagnoses as appears to have been the case with Barbara. We believe that the adoption of this perspective could serve as a template for the generation of testable hypotheses, not only in the case of AN, but for other NPDDs as well. An example follows.

PANDAS is an acronym for paediatric autoimmune neuropsychiatric disorder associated with streptococcus and presenting with OCD and/or a tic disorder (Leonard and Swedo 2001; Snider and Swedo 2003). Individuals with higher B-lymphocyte antigen D 8/17 positivity with post-streptococcal central nervous system autoimmunity may be most susceptible. A high expression of the D 8/17 lymphocyte antigen in autistic patients serves as a marker for compulsion severity (Hollander et al. 1999). Antibodies against human putamen have been noted in adolescents with AN (Harel et al. 2001). There have been reported case studies of group A β-hemolytic streptococcus infection-triggered OCD and AN (Henry et al., 1999). A critical review of the literature regarding PANDAS-AN indicated there is some evidence in support of the concept, but this is limited by methodological problems (Puxley et al. 2008). The prevalence of D 8/17 positive individuals among PANDAS and AN patients is increased (Sokol et al. 2002). Additionally, there have been cases of less specific paediatric infection-triggered autoimmune neuropsychiatric disorders (PITANDs) in AN (Sokol 2000; Sokol and Gray 1997). Is it possible that D 8/17 positivity is a common biological susceptibility marker for autism, PANDAS related OCD and TS, and AN? This suggests the hypothesis: "Post-streptococcal autoimmunity and B-lymphocyte D 8/17 antigenicity are risk factors for AN and related eating

disorders". We speculate for the sake of example. If such a hypothesis were to be validated by case-controlled studies, one could see D 8/17 positivity not only as a risk factor for AN, but also as an endophenotype which might help in searching for a candidate susceptibility gene(s) in AN. Clinically, one could test for D 8/17 positivity in families with a history of AN in order to ascertain those at greatest risk.

Another example of hypothesis generation conceiving of AN as on the spectrum of NPDD is as follows. Deletions of imprinted genes at chromosomal locus 15q11–q13 have been associated with a condition known as Angelman syndrome, of which many individuals may also have autism spectrum disorders, and with Prader–Willi syndrome (PW) (Williams 2005). Individuals with PW exhibit hyperphagia, food seeking, skin picking, hoarding, obsessive-compulsive redoing, seeking symmetry, pursuing exactness, cleanliness, ordering, and arranging (Dykens et al. 1996; Dykens and Shah 2003). An adult with PW and AN has been reported (Counts 2001). Additionally, 15q11–q13 is felt to be one of the more common autism susceptibility loci (Kerbeshian et al. 1990). Maternally derived duplication in the 15q11–q13 area may be linked to a subset of AD patients with savant skills (Nurmi et al. 2003). The savant skills of drawing with perspective and of hyperlexia have been noted in our patient and others (Burd et al. 1987). Hyperlexia has been noted in PW (Burd and Kerbeshian 1989). Hypothesis: "There is a candidate gene susceptibility locus for AN in the 15q11–q13 region". If such an hypothesis could be supported in some cases by clinical studies, one might further explore whether genetic processes such as imprinting could also be playing a role in the pathway to the AN phenotype.

We believe that the concept of NPDD readily incorporates the pAN in our patient. Whether other subtypes of AN, or AN in general fits this construct remains to be seen. We believe the research agenda, hypothesis generation, and treatment approaches for AN could all benefit if a NPDD perspective were to be applied to AN in addition to the already existing rich and fruitful research and clinical agenda.

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## Statement of interest

The authors have not declared any conflicts of interest.

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**CASE REPORT**

# A controlled single case study with repeated fMRI measurements during the treatment of a patient with obsessive-compulsive disorder: Testing the nonlinear dynamics approach to psychotherapy

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**Abstract**

There is increasing evidence that obsessive-compulsive disorder (OCD) is associated with a dysfunction of cortico-striato-thalamo-cortical neuronal circuits. In order to examine treatment-related changes in neuronal processes, a drug-naive female patient with OCD (subtype: washing/contamination fear) and an age- and gender-matched healthy control were repeatedly tested using functional magnetic resonance imaging (MRI) during the presentation of a symptom provocation task. Patient-specific visual stimuli of symptom provoking situations were compared with disgust provoking and neutral pictures. FMRI scanning was conducted at the beginning, during and upon completion of an inpatient treatment. During the treatment period of more than eight weeks (combined behavioural and systemic couple therapy) the patient filled out a therapy process questionnaire (TPQ) which was administered daily. Results show a phase transition-like change characterized by a sudden reduction of clinical symptoms as assessed by the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) in the middle of the treatment period. Before the discontinuous symptom reduction occurred, the dynamic complexity of the TPQ-time series increased which might be indicative for a critical instability of the system. The fMRI results at the beginning of the treatment suggest strong activities in various brain regions, especially in the anterior cingulate cortex. The results of the second and third acquisition revealed comparably smaller OCD-related neuronal responses. The results may indicate that important clinical changes are taking place during the psychotherapy process which correspond to changing patterns of brain activation as well as to critical instabilities and phase-transition like phenomena in the time-series of the patient's daily self-report data.

**Key words:** *Obsessive-compulsive disorder, functional MRI, psychotherapy process, nonlinear phase transition, anterior cingulate cortex*

## Introduction

Whereas some studies report changes in brain activities after successful psychological treatment of OCD, little is known about the dynamics of psychotherapeutic processes and their neural correlates.

Neuroimaging studies showed that the frontocortico-striatal circuitry is affected in OCD. This circuitry seems to be explicative for executive dysfunctions and impulse control disorders (Friedlander and Desrocher 2006; Schiepek et al. 2007). Neurophysiological and brain imaging studies on washing/contamination fear reported variations in various brain regions, e.g., subcortical areas (Desarkar et al. 2007), increased rCBF in the left orbitofrontal cortex and in the anterior cingulate cortex (Rauch et al. 1998, PET-study), as well as increased activity in the ventromedial prefrontal cortex (Mataix-Cols et al. 2004, fMRI-study), in the ventrolateral prefrontal

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cortex, the gyrus parahippocampalis, and the right insula (Shapira et al. 2003, fMRI-study). Gross-Isseroff et al. (2003) reported on communalities between OCD and schizophrenia in the caudate nucleus, orbitofrontal cortex, anterior cingulate, and mediodorsal thalamic nucleus.

Evaluations of cognitive behavioural therapy (CBT) using neuroimaging methods showed reduced responses in the caudate nucleus (NCd) after treatment (right NCd: Baxter et al. 1992 (FDG-PET, nine patients CBT, nine patients fluoxetine, four healthy controls); Nakatani et al. 2003 (fMRI, 22 patients behaviour therapy (BT), 31 healthy controls); left and right NCd: Schwartz et al. 1996 (FDG-PET, nine patients CBT, nine controls)). Pre-treatment associations between orbitofrontal cortex, caudate nucleus, and putamen (Baxter et al. 1992), as well as between orbitofrontal cortex, caudate nucleus, and thalamic structures (Schwartz et al. 1996) decreased after successful CBT. Brody et al. (1998) reported on higher pre-treatment metabolic activity in the left orbitofrontal cortex predicting a better outcome after behaviour therapy, but not after treatment with fluoxetine (FDG-PET, 27 patients). Using a symptom provocation task, Nakao et al. (2005, fMRI study) found reduced activity in the orbitofrontal cortex, anterior cingulate cortex, putamen, insula, temporal and occipital cortex, and cerebellum after successful BT, as well as after successful medical treatment with fluvoxamine.

These partially heterogeneous results indicate that further research on the neurobiological effects of the psychological treatment of OCD is warranted. It is assumed that treatment including CBT may lead to a "normalization" of neurobiological processes. However, relatively little is known about the specific mechanisms of psychological or pharmacological treatment (Etkin et al. 2005) and especially about treatment-related changes. For this purpose, neuroimaging procedures should be applied not only at the beginning and upon completion of the therapy but also during the treatment process.

The aim of this study was to investigate OCD-related BOLD responses and to display variations during the therapeutic process. We assumed that reduced symptom severity should correspond to reduced activity in OCD-related brain regions. With daily ratings, phase-transition-like phenomena between patterns of emotions and cognitions were hypothesized to occur, each transition accompanied by critical instabilities manifesting transient increases in fluctuations or dynamic complexity of the time series. Similar structures of therapeutic change processes were identified in recent process-outcome studies (Haken and Schiepek 2006; Schiepek and Perlitz in press). The phase-transitions observed could be associated with intensive and widespread brain activity compared to brain responses after the transition. As ACC is thought to be connected with conflict monitoring (van Veen and Carter 2002a,b) or – in terms of synergetics – to be a symmetry monitoring system, respectively, dynamic instabilities should be correlated to ACC activity.

## Methods and Materials

### *Subjects*

A 34-year-old female patient with OCD (washing/contamination fear) (DSM IV: 300.3, APA 1994) was investigated. The patient had been unmedicated for several years and devoid of comorbid psychiatric or somatic diagnoses (clinical judgement based on ICD-10 and DSM-IV criteria and on a psychiatric interview). The father of the patient died at the age of 58 years, suffering from alcohol dependence and OCD (controlling compulsions). The younger sister of the patient met the criteria of major depression, eating disorder, and OCD. The obsessive-compulsive symptoms of the patient had begun about 4 years before admission to the psychosomatic hospital.

On a special ward for OCD the patient was treated with combined behaviour therapy and systemic therapy (couple therapy). The duration of the hospital stay was 59 days. The functional MRI data of the patient were compared to those of a healthy, female, control subject (38 years) without any history of neurological or psychiatric disorders.

A written informed consent was obtained from both participants after procedures had been fully explained according to the guidelines of the ethics committee of the University of Munich.

### *Real-time monitoring procedure*

Upon admission, the patient was asked to fill out the Therapy Process Questionnaire (TPQ, Haken and Schiepek 2006) presented on a PC screen at the end of each day, using the internet-based Synergetic Navigation System. Daily ratings were given on seven-point Likert scales or visual analogue scales, and then transformed into time series of the clinical course. A factor analysis of the items was realized in a former study and resulted in the following seven subscales of the TPQ: (1) experience of progress, confidence, and self-efficacy during the ongoing therapy, (2) insight and development of new perspectives, (3) intensity of therapeutic work and intrinsic motivation, (4) social climate and interpersonal relations to other patients, (5) quality of the therapeutic relationship, (6) dysphoric emotions and self-relatedness, (7) symptom severity. The subscales

resulted from a factor analysis which produced seven factors with eigenvalues $>1$ (cumulated explained variance: 59.6%). Cronbach's alpha ranges from .82 (subscale VII) to .94 (subscale I). Reliability and validity measures of the TPQ were reported in detail in Haken and Schiepek (2006).

The real-time monitoring technology is an internet-based service which allows the continuous visualization of raw data and subscale values as time series. For further analysis the dynamic complexity of the time series was calculated and visualized. Dynamic complexity is a combined measure of the intensity of fluctuations, and the distribution of the values over the available range (Schiepek 2003). Fluctuations are calculated by summing up the ratio between the absolute difference of the measurements between two turning points (i.e. the change between ascending and descending values, or vice versa) to the duration of this change process. The fluctuation is sensitive to the frequency and amplitudes of value alternations. The distribution measure is high if the values are not concentrated within small subdivisions of the range, but follow an equal distribution over the range of possible measurement values. The multiplicative combination of both results become evident in the *dynamic complexity* of the time series which was calculated within a gliding window (window width: 7 days) (for details of the algorithm, see Haken and Schiepek 2006).

### *Outcome criteria*

Treatment effects were measured using the Y-BOCS (Goodman et al. 1989a,b), BDI (Beck Depression Inventory, Beck and Steer 1987), SCL-90 (Symptom Check List, Derogatis 1983), and the INK (Incongruence Questionnaire, Grosse Holtforth et al. 2004). The Y-BOCS was administered weekly, the other questionnaires were completed at the beginning and at the end of the hospital stay.

### *fMRI scanning*

The fMRI scans were carried out three times during the stay at days 9, 30, and 57. The second acquisition was done after an intensive period of critical instability of the time series, but just before the flooding treatment was started. The healthy control was also scanned three times at identical time intervals so was the patient. Both subjects were scanned with a 1.5-Tesla MRI scanner (Magnetom Sonata; Siemens, Erlangen, Germany) and a standard head coil. A high-resolution T1-weighted scan was acquired for anatomical referencing. Functional images were obtained with a gradient echo-planar imaging sequence (repetition time: 4000 ms; echo time: 53 ms; 16 axial slices; matrix size: $64 \times 64$; slice thickness: 6 mm; gap: 0.3 mm).

For the processing and statistical analysis of the fMRI data, the Brain Voyager Software Package (Goebel, Maastricht) was used. The first five images were excluded from any further analysis due to inhomogeneities of the magnetic field. The preprocessing of the functional data included high-pass filtering (cutoff three cycles in time course) to low-frequency signal drift inherent in echo planar imaging, slice scan time correction, spatial smoothing (Gaussian filter with FWHM 8.0 mm) and a 3D motion correction. In addition, the functional images were transferred to a standard Talairach brain.

Significant fMRI activity was determined by cross-correlation of MR image pixel intensity with an expected hemodynamic response function. Voxel-wise *t*-tests were used to identify the brain areas in which the percent signal change associated with each contrast was significantly different between patient and control subject under different conditions, respectively (GLM analysis). BOLD responses thresholded at $P<0.001$ uncorrected for multiple comparisons were regarded as significant. Only clusters with more than 50 activated voxels were included in the analyses.

### *Picture material and stimulation paradigm*

The visual stimulation consisted of 30 symptom provoking, 30 disgust provoking, and 30 neutral pictures. Disgust and neutral pictures were taken from the International Affective Picture System (IAPS, Lang 1997/2001). The OCD-relevant pictures had been photographed by the patient: the pictures showed specific triggers for obsessive-compulsive behaviours. The scenes were captured with a digital camera from the patient's home context (see Schienle et al. 2005, for first using this individualized symptom provocation paradigm with OCD patients). All pictures were shown twice each for four seconds in pseudorandomized order. The same picture sequence was presented to the patient and the matched control subject. All pictures were presented to the participants prior to the first fMRI session in order to reduce primacy, surprise or habituation effects.

After the MRI session subjects were asked to rate the pictures by means of five-point scales for (a) emotional valence, (b) arousal, (c) ambiguity, and (d) self-efficacy. The ratings for valence and arousal used the visual symbols of the IAPS standard rating procedure (five steps), the ambiguity and self-efficacy ratings were done on a five-point scale (with 1 = "not at all" and 5 = "very strong").

## Results

### *Therapy effects*

The patient started with a Y-BOCS score of 14 (mild symptom severity) in the first week of treatment. Although this score corresponds to only mild symptom severity, the patient's suffering from OCD was strong, and she was obviously impaired in her daily routines and vocational activities. The score slightly increased up to 17 (moderate severity) until week 3 of treatment and then decreased to a value of 4 in week 5. Between weeks 5 and 8 the Y-BOCS scores remained stable on a low level and reached a score of one (i.e. no clinical OCD) by the end of treatment (Figure 1).

The BDI overall score was 21 at the beginning and 3 at the end of treatment. A pre-post comparison of the SCL-90 showed reduced values in all psychopathological subscales at the end of therapy. The most pronounced pre- versus post-differences were seen in the subscales of obsession/compulsion (raw score: 2.7 vs. 0; *t*-value: 77 vs. 37), depression (raw score: 2.8 vs. 0; *t*-value: 75 vs. 37), and aggression/hostility (raw score: 2.0 vs. 0; *t*-value: 68 vs. 39). The total score was reduced from 1.32 to 0.16 (*t*-value: 66 vs. 46) indicating a significant clinical improvement. The experienced incongruence between personal needs and goals on one hand and the actual fulfilment of these needs and goals on the other hand was measured by the incongruence questionnaire (INK). The incongruence of the subscale "goals to approach" was reduced from 3.54 (pre) to 1.84 (post), the incongruence of the subscale "goals to avoid" was reduced from 3.50 to 1.45, and the overall incongruence was reduced from 3.52 to 1.64.

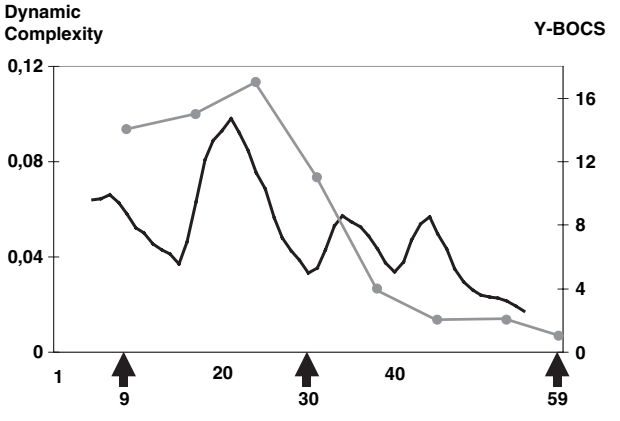


Figure 1. Grey dots and line: the course of the Y-BOCS scores (ratings once a week, scale on the right side). Black line: dynamic complexity, averaged over all 45 items of the TPQ. The dynamic complexity of each item is based on daily ratings and calculated within a gliding window of 7 days (scale on the left side). *X*-axis: days of hospital stay. Black arrows indicate the days where fMRI sessions were taking place.

This is a significant increase in experienced emotional congruence which is seen as an important factor of mental health (Grawe 2004).

### *Clinical course and time series data*

The problems of the patient can be seen in a context of autonomy conflicts with her husband. Symptoms started after a dermatological infection of her husband, and the focus of her symptoms and anxieties concentrated on getting infected from her husband. Before the actual clinical stay, she visited some sessions of couple therapy together with her husband and an ambulatory individual psychotherapy, which was interrupted when a potential separation was discussed as a possible solution by the therapist.

Actual development: After the second couple therapy session the patient decided to file for divorce. This decision was accompanied by feelings of anger. The couple's therapy session took place 2 days before the second fMRI session. The period before this critical event and her decision were characterized by intensive fluctuations and increased complexity of her daily ratings of therapy-related feelings and cognitions (Figure 2). The average dynamic complexity of all items of the TPQ

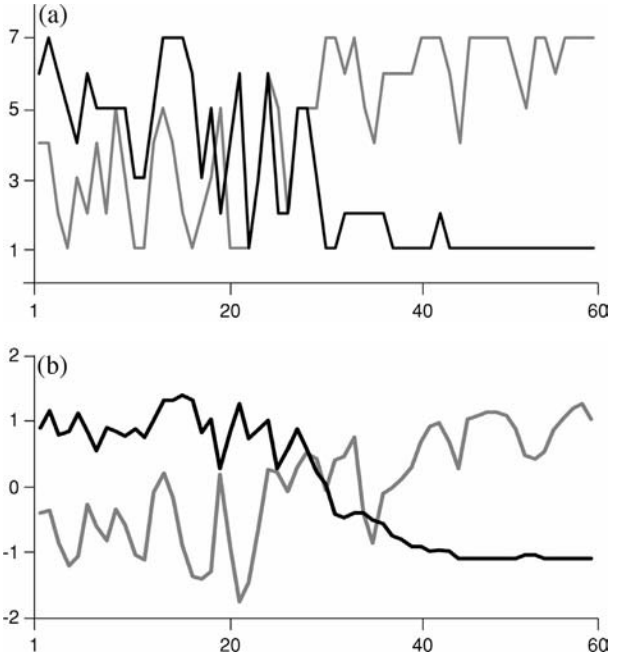


Figure 2. (a) The course of two TPQ items: Concerning my personal goals, I was successful (1 = "not at all", 7 = "very successful" (grey line); Today, I avoided situations provoking obsessions or compulsions (1 = "not at all", 7 = "very much") (black line). (b) The course of two factors (subscales) of the TPQ (items were *z*-transformed and averaged). Factor 1: progress of therapeutic work/confidence/self-efficacy (grey line). Factor 7: symptom severity (black line). *X*-axis: days of hospital stay.

manifested a significant peak between days 15 and 30 (Figure 1). Evidently the patient was destabilized and a phase transition took place, in the period before the second couple therapy session. Twenty-six out of 45 TPQ items manifested statistically increased complexity values during this period.

The flooding procedure started one day after the second fMRI session. Thus, specific psychopathological variations were demonstrated before decisive psychotherapeutic interventions took place (rapid early response Hayes et al. 2007; Lambert and Ogles 2004): alterations in brain activity (see below), increased instability of the therapy dynamics, and the beginning of an essential symptom reduction (mirrored by her daily ratings, see Figure 2) as well as by the Y-BOCS (see Figure 1) occurred before the flooding was started. The increased complexity of her daily ratings followed by changed dynamics (Figure 2) can be seen as a phase-transition in the sense of nonlinear dynamic systems theory. Clinically, the phase-transition concerned a pattern including several components like finishing an important and long-term subliminal decision process, becoming aware of one's emotions, and a clear-cut symptom reduction. Evidently, the system was destabilized before a discontinuous symptom reduction begun which was initiated before the flooding started and the second fMRI session took place.

*Self-report data on the stimulus material*

Figure 3 shows the results of the participants' evaluation of pictures immediately after the fMRI scans. The neutral pictures were rated more positive by the patient (Mean = 4.92) than by the control subject (Mean = 3.75, $t = 11.19$, $P = 0.000$). The patient rated the OCD-related pictures more negative than the disgust-associated pictures (1.73 vs. 2.22, $t = -3.27$, $P = 0.002$). The reverse was true in the healthy subject (OCD-related pictures: 2.57 vs. disgust pictures: 1.90, $t = 6.33$, $P = 0.000$). The OCD stimuli were rated by the patient more positive in the third fMRI session (2.06) compared to the first session (1.46) ($t = 3.84$; $P = 0.001$). Furthermore, arousal ratings differentiated clearly between OCD (4.53), disgust (2.93), and neutral pictures (1.00) (OCD vs. disgust: $t = 6.03$, $P = 0.000$; OCD vs. neutral: $t = 33.87$, $P = 0.000$; disgust vs. neutral: $t = 8.61$, $P = 0.000$). The arousal level of the patient was smallest for the neutral pictures and greatest for the individual OCD-associated pictures. Concerning OCD-related pictures, the arousal decreased from the first session (4.53) to the second session (3.96) and from the second session to the third session (2.00). By contrast, the experienced self-efficacy increased from the second session (1.73) compared to the third session (4.23). For the control person, arousal was most intensive for disgust pictures (disgust: 3.26, OCD: 1.06, neutral: 1.73, first session). The individual scores in the ambiguity scale were not very pronounced, neither for the patient nor for the control subject. Self-efficacy related to OCD pictures differentiated clearly between patient and control. For the control subject it seemed to be easier to cope with OCD pictures (4.92) than with disgust provoking situations (3.03). For the patient her own OCD situations were the most difficult to cope with at the beginning of the therapy (session 1: OCD-pictures: 1.53, disgust pictures: 3.66, $t = 6.80$, $P = 0.000$), whereas at the end of treatment there was no more difference (session 3: OCD-pictures: 4.23, disgust-pictures: 4.26, $t = 0.11$, $P = 0.910$). Interestingly, self-efficacy during disgust provocation seemed greater for the patient than for the control person (mean over all three sessions: 3.91 vs. 3.03, $t = 4.48$, $P = 0.000$).

*Functional MRI data*

At the beginning of the therapy, the patient demonstrated enhanced activations during the presentation of OCD-associated pictures compared to the presentation of neutral pictures in medial frontal brain regions, including the anterior cingulate cortex (BA 32/24), supplementary motor cortex (BA 6), medial frontal gyrus (BA 9), and the medial and left lateral part of the superior frontal gyrus (BA 10). Moreover, symptom-related pictures compared to neutral pictures produced enhanced activations mainly in the following areas: superior frontal gyrus (BA 9/10), inferior frontal gyrus, insula (BA 47), inferior frontal and inferior temporal gyrus (BA 47/38), superior temporal gyrus (BA 38), precentral gyrus (BA 6), inferior parietal lobe (BA 40), thalamus (L > R), and caudate nucleus. In addition, clear differences could be observed in the (pre-) cuneus (BA 7), the fusiform gyrus (BA 37) as well as (secondary) visual-association areas in occipito-parietal brain regions (e.g. superior and inferior parietal lobe (BA 7/39), middle and inferior occipital gyrus (BA 18)). The BOLD responses in these areas were more

Figure 3. Ratings of the pictures which were presented during the fMRI sessions. Left column: patient; right column: healthy control. The diagrams represent the experienced intensities of emotional valence (5 = positive, 1 = negative), arousal (5 = very strong, 1 = no arousal), ambiguity (5 = very strong, 1 = no ambiguity), and self-efficacy (5 = I feel able to handle this situation, 1 = not at all). Results are showing the intensities of neutral, disgust provoking, and OCD provoking pictures, during the first (1), second (2), and third (3) fMRI session.

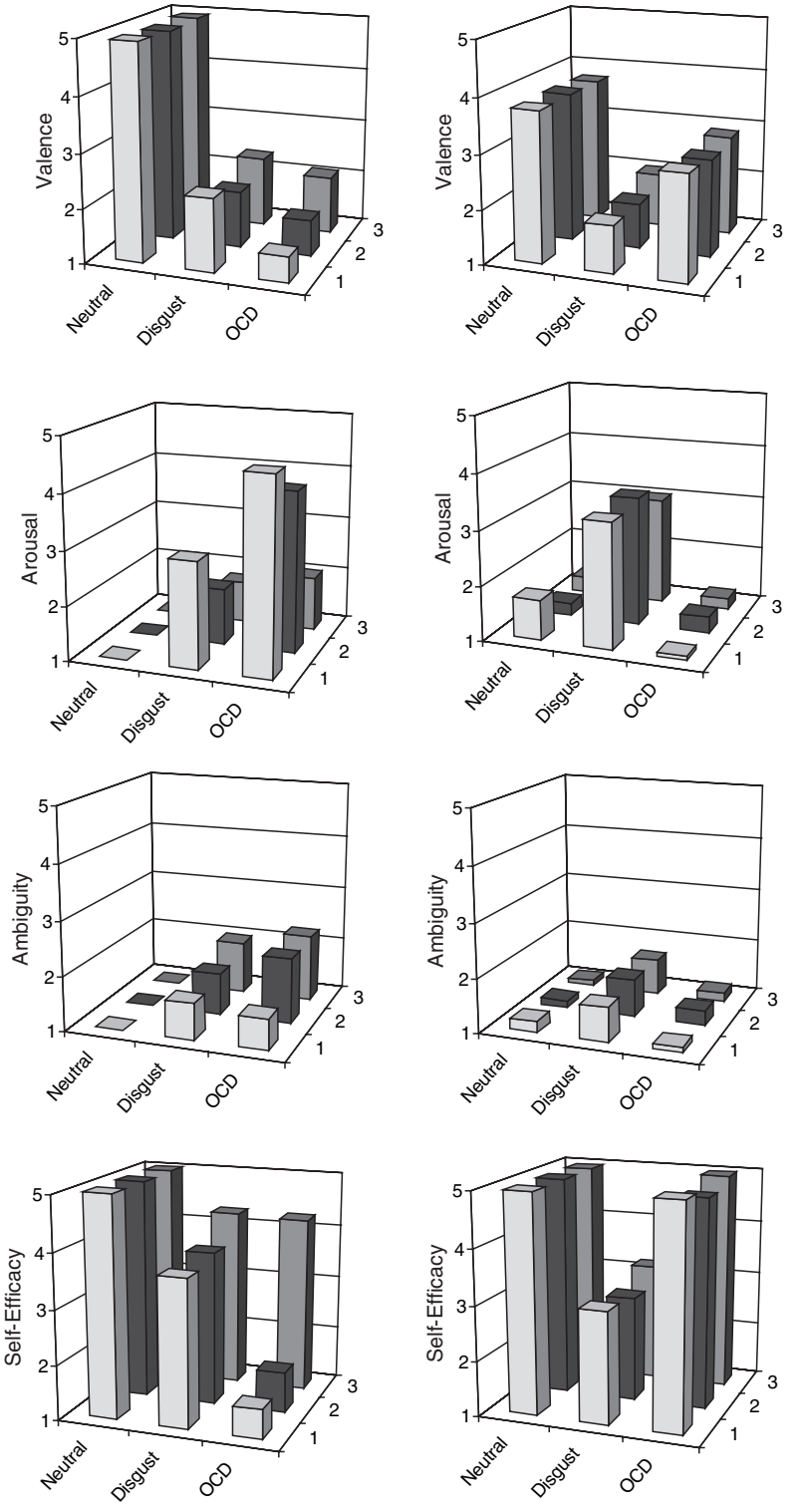


Figure 3 (*Continued*)

pronounced during the OCD-associated stimulation than in the control condition (Figure 4).

The comparison of OCD-specific brain responses at the beginning of the therapy and the second fMRI measurement during the therapy revealed reduced contributions mainly of the medial frontal gyrus (BA 8/6) and anterior cingulate cortex (BA 32/24), superior and middle frontal gyrus (BA 6/10), precentral gyrus (BA 44/45), superior temporal gyrus (BA 22), cuneus (BA 19/18), the thalamus and

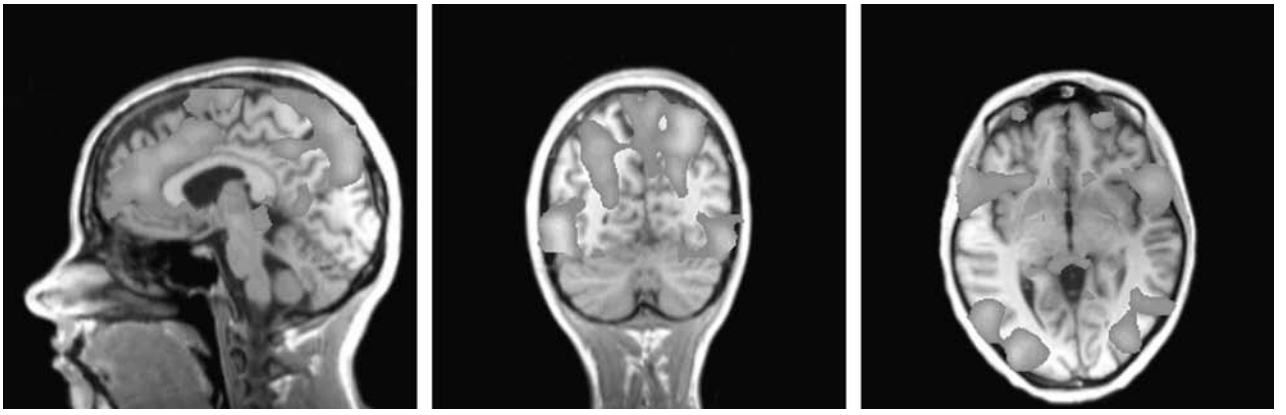

Figure 4. OCD-related functional MRI activation of the patient at the beginning of the therapy (OCD-related pictures minus neutral pictures; $x = 4$, $y = -62$, $z = -3$; $P$(uncor) $< 0.001$).

caudate nucleus in both hemispheres as well as the right fusiform gyrus during the second session (Figure 5).

The OCD-associated BOLD responses (activations during the presentation of OCD-related pictures compared to activations during the presentation of neutral pictures) of the second and third session at the end of therapy revealed only small differences. Slightly enhanced responses were found during the second session compared to the third session in the precuneus (BA 7), and the inferior parietal lobe (BA 40). However, the middle frontal gyrus (BA 8), the left inferior parietal lobe (BA 40), the cuneus (BA 18/19) the superior and middle frontal gyrus, and the cingulate gyrus (BA 32) responded slightly stronger during the third session compared to the second session (Figure 6).

The comparison of disgust-related BOLD responses did not show any significant results. However, when the influence of BOLD responses was examined on a lower significance level ($p < .005$; uncorrected for multiple comparisons), disgust-related activities during the first session were observed in the thalamus, the middle frontal gyrus (BA 46/8), the postcentral gyrus (BA 2), the (pre-)cuneus, the inferior frontal gyrus, the anterior cingulate gyrus (BA 32) and the fusiform gyrus. The activations in the superior frontal gyrus (BA 10), the thalamus, the postcentral gyrus (BA 3), the precuneus, the inferior parietal lobe, and the fusiform gyrus (BA 20/36) slightly decreased between the first session and the second session. A comparison of the second and the third session differences in the left middle frontal gyrus (BA 46/10), and the left inferior frontal gyrus showed that these brain areas were stronger activated during the second session. However, slightly increased brain activations in the third session compared to the second session were evident in the left thalamus, the (pre-)cuneus (BA 30/31), the right superior temporal gyrus (BA 22), the left inferior frontal gyrus (BA 20), and the left insula (BA 13).

The healthy subject showed comparable BOLD responses during the presentation of OCD-related pictures and neutral pictures. Slightly enhanced activations during the neutral condition compared to the OCD-related pictures where shown in the left

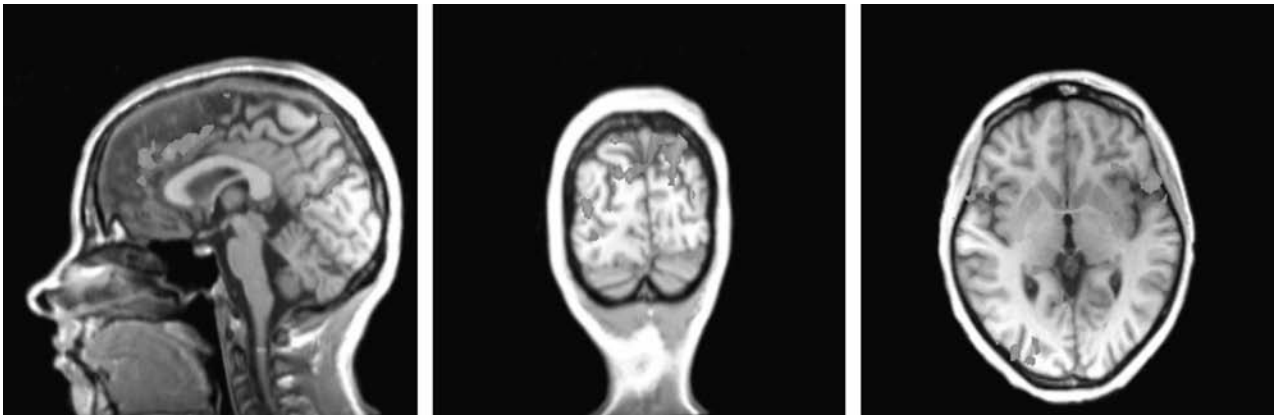

Figure 5. OCD-related BOLD responses in the patient at the beginning of the therapy compared to the BOLD responses during the second session (OCD-related pictures minus neutral pictures; $x = 1$, $y = -82$, $z = 1$; $P$(uncor) $< 0.001$).

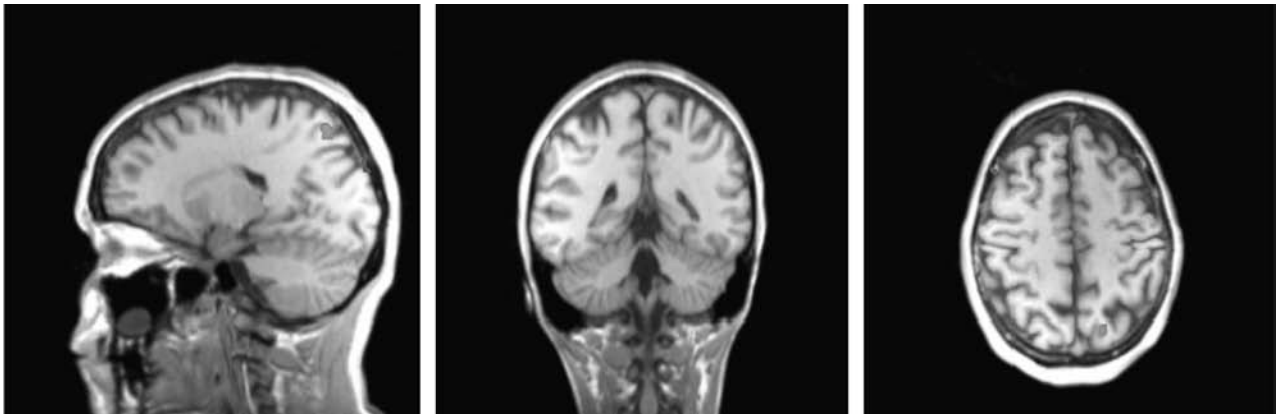

Figure 6. OCD-related BOLD responses in the patient during the second session compared to the BOLD responses at the end of therapy (OCD-related pictures minus neutral pictures; $x = -18$, $y = -37$, $z = 46$; $P$(uncor) $< 0.001$).

inferior occipital gyrus. In addition, the superior frontal gyrus (BA 6), the middle frontal gyrus (BA 8), the inferior frontal gyrus (BA 44/45), and the right superior temporal gyrus (BA 13) were stronger activated during the OCD condition compared to the neutral condition (Figure 7).

In order to compare the MRI activity after the presentation of disgust-related pictures to brain responses after the presentation of neutral pictures in the healthy control subject, the significance level was lowered to $P < 0.005$ (uncorrected for multiple comparisons). Disgust associated information revealed enhanced BOLD activity in the left medial and bilateral lateral part of the superior frontal gyrus (BA 8/9), medial frontal gyrus (BA 6), the precuneus (BA 7), the cuneus (BA 17), the left precentral gyrus, the right postcentral gyrus (BA 2/3), the left caudate body, and the right superior temporal gyrus.

These disgust-specific responses (disgust minus neutral condition) hardly changed even when presented several times in functional MRI sessions. Slightly enhanced responses during the first session compared to the second session were shown in the medial frontal gyrus (BA 6), the precentral gyrus (BA 6), and the left caudate body. The disgust-specific responses of the second MRI session were somewhat enhanced in the left precuneus (BA 7) and the inferior frontal gyrus (BA 44) compared to those activations in the third MRI session.

## Discussion

The aim of the study was to identify the association between brain activity and changes in the subjective experience of psychotherapy. Daily ratings of the therapy processes were done using an internet-based Real-Time Monitoring device (Synergetic Navigation System) which allowed ongoing data analyses of the resulting time series. Three repeated fMRI sessions were performed (1) before and at the end of the treatment period as well as (2) immediately after a critical instability.

Concerning functional MRI data, the most pronounced changes in brain activity occurred between the first and the second session. As these changes happened before the flooding procedure was started,

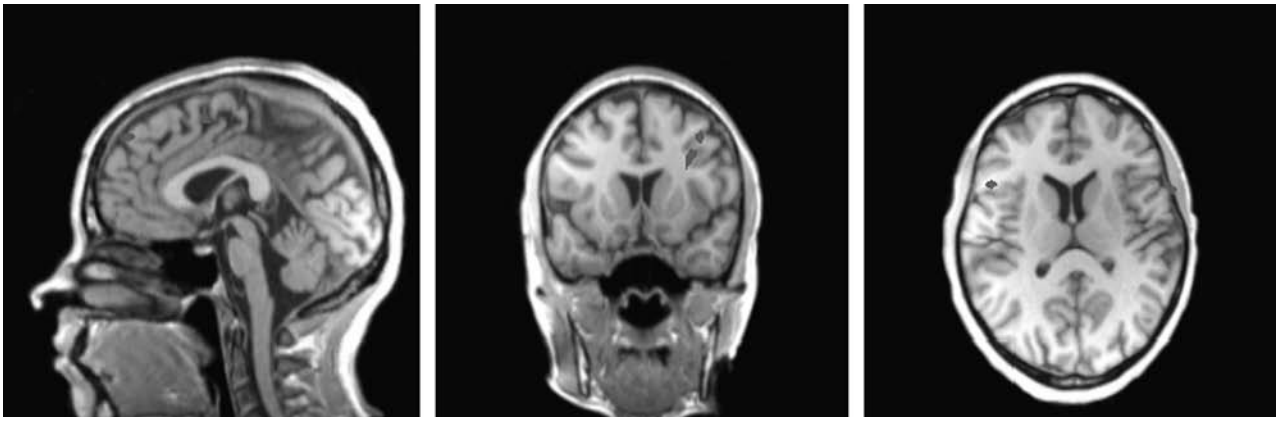

Figure 7. Functional MRI activation during the presentation of the OCD-related pictures in the healthy control subject (first session, OCD-related pictures minus neutral pictures; $x = -2$, $y = 10$, $z = 13$; $P$(uncor) $< 0.001$).

these variations may indicate early rapid responses in psychotherapy (Lambert and Ogles 2004; Hayes et al. 2007). They occurred after a preceding instability. Marked alterations in brain activity were to be observed before or during symptom reduction took place, not afterwards.

Alterations in brain activity involved widespread areas, e.g., the medial frontal brain regions including anterior cingulate cortex, superior and middle frontal gyrus, inferior frontal and precentral gyrus, superior temporal gyrus, superior parietal lobe, cuneus, thalamus and caudate nucleus in both hemispheres, as well as the right fusiform gyrus. Activations in parietal structures like the precuneus or gyrus supramarginalis matched the results of other studies using OCD-specific stimulation in comparison to emotion provoking material (disgust, fear) (e.g., Schienle et al. 2005, fMRI-study, 10 patients, 10 healthy controls). Any activation in the dorsolateral-caudate-striatum-thalamus circuitry has repeatedly been demonstrated to "normalize" after psychotherapeutic treatment (e.g., Baxter et al. 1992; Schwartz et al. 1996).

The function of the anterior caudate nucleus in OCD is less clear. Some studies have shown that the caudate nucleus is part of the modulatory control model of OCD and could play a role in the washing/contamination fear syndrome (e.g., Rauch et al. 1998; Saxena et al. 1998; Friedlander and Desrocher 2006; Schiepek et al. 2007 Whiteside et al. 2004). The cingulate cortex comprises various functions like somatosensoric integration, mediation of affective and cognitive processes, control of attention, and processing of painful stimuli. Additionally, it plays an important role as conflict monitoring system: it is sensitive to ambiguous or conflicting information (van Veen and Carter 2002a,b; Davidson et al. 2003), is involved in decision processes (Sanfey et al. 2003; King-Casas et al. 2005), and its activation is predictive to treatment outcome in depression (e.g., Mayberg et al. 1997; Pizzagalli et al. 2001; Etkin et al. 2005; Evans et al. 2006). In terms of complexity theory, it could be an indicator of symmetry states of brain functioning, which is characterized by two or more dynamic patterns or attractors in competition. Therefore, the ACC activation at the beginning of the therapy could either be part of the pathology or it could be indicative for the critical instability of the cognitive-affective system of the patient (or both). The dynamic complexity of the TPQ data decreased from 0.066 (the days around the first fMRI session) to 0.033 (around the second session) which means a reduction by 50%. The second fMRI measurement was conducted during a local minimum of critical fluctuations. Whether the change in cingulate activation could be attributed to an altered critical symmetry state of the neural self-organization before versus after the phase-transition or to changes in symptom severity cannot be decided within a single case study. Nevertheless, this seems to be an interesting question for further research. Since it could be an indicator for the instability hypothesis, that Y-BOCS scores were similar during fMRI sessions one and two, compared to a 50% reduction in dynamic complexity.

### *Limitations*

There are several limitations to our results. The data were obtained from a single patient and a single control subject and can, therefore, only provide a first approach to this issue. The investigation of a single control subject can be misleading since effects and potential confounders introduced by one healthy subject are not definable or controllable. However, the brain activity of the patient in our study showed neural responses dependent on her subjective experience and independent of the results of the healthy subject. These results are in line with those of previous studies in literature. The healthy control did not show alteration between sessions concerning the evaluation of the pictures. As expected, brain responses of the healthy control were pronounced during the presentation of disgust-related pictures. Small effects were demonstrated when the pictures did not produce strong affective responses.

Although there is an increasing interest in the neuropsychology of positive emotions (e.g., Ryff and Singer 2003), their role within the psychopathology of OCD is not clear. Hence, no positive pictures were integrated in the study.

Statistical analyses of fMRI data were done without correction for multiple comparisons, but the statistical threshold is not unusual for small sample sizes. Nevertheless, the results should be considered preliminary.

Despite these limitations we were able to demonstrate a clear effect of psychotherapy on neural responses contributing to the understanding of neurobiological bases of OCD and therapy processes.

## Conclusion

It seems to be worthwhile having a closer look at the neurobiology of psychotherapy processes, using combined strategies of functional brain imaging and time-series data. We were able to demonstrate marked changes in brain activation during a psychological treatment, associated with a dynamic

phase-transition followed by a pronounced symptom reduction. The results emphasize the importance of an individualized symptom provoking paradigm as well as the application of repeated assessments of functional MRI. Real-time monitoring technology can help to determine important variations in therapy processes in order to analyse symptom reduction, complexity changes or phase transitions.

The theory of a self-organizing brain undergoing cascades of cognitive-affective phase-transitions during the process of psychotherapy could be an interesting framework for the creation of hypotheses, data interpretation, and modelling brain dynamics (Friston et al. 2003; Haken 2002; Haken and Schiepek 2006).

## Acknowledgements

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## Statement of interest

The authors have not declared any conflicts of interest.

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**CASE REPORT**

# Delusion of pregnancy associated with antipsychotic induced metabolic syndrome

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**Abstract**
Metabolic syndrome is currently the research topic of several studies. Although physical manifestations of metabolic syndrome have been described, the psychological and psychiatric impact of metabolic syndrome has not been studied to date. We report the first case of antipsychotic-induced metabolic syndrome which was associated with development of delusions of pregnancy in a post-menopausal woman.

**Key words:** *Delusion of pregnancy, obesity, metabolic syndrome*

## Introduction

Metabolic syndrome, as originally elucidated by Reaven (1988), was originally proposed to explain the link between insulin resistance and hypertension in the causation of cardiovascular disease (Levitt and Lambert 2002). There are several physical manifestations of metabolic syndrome (Bonow and Eckel 2003); however, the psychological and psychiatric impact of metabolic syndrome has not been studied to date, which may be important not only for predicting outcome, but also may determine presentation of psychopathology (Bitton et al. 1991; Wirshing et al. 2002). Delusion of pregnancy is one such rare symptom seen due to organic, functional or drug-induced causes (Qureshi et al. 2001). An earlier report had described delusion of pregnancy in young girls due to drug-induced lactation and amenorrhea accompanied by breast changes (Cramer 1971). We report the first case of antipsychotic-induced metabolic syndrome which was associated with development of delusions of pregnancy in a post-menopausal woman.

## Case report

A 48-year-old, post-menopausal, divorced mother of one child, diagnosed with paranoid schizophrenia for last 16 years and treated with different antipsychotics, with moderate response, was brought to us claiming that she was pregnant for the last 7 months to her ex-husband (despite not having any contact or sexual intercourse with her husband for last 8 years), because her abdomen was enlarging gradually. Detailed history revealed near-complete resolution of positive symptoms of psychosis (no delusions or hallucinations) with the exception of negative symptoms. Clozapine had then been started 10 months previously after cross tapering and stopping other antipsychotics. Since then, her son revealed that she had developed obesity, especially around the waist. Three months after initiation of clozapine, she had started claiming that she could sense fetal movements inside her abdomen and requested safe delivery of the child. Investigations revealed BP 126/94 mmHg, weight 72 kg, height 154 cm, waist circumference (WC) 88 cm, and BMI 30.38 kg/m$^2$. FSH, LH, serum prolactin and serum insulin were normal for her age, repeated pregnancy tests were negative and abdominal ultrasound revealed no abnormalities. She had no lactation. Serum biochemistry revealed triglyceride (TGL) level of 203 mg/dl, HDL of 42 mg/dl and FBS level of 96 mg/dl.

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## Discussion

As the patient had been on treatment with clozapine 350 mg/day prior to admission and she met IDF criteria (IDF 2005) for metabolic syndrome (decreased HDL, increased WC and TGL), she was diagnosed as having delusions of pregnancy due to antipsychotic-induced metabolic syndrome, which is itself uncommon in schizophrenia. It is also possible that these delusions were misinterpreted by the patient as a result of her increasing abdominal girth. However, the role of metabolic syndrome (as increasing waist circumference and weight) cannot be discounted in the etiology of delusion formation.

She was started on amisulpride 400 mg/day with partial improvement noted at 6 months follow-up. A diagnosis of ovarian disease was initially entertained, but later discarded due to normal FSH, LH and insulin values.

Although the epidemiology of delusions of pregnancy is not well known, it has been fairly commonly reported, both among male (Chaturvedi 1989; Bitton et al. 1991; Shanker 1991; Miller and Forcier 1992; Michael et al. 1994; Radhakrishnan et al. 1999) and female (Cramer 1971; de Pauw 1990; Dutta and Vankar 1996) patients. It has to be clinically differentiated from pseudocyesis, simulated pregnancy, pseudo-pregnancy and Couvades syndrome (Bonow and Eckel 2003). We believe that our patient had pseudocyesis rather than pseudo-pregnancy as all investigations were normal.

Coenaesthopathological processes, in which combination of primary somatic sensations arising from the abdomen and other parts of the body due to central obesity (Cramer 1971; Bitton et al. 1991) and endocrinological changes resulting in metabolic syndrome induced by antipsychotic treatment (Cramer 1971; de Pauw 1990; Dutta and Vankar 1996), were misinterpreted as signs of pregnancy leading to our patient developing delusions of pregnancy.

## Conclusion

Along with physical aspects, psychiatric aspects of metabolic syndrome need to be studied further systematically, in terms of psychopathology, i.e. delusion, somatic problems, dysmorphophobia and poor self esteem among others.

## Acknowledgements

None.

## Statement of interest

The authors have not declared any conflicts of interest.

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**CASE REPORT**

# Worsening of bruxism with atomoxetine: A case report

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**Abstract**
Atomoxetine is the only FDA-approved non-stimulant to treat ADHD. We report a case of Atomoxetine-related exacerbation of nocturnal bruxism in a 12-yr old boy with ADHD, subsiding on discontinuing the drug, but re-worsening with retrial, finally subsiding upon adding Buspirone. This is the first report of its kind showing such an association, not included in any pre- or post-marketing data. Clinical implications and possible mechanisms are discussed.

**Key words:** *ADHD, adverse effects, side effects, childhood ADHD*

Bruxism, or pathological grinding of teeth, is a common problem in paediatric population. It can be a serious problem, leading to severe abrasion of teeth or their mobilization in the alveolar ridge, as well as facial pain and damage to temporomandibular joint. Atomoxetine, the only non-stimulant currently FDA-approved for ADHD in children, has shown efficacy in more than 10 controlled trials (Pliszka et al. 2006). The common adverse effects with atomoxetine are nausea, vomiting, fatigue, decreased appetite, abdominal pain and somnolence.

We report a case of exacerbation of nocturnal bruxism while on atomoxetine therapy, which was confirmed on re-challenge. To the best of our knowledge, this is the first case report of atomoxetine-related bruxism in the existing literature.

"A", a 12-year-old boy, presented with a 5-year history of poor concentration, distractibility, poor scholastic performance and inability to perform any task within a given time period. He had developmental delays in motor and language areas. He also had history of nocturnal bruxism, which would vary in frequency, from once in 3–5 days, lasting about 5 min, being very mild so as not to bother him or his family members. IQ testing revealed mild mental retardation. After detailed clinical assessment, a diagnosis of ADD was made. He was referred for behavioural therapy, but did not show much improvement. Subsequently, he was started on atomoxetine 10 mg/day, with significant improvement in attention reported by the parents within 3 weeks. At week 4, the parents noticed that the boy's nocturnal bruxism had worsened. It would occur daily at midnight and would last for 15–20 min. The teeth grinding sound was so loud as to wake up others in the room. No daytime abnormal movements were noted. Atomoxetine was stopped immediately, with returning of intensity and frequency of bruxism to baseline. After remaining drug free for 3 weeks, atomoxetine was restarted. Again, the child showed exacerbation of bruxism as before. Subsequently buspirone 10 mg/day was added to ongoing therapy with marked improvement in bruxism. At 12 weeks follow-up, frequency of bruxism was once in 7–10 days, and of mild intensity. The probability of an adverse drug reaction was assessed using the Naranjo probability scale (Naranjo et al. 1981), which indicated a probable association between atomoxetine and bruxism.

A pubmed search revealed no reports of bruxism with atomoxetine. Full prescribing information also did not describe this adverse effect either in pre- or post-marketing data (Strattera (atomoxetine)

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Prescribing information 2007). In this case, we established the temporal relationship between atomoxetine and exacerbation of nocturnal bruxism, confirmed upon rechallenge with atomoxetine. Its pathophysiology, however, is debatable and the literature is controversial regarding the mechanism for bruxism. Substances related to dopaminergic, serotonergic and adrenergic systems suppress or exacerbate bruxism activity (Winocur et al. 2003). Mechanism of action of atomoxetine in the control and maintenance of ADHD symptoms is thought to be through the highly specific presynaptic reuptake inhibition of norepinephrine (Manos et al. 2007). Further systematic studies are needed to understand this phenomenon better.

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None.

## Statement of interest

The authors have not declared any conflicts of interest.

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**CASE REPORT**

# Augmentative transcranial magnetic stimulation (TMS) combined with brain navigation in drug-resistant rapid cycling bipolar depression: A case report of acute and maintenance efficacy

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**Abstract**

The efficacy of transcranial magnetic stimulation (TMS) has been poorly investigated in the acute and maintenance treatment of bipolar depression. The present case supports the efficacy of low-frequency repetitive TMS (rTMS) of the right dorsolateral pre-frontal cortex (RDLPFC) combined to brain navigation in a drug-resistant, bipolar depressed subject with rapid cycling. While continuing the pharmacological treatment at stable doses, the patient was stimulated for 3 weeks at 1 Hz, 110% of motor threshold, 300 stimuli/day showing a significant improvement on the Hamilton Depression Rating Scale ($HDRS_{21}$), the Montgomery–Asberg Depression Rating Scale (MADRS) and the Clinical Global Impression, improvement scale (CGI-I) total scores. On completion of the 3-week rTMS, the patient was treated with periodic maintenance sessions of rTMS at the same parameters of acute phase for an additional 6 months, at the end of which the therapeutic gains were maintained according to rating scales scores. Larger controlled trials assessing the acute and maintenance efficacy of rTMS in bipolar depression are needed.

**Key words:** *Transcranial magnetic stimulation (TMS), right dorsolateral pre-frontal cortex (RDLPFC), brain navigation, bipolar depression*

## Introduction

Transcranial magnetic stimulation (TMS) allows a non-invasive electrical stimulation of the cerebral cortex by means of magnetic fields generated by a handheld coil. Traditionally used in neurophysiology as a research tool, TMS has since been applied in a variety of psychiatric disorders as a potentially therapeutic intervention.

Recent meta-analyses showed mixed results for rTMS in major depressives (Holtzheimer et al. 2001; Burt et al. 2002; Martin et al. 2003; Couturier 2005), and major concerns on published papers include the wide variability of used parameter settings, the limited samples of patients in randomised trials, the small number of sham-controlled studies and the complex reproducibility of results.

With regard to studies specifically performed on bipolar depression, three controlled studies have been published to date (Dolberg et al. 2002; Nahas et al. 2003; Tamas et al. 2007), two of which limited by the small samples. The larger study among these, a single-blind randomised sham-controlled trial (Nahas et al. 2003), performed for 2 weeks in 23 bipolar depressed/mixed patients, failed to find a statistically significant superiority for left prefrontal rTMS at 5 Hz over sham stimulation. In a subsequent maintenance study (Li et al. 2004), performed in seven bipolar depressed patients who had previously responded to acute rTMS, three out of seven subjects completed one full year of weekly maintenance rTMS, maintaining the same level of improvement achieved at the end of the acute treatment.

As reported in the present case, it is of clinical interest to investigate potential stabilizing effect of rTMS in bipolar disorder, a condition in which recurrence prevention is one of the most critical issues for clinicians to manage.

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## Case report

Mrs MC, a 60-year-old retired woman, married and with one daughter, was referred to our centre for a bipolar disorder type I, according to DSM-IV-TR criteria, with poor response to pharmacological treatment. On referral, the patient reported that the disorder had begun at the age of 40 with a depressive episode; the patient subsequently experienced frequent brief depressive episodes and a few mixed episodes that were treated with a combination of antidepressants plus lithium with poor response. During the last 3 years, the patient had an average of four full episodes (three depressive episodes and onr mixed episode) and several subclinical mixed episodes every year.

When we first visited the patient, she was depressed and her symptoms were quantified with $HDRS_{21}$ and MADRS scores of middle severity (19 and 22, respectively), and she was rated as 'markedly ill' on the Clinical Global Impression, severity of illness, scale (CGI-S). No manic/hypomanic symptoms were present as indicated by the Young Mania Rating Scale (YMRS). During the course of the illness, the patient had been treated with different combined therapies (Table I) for adequate periods (at least 8 weeks) including different selective serotonin reuptake inhibitors (paroxetine up to 40 mg/day, sertraline up to 200 mg/day) and serotonin norepinephrine reuptake inhibitors (venlafaxine up to 225 mg/day, duloxetine up to 90 mg/day) combined to mood stabilizers (lithium up to 900 mg/day). The patient's last treatment consisted of bupropion (150 mg/day) plus valproate (500 mg/day, plasma level of 64 μg/ml), and she had been on this treatment at the above-specified dosages for 3 months – i.e. the duration of the current major depressive episode (MDE) – without any symptom improvement.

The patient was then asked to undergo an investigational treatment with rTMS, conceived as a 3-week open-label stimulation of the RDLPFC at 1 Hz, 110% of the motor threshold. After giving her written informed consent, the patient was stimulated for 15 subsequent work-days, with a single session consisting of five trains of 60 stimuli separated by 1 min of pause, and a total of 300 stimuli per session (4500 stimuli over 3 weeks). During the stimulation period, the patient was maintained on her previous pharmacological treatment at the same dosage of the last 3 months. Before starting the stimulation, a magnetic resonance image (MRI) of patient's brain was obtained and installed on a computer software of Brain Navigation in order to target the stimulation area (RDLPFC) with extreme precision and reliability during the treatment period. In fact, once the registration of patient's RDLPFC was performed at baseline, Brain Navigation allowed to stimulate exactly the same area during each session of rTMS.

Table I. Patient's demographic and clinical features including score reduction in $HDRS_{21}$, MADRS, CGI-I and YMRS during the acute and maintenance phase of rTMS.

| Demographic and clinical variables | |
|---|---|
| Age, 60 | |
| Sex, female | |
| Marital status, married with 1 daughter | |
| Occupational status, retired | |
| Age at onset, 40 | |
| Age at first treatment, 41 | |
| Diagnosis, bipolar disorder type I, rapid cycling (DSM-IV-TR) | |
| Mean number of mood episodes/year (depressive and mixed) during the last 3 years of illness, 4 | |
| Previous pharmacological treatments, | Duration |
| Clomipramine (150 mg/day) plus lithium (900 mg/day) | 48 weeks |
| Imipramine (200 mg/day) plus lithium (900 mg/day) | 30 weeks |
| Paroxetine (40 mg/day) plus lithium (900 mg/day) | 16 weeks |
| Sertraline (200 mg/day) plus lithium (900 mg/day) | 24 weeks |
| Venlafaxine (225 mg/day) plus lithium (900 mg/day) | 8 weeks |
| Duloxetine (40 mg/day) plus lithium (900 mg/day) | 10 weeks |
| Bupropion (150 mg/day) plus valproate (64 μg/ml) | 12 weeks |
| Evaluation scales total scores at baseline, $HDRS_{21}$ 19, MADRS 22, YMRS 3 | |
| Evaluation scales total scores at the end of the first week of rTMS | |
| $HDRS_{21}$ 17, MADRS 20, YMRS 4, CGI-I 'minimally improved' | |
| Evaluation scales total scores at the end of the second week of rTMS | |
| $HDRS_{21}$ 14, MADRS 17, YMRS 3, CGI-I 'modeately improved' | |
| Evaluation scales total scores at the end of the third week of rTMS | |
| $HDRS_{21}$ 8, MADRS 11, YMRS 2, CGI-I 'very much improved' | |
| Evaluation scales total scores at 6-month follow-up visit after maintenance sessions of rTMS, | |
| $HDRS_{21}$ 7, MADRS, 11 YMRS 3, CGI-I 'very much improved' | |

*Dosages of different pharmacological agents into brackets are referred to full or best tolerated doses.

After recording patient's motor threshold in order to set the intensity of the stimulation, she was treated with 3 weeks of rTMS and her clinical conditions were assessed at the end of each week through CGI-I, $HDRS_{21}$, MADRS and YMRS. Safety and tolerability were assessed at each session after baseline using spontaneously reported events.

After 3 weeks of stimulation, patient' symptoms were markedly improved as confirmed by a $HDRS_{21}$

score of 8, a MADRS score of 11 and a CGI-I rating of 'very much improved'. During the stimulation cycle, the patient did not show any manic/hypomanic activation and no side effect but slight headache during the first 3 days. Of clinical interest, the more significant score reduction on rating scales occurred during the third week of treatment (Table I).

At the end of the acute treatment, the patient was asked to continue the stimulation for an additional 6 months with periodic sessions of rTMS every other week (two consecutive sessions every 15 days) at the same parameters of the acute phase. After 6 months of maintenance rTMS, during which no change was made in the pharmacological treatment, the patient maintained the same level of improvement obtained at the end of the acute phase without reporting any side effect, as assessed during the monthly follow-up visits and confirmed by $HDRS_{21}$ and MADRS scores of 7 and 11 respectively.

## Discussion

With regard to the acute phase outcome, some considerations may be put forward in light of the stimulation parameters used. The choice to trial low frequency rTMS was motivated by theoretical reasons such as a lower risk of accidental seizure and a better tolerability (Loo and Mitchell 2005). In terms of stimulation site, it has been pointed out in neuropsychological and imaging studies a contrasting role in mood regulation between right and left hemispheres (Loo and Mitchell 2005), and the hypothesis that the RDLPFC stimulation at low frequency may produce the same antidepressant effect as the left DLPFC stimulation at high frequency is still considered valid. In addition, several studies performed on major depressives have targeted the RDLPFC as stimulation area (e.g., Stern et al. 2007; Tamas et al. 2007). With regard to intensity, studies performed at higher intensities of stimulation, as was done in the present case, were more likely to show positive results (Gershon et al. 2003). Of note, a longer duration of treatment (3 weeks) was used in the acute phase of the present case in comparison to the traditionally reported duration in most acute trials (2 weeks). As a matter of fact, the most significant clinical improvement occurred during the third week of treatment with rTMS in the described case without relevant differences in terms of symptom clusters (e.g., melancholic versus anxiety/somatization items). Some authors, in fact, stressed that a treatment period longer than 2 weeks may be necessary for optimal outcomes. In this perspective, a recent small double-blind, sham-controlled study of slow rTMS in bipolar depressives indicated that 4-week active stimulation of the RDLPFC produced continued improvement even after the stimulation period (Tamas et al. 2007). It may be speculated that is not only the number of stimuli per session to be relevant for outcome, but the duration of treatment as well, and that a reduced number of stimuli per session within a higher number of sessions may translate into a better efficacy and safety.

With regard to 6-month results, there are few maintenance studies – particularly in bipolar depression – to refer to in order to provide a possible explanation. Recently, two open studies examining the maintenance effect of rTMS in patients with unipolar (O'Reardon et al. 2005) or bipolar depression (Li et al. 2004) reported positive results. However, both studies used different parameters than the present case in term of frequency (high frequency versus low frequency), target area (left DLPFC vs. right DLPFC), number of stimuli per session (1600–2000 vs. 300) and frequency of maintenance sessions (every week versus every other week). Basically, a less aggressive protocol was used in the present case than in Li and O'Reardon' studies. In addition, the described patient presented not only a diagnosis of MDE in bipolar disorder but was also characterized by a high recurrence index with several episodes in her recent clinical history and, in this perspective, the therapeutic gains seem of particular clinical interest. On this basis, it may be speculated that once the response is achieved, this may be maintained with a limited number of maintenance sessions. Finally, in both the acute and maintenance treatment phases of the present case, rTMS was combined to Brain Navigation which might have contributed to treatment efficacy. Nevertheless, the provided hypothesis on the efficacy of rTMS in the acute and maintenance phases of the reported case should be considered speculative given the lack of large double-blind controlled trials on the argument.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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**VIEWPOINT**

# A different hypothesis on hyponatremia in psychiatric patients: Treatment implications and experiences

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**Abstract**

Polydipsia, chronic or intermittent, with or without hyponatremia, frequently occurs among chronic patients with schizophrenia. The pathogenesis of polydipsia remains poorly understood. The key assumption of our hypothesis is that in some of these patients, polydipsia and hyponatremia are consequences of patients' adjustment to a prolonged intake of an insufficient diet, dominantly poor in potassium. Deficits of potassium, without significant hypokalemia, may cause impairment of the urine-concentrating ability with polyuria-polydipsia. A fall of intracellular tonicity, dominantly due to a decreased amount of $K^+$ and attendant anions in cells, should be accompanied with a fall of extracellular osmolality. Because of the diminished content of ions that may diffuse out of cells and because osmotic equilibrium between the ECF and ICF compartments cannot be established in a short period of time, these patients have a diminished ability to adapt to an excessive intake of fluids. These mechanisms might be related to the development of polydipsia and water intoxication in patients with different mental and somatic disorders. The experiences with the therapeutic effects of diets containing an sufficient amount of potassium in two patients with schizophrenia are described. Further investigations are needed, and we suggest a possible approach to test our hypotheses.

**Key words:** *Hyponatremia, polydipsia, psychiatric patients, potassium*

## Introduction

Polydipsia, chronic or intermittent, occurs frequently among chronic psychiatric patients (de Leon et al. 1994). The prevalence is at least 20%, and half of these patients probably develop hyponatremia (de Leon 2003). The majority of psychiatric patients with "primary polydipsia", as sometimes termed – polydipsia not explained by medical causes – suffer from schizophrenia (Mercier-Guidez and Loas 2000), although the condition could be associated with alcoholism, mental retardation, affective disorders, organic brain disorders, anorexia and personality disorders. It can be loosely characterized as a progressive process with stages from simple polydipsia with accompanying polyuria to polydipsia with water intoxication and physical complications secondary to ingestion of fluids in large quantities (de Leon et al. 1994). Enuresis, incontinence, bladder dilation, hydronephrosis, renal and congestive heart failure, osteoporosis and associated pathological fractures (Halbreich and Palter 1996) are sometimes consequences of the condition. It may also lead to different symptoms, such as nausea, vomiting, delirium, ataxia, seizures, and sudden death, which are mostly referred to water intoxication and hyponatremia (de Leon et al. 1994).

The pathogenesis of polydipsia remains poorly understood, and similarly its treatment remains a clinical challenge. Polydipsia is still commonly considered a side effect of antipsychotic treatment (Jessani et al. 2006), because the prolonged D2 blockade might have influence on drinking behaviour (Meerabux et al. 2005). On the other hand, polydipsia was first recognized in patients with schizophrenia in the 1930s (Illowsky and Kirch 1988), and it is estimated that the frequency has changed little over the years.

Among the possible causes of hyponatremia is the syndrome of inappropriate antidiuretic hormone

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secretion (SIADH), when patients secrete abnormally high levels of the antidiuretic hormone (arginine vasopressin, AVP) relative to plasma osmolality, resulting in inappropriate free-water retention and a fall in serum $Na^+$ concentration. Although it has been reported that patients with schizophrenia on antipsychotic medications have lower plasma levels of AVP than volunteers, it has been also reported that these patients have, for a level of plasma osmolality, increased levels of AVP (Goldman et al. 1988) and that in these patients AVP is secreted even when the plasma osmolality falls below the limit of 270 mOsm/kg (Malidelis et al. 2005). Furthermore, the roles of other hormones associated in osmoregulation, such as angiotensin II and atrial natriuretic peptide, remain ambiguous (Verghese et al. 1998) or completely uninvestigated (e.g., aldosterone).

However, psychiatric patients with polydipsia usually have euvolemic hyponatremia. They generally have no oedema, hypertension, hypocalcaemia or hypokalemia. The question is: why is "sodium diluted" and other elements in the blood are not diluted? In other words, the condition cannot be explained solely on a dilutional basis, and in such circumstances alternative explanations are needful (Verbalis 2006).

## Basic postulations

Hyponatremia might be associated with malnutrition (Cawley 2007) or a changed diet (Adrogué and Madias 2000). Because of different reasons psychiatric patients often have insufficient nutrition. However, the possible importance of malnutrition in the development of hyponatremia in these patients has not yet been investigated. Importantly, hyponatremia is often intermittent, even in patients who are administered a stable medication regime for certain a period of time, without obvious stress and without changes in smoking habit. Thus, our hypothesis is that in some of these patients the condition might be related to diet, and that changes of diet may have a therapeutic effect.

Differences in concentrations of anions and cations in extracellular (ECF) and intracellular (ICF) compartments depend on different factors such as selective permeability of the cell membrane, the presence of proteins and other large anions and the functioning of the sodium–potassium exchange pump. These factors are responsible for the resting membrane potential (Seeley et al. 1992). It is necessary that osmotic equilibrium is established between the ECF and ICF. Although many substances in body fluids have some influences, intracellular osmolality primarily depends on potassium ($K^+$) ions and organic solutes other than urea, whereas the extracellular osmolality primarily depends on the sodium ions level ($Na^+$) plus its attendant anions, chloride and bicarbonate. Plasma sodium levels depend on the concentration of other ions and organic solutes in a complex way. Complex equations on that issue exist (Nguyen and Kurtz 2004). Basically, a fall in ECF tonicity causes water to move into cells, expanding the ICF volume. The preservation of cell volume is possible, but cells must lose $K^+$ ions plus an anion and/or lower their content of organic solutes (Edoute et al. 2003). However, we have postulated that the opposite process may be an explanation. If a patient has an insufficient diet, poor with potassium, then a fall of intracellular tonicity (dominantly due to a decreased amount of $K^+$ and attendant anions in cells) should be accompanied with a similar fall of extracellular osmolality. In fact, a fall of extracellular osmolality would be dominantly accomplished with the loss of sodium, but also with relatively increased activity of AVP. In such circumstances, levels of urine sodium should be increased (Figure 1). Thus, the key assumption of our hypothesis is that in these patients, polydipsia and hyponatremia are consequences of patients' adjustment to a prolonged intake of an insufficient diet, dominantly poor with potassium, and that normal levels of plasma potassium do not mean that there are no deficits of that ion in the organisms. We certainly do not neglect the importance of drugs, other ions or proteins, possible genetic influences, or changes of hormonal and renal function, on the contrary.

Furthermore, we have postulated that if there is a chronic deficit of $K^+$ in a polydipsic patient a diminished content of different anions and anionic proteins should exist in ICF. The result of such a diminished content of anions in ICF might be that, during the excessive intake of fluids, the cell has diminished possibilities to lose anions. Depending on the possibility of molecules to diffuse, a relatively fast fall in the concentration of anions that may diffuse should be expected. In ICF, the proportions of anions that may and may not diffuse would be changed more than in a normal person after taking liquids. The results are that an osmotic equilibrium between the ECF and ICF compartments cannot be established in a short period of time without the development of oedema, simply because days are needed for slow adaptation – the diminishing of the content of anions that may not diffuse. In such circumstances a rapid fall of plasma $Na^+$ levels should be expected too. The increased electrical gradient would cause some $Na^+$ to diffuse in the cell. However, because of its increased concentration gradient, some of $K^+$ would diffuse out the cell. That is perhaps a reason why the hypokalemia would

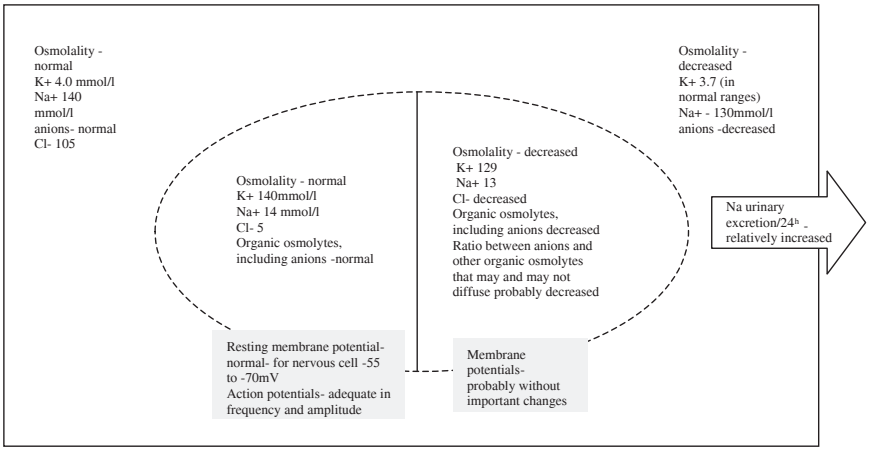


Figure 1. stability of the cell's volume and the electric activity. The ellipse represents the cell membrane in the imaginary situations. The normal situation is shown on the left, and the situation of chronic euvolemic hyponatremia is shown on the right. The most effective osmoles in the ECF and ICF compartment are shown. The volume of the cell in both situations is the same. (A) Postulations on osmotic equilibrium. The important deficit of $K^+$ (on the right) is accompanied with a decreased content of attendant anions, and the consequence is the decreased osmolality in ICF. The constant cell volume is an imperative task closely related to the maintaining of osmotic equilibrium. An organism would achieve that task dominantly by the extrusion of $Na^+$ and attendant anions. (B) Postulations on membrane potentials. Although the creation of resting membrane and action potentials depend on the intra- and extracellular concentrations of different ions, they primarily depend on potassium and sodium concentrations on both sides of cell membrane. The resting membrane is more permeable to $K^+$ than to the other ions, and this permeability is the dominant source of the resting potentials. That is why the equilibrium (or the Nernst potential) for $K^+$ (when the $K^+$ influx $=K^+$ efflux), calculated by the Nernst equation for body temperature of 37°C ($E = -61$ mV $\times \log [K^+]_i/[K^+]_e$), is rather similar to the resting membrane potential. In both the presented imaginary situations the Nernst potential for $K^+$ is practically the same, $-94$mV (inside of cell). The development of action potentials, when the cells' membranes become highly permeable to $Na^+$, dominantly depend on $Na^+$ concentrations in ICF and ECF. By the use of the Nernst equation, the calculated Nernst potential for $Na^+$ in both of the presented situations is $+61$ mV. Therefore, it seems possible that important changes of osmolality and ions concentrations are not necessarily accompanied by important changes in the cell's electrical activity.

be observed less often in more serious cases. Thus, the conclusion might be that these patients have expressly diminished possibilities of being able to adapt to the excessive intake of fluids.

Some previous observations are worth mentioning.

Earlier studies have shown that $K^+$ deprivation induced polyuria and impairment of the urine-concentrating ability in humans and experimental animals. Results from the animal study by Amlal et al. (2000) have shown that a $K^+$ deprivation induced urinary-concentrating defects as early as 12 h of $K^+$ deprivation and that the syndrome of polyuria-polydipsia follows after 24 h of $K^+$ restriction. Rats from the same study fed a $K^+$-free diet developed significant hypokalemia at 6 days. Importantly, these results suggest that a deficit of potassium in the organism is sufficient to cause polyuria-polydipsia, even without obvious hypokalemia (Amlal et al. 2000). Furthermore, it has been reported that in hyponatremic patients, $K^+$ administration increased sodium levels (Nguyen and Kurtz 2004), and that in food-deprived goats with hyponatremia additional supplementation with KCl may maintain their plasma concentration of sodium (Holtenius 1990).

In case reports on hyponatremia induced by different psychotropic medications, only rarely has the diet been mentioned. Miehle et al. (2005) have reported the case of SIADH with hypokalemia in an elderly patient associated with the use of citalopram and, importantly, with reduced food intake for 5 days. Are similar cases the consequences of drugs' impact on hormonal or renal functions, or associated with nausea and decreased food intake? Either way, older age is a risk factor for the development of hyponatremia (Adrogué and Madias 2000), and also for the development of hyponatremia associated with the use of selective serotonin reuptake inhibitors (SSRIs) (Bouman et al. 1998), and people from that population often have inadequate diet (Lee and Frongillo 2001).

## Experiences

We followed the importance of potassium-rich food in two male hyponatremic, otherwise healthy, patients with chronic schizophrenia who had no delusions related to fluid intake. The patients were receiving treatment with conventional antipsychotics (haloperidol, fluphenazine, promazine), and it is considered that such treatment has little if any influence on curbing polydipsia (Goldman and Hussain 2004). Both patients smoke a pack of cigarettes daily (with no obvious changes of that habit during a prolonged period), and the second

patient was treated with biperiden. These factors might be linked to an exaggeration of the condition (Goldman and Hussain 2004). No other drugs that may cause polydipsia and/or hyponatremia (e.g., lithium, carbamazepine, diuretics or antihypertensive drugs) were used. Doses of all drugs were stable, the patients were not exposed to obvious stress, forced water restriction was not used, and smoking was not restricted during the follow-up. Therefore, the only important change during that period was the change of diet. Because these patients sometimes do not eat, their food intakes were supervised. They had daily caloric intakes between 2200 and 2500 kcal, and at least 1000 kcal was in the form of food rich in potassium (e.g., meat, fish, bananas, tomato soup). In the first patient with hyponatremia (at 07:00 Na 127 mmol/l; K 3.8 mmol/l; at 17:00 Na 128 mmol/l, K 4.1 mmol/l, and diurnal weight gain of 6.1%), stabilization was recorded after 2 weeks (at 07:00 Na 136 mmol/l, K 5.0 mmol/l; at 17:00 Na 138 mmol/l, K 4.9 mmol/l, and diurnal weight gain of 1.3%) and after 5 weeks (at 07:00 Na 144 mmol/l, K 4.5 mmol/l; at 17:00 Na 141 mmol/l, K 4.5 mmol/l, and diurnal weight gain of 1.26%). In the second patient with hyponatremia (at 07:00 Na 139 mmol/l, K 3.8 mmol/l; at 17:00 Na 130 mmol/l, K 4.6 mmol/l, and diurnal weight gain of 4.5%) stabilization was recorded after 1 week (at 07:00 Na 141 mmol/l, K 5.1 mmol/l; at 17:00 Na 136 mmol/l, K 4.3 mmol/l, and diurnal weight gain of 1.5%). Diurnal weight gains were monitored because they often significantly correlate with urine excretion (Vieweg et al. 1991).

## Remarks and questions

In patients with hyponatremia, normal plasma potassium levels do not mean that patients have no deficits of that ion in the body. The authors of recently published articles on the treatment of acute or chronic hypotonic hyponatremia have mentioned different approaches, have mentioned the formulas for calculating the needed quantities of $Na^+$, but have not mentioned potassium or food (Costanzo et al. 2004; Douglas 2006; Parenti et al. 2007), except if hypokalemia is present (Adrogue and Madias 2000). Basically, the treatments are concentrated on "visible" changes in ECF. The possibility that the key changes in ICF only have to be accompanied with the changes in ECF is rather neglected in usual clinical practice.

One may expect decreased urinary excretion of $K^+$ in these patients. These patients probably have relatively lower potassium excretion than patients without it or normal persons. However, potassium excretion is under the control of aldosterone, and aldosterone activity depends on plasma potassium levels. Therefore, patients with polydipsia-hyponatremia usually have no hypokalemia, and most probably have urinary $K^+$ excretion in normal but broad ranges, 25–125 mmol in 24 h.

The question is why hypokalemia is not ubiquitous but rather uncommonly present if the patient's intake of potassium is lower than is necessary? Healthy persons or animals on a diet without potassium would have a normal urinary excretion of potassium during the next few days, and after that initial period a decrease of plasma potassium levels and a decrease of urinary excretion would be developed. The prolongation of such a test would cause the death of an organism. However, according to our hypothesis, the process of the development of hyponatremia is rather different. We assume that these patients have lower contents of potassium in their diet during prolonged periods of time, and that these contents are insufficient to achieve normal cell tonicity, but are compatible with life. Indeed, the main questions which we would like to address to physiologists are the following: can a certain quantity of potassium in food be sufficient for survival, but insufficient for normal functioning, and how would an organism adapt to such changes of potassium intake?

One may ask, if potassium has the key role, why KCl was not used during follow-up? Here, it should not be forgotten that the diminished content of $K^+$ in ICF must be accompanied by a diminished content of different anions, which are dominantly organic molecules. In other words, if we want to treat "visible" hyponatremia in these patients, we should normalize $K^+$ concentrations in ICF. The normalization of $K^+$ concentrations in ICF should be accompanied by the normalization of contents of organic anions (e.g., re-synthesis of anionic proteins). Thus, in our opinion, the stable therapeutic effects of different ingredients of food with a sufficient amount of potassium would be more certain than treatment with KCl.

Unfortunately we had no capacity to use more sophisticated laboratories, or to test our hypothesis in more patients while taking standardized meals. However, we are absolutely aware that this mechanism can probably explain the development of polydipsia-hyponatremia only in a certain population of patients. In some patients the condition is clearly associated with delusions (e.g., "... I drink a lot, because I have to wash my dirty intestines ..."), in others with the use of certain drugs (e.g., carbamazepine, different antidepressives) (Holtschmidt-Täschner and Soyka 2007; Koelkebeck et al. in press). Yet our hypothesis gives rise to many general and specific "psychiatric", questions. For example,

can a decreased food intake, and especially intake of potassium, be an important factor in the development of hyponatremia regardless of the primary diagnosis (e.g., in elderly, on intensive care units, after surgery, in patients with tumours, in psychiatric patients)? Can we prevent or improve the treatment of hyponatremia with food rich in potassium? Can we expect a stable therapeutic effect without a normalization of the contents in ICF? In frames of psychiatric care, it has been reported that the intensity of polydipsia and hyponatremia is significantly related to hospitalization length (Schnur et al. 1997). Does that mean that the menus of these patients may influence the outcomes? If yes, that fact most probably would not only be related to some psychiatric patients.

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## Statement of interest

The authors report no financial affiliations or other relationships relevant to the subject of this manuscript

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**VIEWPOINT**

# Trazodone generates m-CPP: In 2008 risks from m-CPP might outweigh benefits of trazodone

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**Abstract**
Since deleterious effects of *m*-CPP, the primary catabolic metabolite of trazodone, were last reviewed 2 years ago, research data continue to accrue showing that clinically significant levels of *m*-CPP (a) are generated in patients using trazodone for sleep and (b) are present 24 h a day and (c) have potentially serious ill effects. This commentary argues that the documented potential for harm and multiple risks of *m*-CPP outweigh potential benefits of trazodone, given the development and marketing of many safer alternatives since trazodone's introduction in the 1980s.

**Key words:** *Anxiety, fibrosis, inflammation,* m-*CPP, trazodone*

## Introduction

In 2008 trazodone is still commonly recommended as an antidepressant (Quaseem et al. 2008) and sleep aid (Flannagan et al. 2007; Roth 2008). Its current clinical use is mainly as sleep aid. This commentary reviews recent data leading to a conclusion that continued trazodone use should be re-considered and curtailed. Trazodone's primary metabolic catabolite is *m*-clorophenylpiperazine, *m*-CPP. A previous review (Kast 2007) concluded that *m*-CPP risks might not be worth the benefits of trazodone, this update now concludes the same. Wider awareness of the large database that continues to accrue, showing potential harm of *m*-CPP, is important.

Known by various trade names (Desyrel, Molipaxin, Trittico, Thombran and Trialodine) trazodone is cheap, available generically, and relatively safe in single-drug overdose. The central problem of trazodone is its hepatic catabolism by CYP3A4 to *m*-CPP, as shown in Figure 1. Basic pharmacological and pharmacokinetic properties of *m*-CPP are listed in Table I.

*m*-CPP is generated also from the trazodone-related antidepressant nefazodone (Barbhaiya et al. 1996). Nefazodone was withdrawn from the market in most countries in 1996 due to hepatotoxicity.

Initial, middle, or late insomnia are core features of depression. The selective serotonin reuptake inhibitors, SSRIs, though comprising the mainstay of current treatment of depression can fragment sleep further even when they are effective in alleviating the mood disorder (Pandi-Perumal et al. 2008). In current clinical practice, trazodone, as a sleep-enhancing antidepressant, is often added to counter or correct SSRI induced sleep fragmentation.

High and clinically significant daytime *m*-CPP levels (about 100 ng/ml or a tenth of plasma trazodone levels) continue to be documented during in normal humans taking a common trazodone dose for sleep, 150 mg once at bedtime (Mercolini et al. 2008). This is comparable to levels attained when anxiety or panic attacks are provoked by i.v. *m*-CPP in studies with normal human volunteers (Van Veen et al. 2007).

## An odd street drug-of-abuse

*m*-CPP is becoming a street drug-of-abuse. Some patients report pleasant or desirable feelings after

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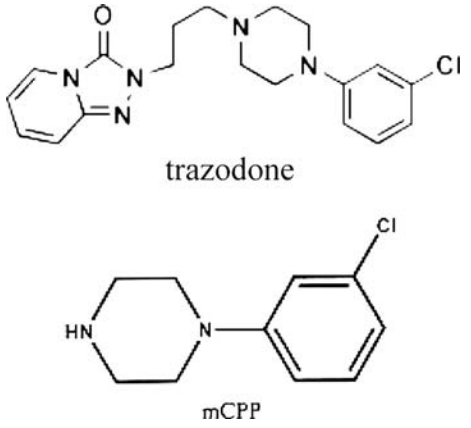


Figure 1. Chemical structures of trazodone and *m*-CPP.

ingesting street *m*-CPP but most people report headaches, anxiety, panic, confusion, and depressed mood (Kovaleva et al. 2008). Patients presenting with acute *m*-CPP ingestion often report dysphoria and show great distress without being able to further specify or add detail. A similar clinical picture is often obtained after research administration of *m*-CPP to young healthy human volunteers (Gijsman et al. 1998; Feuchtl et al. 2004).

Street *m*-CPP tablets, sold as "LSD", "ecstasy", "dumpers", "rainbows", and other names, are usually characteristically multicolored tablets containing 20–40 mg *m*-CPP with unknown contaminants and excipients. A common patient dose is several of these. Expected *m*-CPP blood levels from street ingestion is unknown. Research volunteers with prior experience of the real street drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA) report that *m*-CPP in doses from 17.5 to 52.5 mg/70 kg produced MDMA-like stimulant and hallucinogenic effect (Johanson et al. 2006). *m*-CPP is appearing as an adulterant in cocaine and other street drugs-of-abuse (Staack et al. 2007; Kovaleva et al. 2008).

### *m*-CPP stimulates 5-HT2B and 5-HT2C receptors

*m*-CPP as potent and clinically relevant agonist at 5-HT2B and 5-HT2C receptors was reviewed previously (Kast 2007). Agonism at 5-HT2B or 5-HT2C is expected to be destructive psychiatrically and somatically. Additional observations since 2006 confirm and extend that data:

(a) *m*-CPP agonism at 5-HT2B and 5-HT2C receptors was shown to be either anxiolytic or anxiogenic, depending on specific brain location of *m*-CPP infusion in mice (Nunes-de-Souza et al. 2008).

(b) It is the 5-HT2B agonism by pergolide in humans treated for parkinsonism that generates and is the direct cause of tricuspid and mitral valve fibrosis in a fifth of those so treated (Junghanns et al. 2007; Görnemann et al. 2008). Several hundred percent increased risk of clinically significant cardiac valve fibrosis after pergolide was seen (Schade et al. 2007), leading to pergolide's withdrawal from the US market. It is not unreasonable to suspect similar pro-fibrosis *m*-CPP mediated consequences of trazodone use.

(c) 5-HT2B receptor density on pulmonary artery endothelium is increased in pulmonary hypertension. Stimulation of 5-HT2B receptors is crucial for the associated pulmonary artery fibrosis (Launay et al. 2002). A long history associates fibrosis of liver, lung, cardiac valves, and retroperitoneal space with 5-HT2B agonism (Ruddell et al. 2006; Kast et al. 2007).

(d) 5-HT2C agonists stimulate neurons in the suprachiasmatic nucleus, generating a light-mimetic effect, further disrupting day-night cycles (Varcoe et al. 2008).

Table I. Some basic pharmacology and pharmacokinetics of *m*-CPP. "Tobacco" is underlined as a strong CYP 3A4 inducer only because of its ubiquity.

| | |
|---|---|
| $T_{1/2}$ | 3–6 h |
| $T_{max}$ | Wide inter-individual variation (h) |
| $C_{max}$ | Unknown |
| Generated by | Hepatic metabolism of trazodone or nefazodone by CYP3A4 |
| Catabolism | By CYP 2D6 to inactive hydroxy-*m*-CPP (renal excretion) |
| Some common, strong, hepatic CYP3A4 inhibitors | Indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin |
| Some common hepatic CYP3A4 inducers | Efvirenz, nevirapine, barbituates, carbamazapine, modafinil, pioglitazone, troglitazone, tobacco, rifabutin, phenytoin |
| Average daytime plasma concentration after bedtime dose of 150 mg trazodone | 100 ng/ml |

### *m*-CPP as anxiogen

*m*-CPP is a potent anxiogen in humans and rodents, as previously reviewed (Kast 2007). Empirical data supporting the anxiogenic nature of trazodone and *m*-CPP continues to accrue:

(a) A 6-week comparison of trazodone to sertraline found both similarly separated from placebo in lowering depression, but anxiety burden was higher in the trazodone group (Munizza et al. 2006). The occasional patient will have an intolerable anxiety response to trazodone (Munizza et al. 2006).
(b) Concordant rodent studies show anxiogenic properties of *m*-CPP and anxiolytic properties of other experimental selective 5-HT2C antagonists (Bagdy et al. 2001; Harada et al. 2006).
(c) De novo appearance of psychycotic signs and symptoms is rarely noted after trazodone use (Mizoguchi et al. 2005).
(d) Studies into the nature of anxiety and panic continue to use i.v. *m*-CPP to generate signs and symptoms of these disorders in normal human volunteers (Van Veen et al. 2007; Bagady et al. 2002). *m*-CPP levels in these studies (40–100 ng/ml (Van Veen et al. 2007; Bagady et al. 2002)) are comparable to those seen in patients treated for insomnia with trazodone (10 ng/ml *m*-CPP after a single 100 mg trazodone dose in healthy volunteers (Patel et al. 2008), 100 ng/ml in patients treated chronically with 150 mg trazodone at bedtime (Mercolini et al. 2008)). It cannot be excluded that in anxiety generation slope of *m*-CPP concentration change is important as well as actual serum level.

### Additional *m*-CPP toxicities

Dose-proportional memory impairment is seen in rats given *m*-CPP (Khaliq et al. 2008). We should worry about similar decrements in humans until formal study can allay such fears.

5-HT2B agonism in a neuroectodermal cell line was shown to result in increased TACE, tumour necrosis factor-alpha converting enzyme (Schneider et al. 2006). TACE is responsible for conversion of outer cell membrane-bound TNF-alpha to soluble, circulating, TNF-alpha. Increased soluble TNF was demonstrated after 5-HT2B agonists (Schneider et al. 2006). If this were to occur in humans, unfortunate pro-inflammatory and fibrogenic consequences can be expected.

Post-mortem study of brain tissue of depressed patients shows evidence of oxidative stress (Michel et al. 2008). We have several lines of evidence that trazodone might increase this brain oxidative stress. Mice exposed to 4 h of stress by watching, hearing, and proximity to cagemates exposed to unpleasant foot electric shocks, similar to depressed patients in the post-mortem study above showed increased brain lipid peroxidation products even though they themselves received no shocks or any noxious stimuli other than proximity to suffering cagemates (Matsumoto et al 1999). Adding *m*-CPP to these stressed mice potentiated increases in lipid peroxidation products (Matsumoto et al 1999). Concordant with these findings are in vitro evidence of oxidative stress from nefazodone and trazodone by mitochondrial potential collapse and glutathione depletion (Dykens et al 2008).

### Conclusion

The outlined deleterious effects of trazodone's primary metabolite *m*-CPP probably make trazodone's ongoing use generally inadvisable. Safer alternatives exist for depression and insomnia treatment.

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### Statement of interest

None.

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**LETTER TO THE EDITOR**

# Ashwagandha for anxiety disorders

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**Key words:** *Ashwagandha, Withania somnifera, anxiety, treatment*

Sir,

In their extensive review and treatment guideline, Bandelow et al. (2008) described various pharmacological interventions for the management of anxiety disorders. They listed Ginkgo biloba and kava kava among the herbal preparations that have been studied. However, they did not refer to an important herb, Ashwagandha (ASW), which has for long been used in traditional medicine to treat disorders related to stress (Handa 1995; Satyavati 1995).

ASW is obtained from the root of the plant *Withania somnifera*. The plant grows wild but is also cultivated for medicinal use in many parts of India as well as in other Asian countries. Preclinical studies suggest that ASW contains an ingredient with GABA-mimetic activity (Mehta et al. 1991; Kulkarni et al. 1993; Cott et al. 1994). ASW also downregulates 5-HT1 and upregulates 5-HT2 receptors in the rat brain; these changes are accompanied by a decrease in behavioural indices of anxiety and depression (Tripathi et al. 1998). In animal models, ASW glycowithanolides have an anxiolytic effect comparable to that of lorazepam (Bhattacharya et al. 2000).

At least three studies have examined the efficacy of ASW in anxiety disorders. In a preliminary investigation, the safety and efficacy of an ethanolic extract of ASW were examined in 50 patients with anxiety disorders. By the end of the first month of treatment, 36 patients showed moderate to excellent improvement at a dose of 1 g/day; in about half of these cases, these statistically significant benefits developed within the first 2 weeks, itself. These 36 patients were continued on ASW for up to 18 months, after which the medication was uneventfully withdrawn. ASW remained effective and was very well tolerated (data available from the author on request).

In a double-blind, placebo-controlled clinical trial, we (Andrade et al. 2000) randomized 40 patients with ICD-10 anxiety disorders to receive either flexibly-dosed ethanolic extract of ASW (1.0–2.5 g/day) or placebo for 6 weeks. About two-thirds of the sample was diagnosed with generalized anxiety disorder. By the end of the second week of treatment, 12 of 17 ASW patients but only six of 16 placebo patients met criteria for response. At the end of the study, 15 of 17 ASW patients versus only eight of 16 placebo patients had responded ($P<0.05$). Mean Hamilton Anxiety Scale scores dropped from about 18.0 in the two groups at baseline to 8.5 with ASW versus 12.2 with placebo at the 6-week treatment endpoint. ASW was well tolerated and had a placebo level of adverse effects. At the end of the study, abrupt withdrawal of ASW did not precipitate withdrawal phenomena. Thus, ASW appeared to have several of the advantages but none of the disadvantages of conventional anxiolytic drugs such as antidepressants and the benzodiazepines.

Auddy et al. (2008) described a 2-month, double-blind, randomized controlled study which examined benefits with a standardized extract of ASW in 130 subjects who received a descriptive diagnosis of chronic stress in an Ayurvedic hospital. ASW was dosed at 125, 250, or 500 mg/day; a fourth group of patients received placebo. All doses of ASW attenuated modified HAM-A scores significantly more than did placebo at the 1-month follow-up; there was

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further improvement at the 2-month endpoint. The benefits with ASW appeared to be dose-dependent; placebo patients improved little. Furthermore, relative to placebo, ASW significantly lowered heart rate (by 6–8%), systolic blood pressure (by 2–3%), and diastolic blood pressure (by 5–6%); there were significant reductions in serum cortisol, fasting blood sugar, serum lipid, and C-reactive protein levels, as well.

All of these three studies were industry-initiated and none was of sufficient quality for recommendations to be based upon; nevertheless, it does appear that ASW merits study as a possibly safe and effective treatment of anxiety disorders. At the very least, clinicians should be aware of the use of ASW in complementary and alternative medicine. Finally, other herbal treatments for anxiety are also available in India and elsewhere in the world; these treatments may also merit scientific study.

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No financial support was received from any source for the preparation of this letter. I have no financial interest which would either favor or disfavour the thoughts and conclusions advanced in this letter. I have no financial interest which would influence any of the contents of this article. About 10 years ago, I received a very small (approximately USD 2000) but completely unrestricted grant from Gufic, an Indian pharmaceutical company, to study the clinical efficacy of its proprietary formulation of Ashwagandha in anxiety disorders. I describe my study in this letter.

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**LETTER TO THE EDITOR**

# Mirtazapine and hyperpigmentation

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**Key words:** *Adverse effects, antidepressants, depression*

Sir,

Among the antidepressants, mirtazapine has a unique structure and mechanism of action, which could be best described as a nonadrenergic and specific serotonergic antidepressant. Common side effects of this drug are somnolence, dry mouth, increased appetite and weight gain (Montgomery 1995). We report a case of a woman with depressive disorder who was being treated with mirtazapine and developed hyperpigmentation in photo-sensitive areas. To our knowledge, this is the first reported side-effect in the existing literature.

Mrs A, a 38-year-old fair-complexioned married woman presented to us with a history of sadness, anxiety, disturbed biological functioning and low confidence levels of 2 years duration. Her symptoms met DSM-IV criteria for major depressive disorder. In the initial phase of her illness, for approximately 18 months, she was drug nave and had undergone a few sessions of psychotherapy without any improvement. Subsequently, she was started on imipramine 25 mg/day but this medication was discontinued after 15 days because of severe dryness of mouth and constipation.

She was then started on mirtazapine 7.5 mg/day which was increased to 15 mg/day over a period of 2 weeks. She tolerated this drug very well and showed good clinical response with improvement in sadness of mood and biological functioning. She, however, had a recurrence of depressive symptoms when the dose of mirtazapine was reduced to 7.5 mg. She was therefore stabilized on a daily dose of 15 mg of mirtazapine. After 8 months of mirtazapine therapy, she and her family members noticed a darkening of her skin complexion in the exposed areas of her body such as face, extensor part of forearm and posterior aspect of the neck. However abnormal pigmentation was absent in the sclera, nails and teeth. There was no history of any dermatological disorder preceding the administration of mirtazapine therapy. She did not have any endocrinological abnormalities and all her routine investigations were within normal limits including serum iron and blood glucose levels. Mrs A denied having used new soap, cream or different kind of food. She also denied more exposure to sunlight than usual and was not pregnant.

Dermatological consultation confirmed it as a case of hyperpigmentation. She refused to give consent for a skin biopsy stating cosmetic reasons. Mirtazapine was discontinued completely and it was replaced with sertraline 100 mg/day. She showed mild improvement in her hyperpigmentation over the next 6 months. The probability of an adverse drug reaction was assessed using the Naranjo probability scale (Naranjo et al. 1981).This indicated a possible association between mirtazapine and hyperpigmentation.

A literature review and PubMed search found that, besides tricyclic antidepressant drugs (Metelitsa et al. 2005), there have been reports of hyperpigmentation with citalopram (Inalz et al. 2001) and sertraline (Ghanizadeh 2007). A similar search (using the keywords mirtazapine, pigmentation and hyperpigmentation) found no published reports of hyperpigmentation with mirtazapine. Our finding is important: firstly because atypical antidepressants

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are now routinely prescribed over tricyclic antidepressants and secondly because the side effect did not appear to be fully reversible even after 6 months of follow-up. Secretion of the melanocyte stimulating hormone (α-MSH) is closely linked to serotonin and dopamine and, through its actions on tyrosinase and melanin synthesis, may be related to hyperpigmentation (Ghanizadeh 2007). A more conclusive association can be determined by rechallenge to mirtazapine: however, since the subject would not consent to rechallenge this was not carried out. Further studies are needed to try and determine the exact biological mechanism for the emergence of hyperpigmentation.

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**Statement of interest**

The authors have not declared any conflicts of interest.

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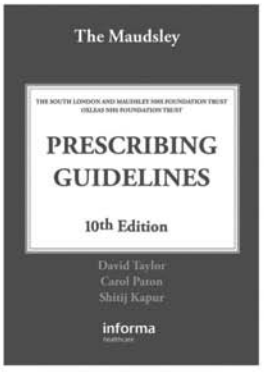


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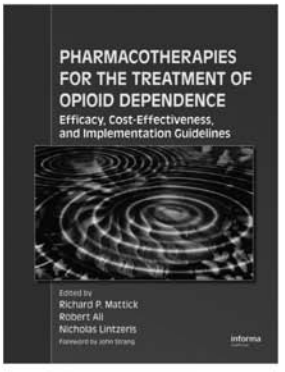


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