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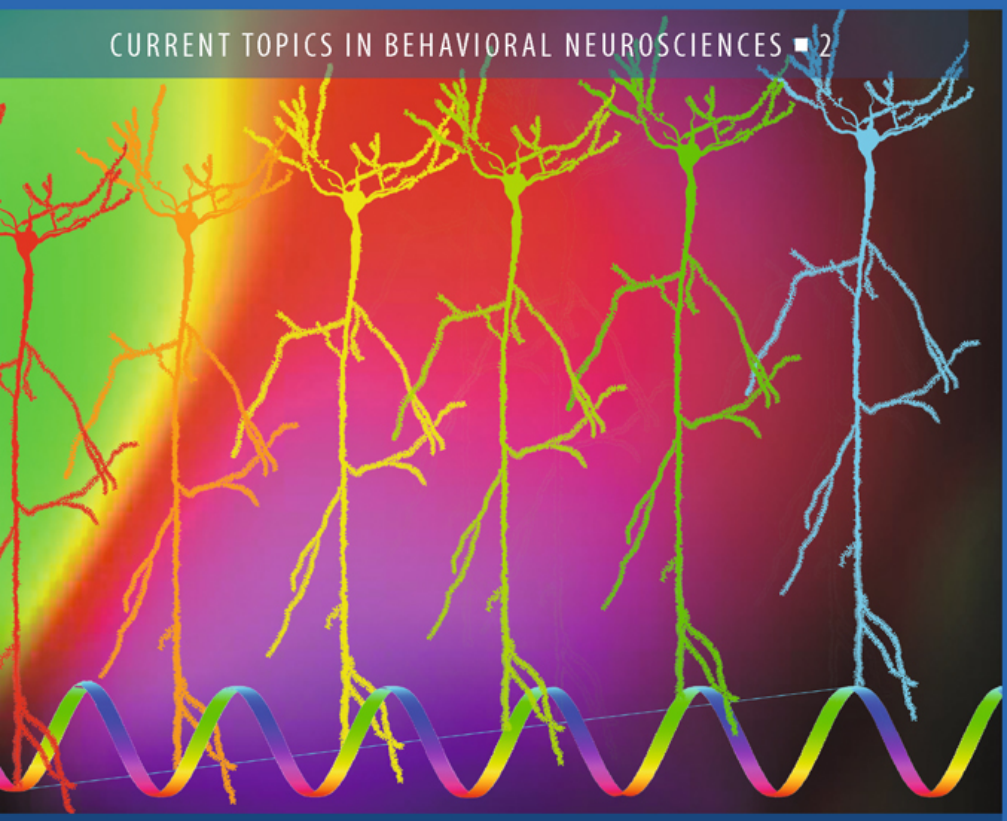

Behavioral Neurobiology of Anxiety and Its Treatment

M.B. Stein
T. Steckler
Editors

Springer

Current Topics in Behavioral Neurosciences

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Murray B. Stein • Thomas Steckler
Editors

Behavioral Neurobiology of Anxiety and Its Treatment

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Preface

The book is part of a series on Current Topics in Behavioral Neurosciences, which has as its focus anxiety and its treatment. We have brought together a distinguished cadre of authors with the aim of covering a broad array of topics related to anxiety disorders, ranging from clinical diagnosis, epidemiology, preclinical neuroscience, and animal models to established and innovative therapeutic approaches. The book aims at bridging these disciplines to provide an update of literature relevant to understanding anxiety, its consequences, and its management. Following is a brief overview of the chapters and their content, meant to serve as a guide to navigating the book.

The first section covers clinical aspects of anxiety disorders. Joe Bienvenu and colleagues provide an incisive overview of diagnostic considerations in the anxiety disorders in which they emphasize the strengths and shortcomings of our current nosologic systems. This is followed by a review and update of the epidemiology of anxiety disorders by Ron Kessler and colleagues, which provides an authoritative survey of anxiety disorder incidence, prevalence, and risk factors. This is complemented by a comprehensive review of the literature on disorders that co-occur with anxiety disorders by Kathleen Merikangas and Sonja Alsemgeest Swanson. Their review highlights the tremendous comorbidity that occurs not only within the anxiety disorders, but also with other mental and physical health conditions.

The second section is devoted to clinical neuroscience topics that are germane to anxiety disorders. Katharina Domschke and Jürgen Deckert provide a summary of genetic findings in the anxiety disorders. Newton Sabino Canteras and colleagues describe neuroanatomical structures and circuits relevant to anxiety and anxiety disorders. This section is concluded by José Miguel Pêgo and colleagues with a critical overview of the neuroendocrinology of anxiety and stress, tying this together with findings in anxiety and stress disorders.

The third section focuses on animal models. Dallas Treit and colleagues provide an overview on preclinical tests of anxiety-related behavior and discuss the validity of these tests and their sensitivity to pharmacological intervention. This is followed by a review by Laura Jacobson and John Cryan on genetic animal models of anxiety, a discussion of the strengths and weaknesses of such models, and their

contribution to our understanding of the neurobiological and genetic basis of anxiety disorders.

The fourth section is devoted to translational science that can inform our understanding of anxiety disorders. Victoria Risbrough discusses behavioral correlates of anxiety disorders with a special emphasis on work that has used emotion-(usually fear-) potentiated startle to test the integrity of particular anxiety-relevant circuits in humans. Marlies van Duinen and colleagues provide a review of the so-called challenge studies in anxiety, in which subjects are exposed to substances (e.g., pharmacological agents) intended to evoke anxiety as a test of their specific properties. Their particular emphasis in this chapter is on challenges to and responses of the respiratory system which, arguably, have been best studied in the anxiety disorders, particularly panic disorder. Amit Etkin subsequently provides a comprehensive review of the functional neuroimaging literature in anxiety disorders. He examines the similarities and differences across anxiety disorders that have emerged from this literature, much of it using functional magnetic resonance imaging (fMRI). Finally, in a tour de force of the merits of translational research, Dennis Choi and colleagues provide a rationale for the pharmacological enhancement of behavior therapy for anxiety disorders.

The fifth section covers the preclinical pharmacology of systems that are relevant to anxiety, as well as some of the molecular targets for potential anxiolytics with novel mechanism of action. Claire Durant and colleagues set the scene with a review of the clinical neurochemistry of anxiety disorders, emphasizing the different neurochemical systems that are targeted by currently used and newly developed anxiolytic drugs. This is followed by an authoritative overview by John Atack on GABAergic approaches, delineating the potential of GABA-A subunit-specific compounds to treat anxiety disorders. Anton Bespalov and colleagues then discuss the importance of antidepressant drugs in the treatment of anxiety disorders, especially the conundrum that preclinical animal models often fail to detect anxiolytic-like effects of antidepressants despite their clinical utility. Their interesting proposal is to also consider cognitive components of anxiety-related behavior and to expand the armamentarium of preclinical models relevant to anxiety to more readily detect the efficacy of antidepressant drugs. Will Spooren and colleagues subsequently review the emerging field of compounds acting at the different metabotropic glutamate receptors as an innovative avenue for the development of novel anxiolytic drugs, followed by a summary by Thomas Steckler on the status of neuropeptidergic approaches to the treatment of anxiety disorders, especially focusing on clinical proof-of-concept studies with small-molecule, nonpeptidergic antagonists of the CRF1, NK1 and 3, and CCK2 receptors, but also on the atrial natriuretic and oxytocin systems. This section concludes with a state-of-the-art overview by Fabricio Moreira and Carsten Wotjak on cannabinoids and anxiety, and modulation of the endogenous cannabinoid system as yet another promising therapeutic approach in the quest for novel, more efficacious and better tolerated anxiolytic drugs.

The sixth and final section of the book reviews the clinical pharmacology of anxiety disorders. This section is organized such that each chapter provides an authoritative review of the literature on the pharmacological management of the

most common anxiety disorders. David Baldwin and colleagues review the pharmacotherapy of generalized anxiety disorder (GAD) and then Jeffrey Lightfoot and colleagues do the same for panic disorder. Keith Ganasen and Dan Stein summarize the evidence base for the pharmacotherapy of social phobia (also known as social anxiety disorder), highlighting areas where knowledge is strong and where it is especially sparse. Lakshmi Ravindran and Murray Stein review the evidence for pharmacological treatment of posttraumatic stress disorder (PTSD), a topic that has been especially controversial and in the news of late, given recent military conflicts around the world and the increased awareness of their impact on health of combatants and civilians alike. Blair Simpson closes this section with a summary of the pharmacotherapy of obsessive compulsive disorder (OCD), a particularly chronic and difficult-to-treat anxiety disorder for which new treatments are sorely needed, but have been slow in coming.

These chapters, individually and in their aggregate, provide a broad summary and synthesis of behavioral neuroscience findings in anxiety and a detailed update on its treatment. As we look forward to the future – as many of the authors have done in their chapters – we are particularly excited about recent advances in the translational neuroscience of anxiety which promise to lead to the development of more potent therapies for the patients who need them.

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Pharmacological Therapy

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Anxiety Disorders Diagnosis: Some History and Controversies

O. Joseph Bienvenu, Lisa A. Wuyek, and Murray B. Stein

Contents

Abstract Treatment of and research on anxiety disorders depends on the adequate conceptualization and measurement of these conditions. We review the history of the nosology of anxiety disorders and note that divisions of "neurosis" have inadvertently taken attention away from what is shared among conditions now classified separately. We note the changes in the definition of agoraphobia over time and the striking differences between DSM-IV and ICD-10 definitions. We mention ongoing controversies in the diagnoses of posttraumatic stress disorder, acute stress disorder, and generalized anxiety disorder. Finally, we discuss

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controversies surrounding the proper placement of obsessive-compulsive disorder and putatively related conditions in future diagnostic classifications. We hope that reviewing controversial aspects of diagnosis is useful to clinicians and researchers interested in the neurobiology of anxiety disorders.

Keywords Anxiety disorder · Nosology · Classification · Agoraphobia · Posttraumatic stress disorder · Acute stress disorder · Generalized anxiety disorder · Obsessive-compulsive disorder

1 Introduction

In this chapter, we intend to familiarize readers with some of the historical origins of the anxiety disorders section of the Diagnostic and Statistical Manual of Mental Disorders (DSM), fourth edition (APA 1994), noting some differences between this and the similar section in the International Classification of Diseases (ICD), tenth edition (WHO 1992, 1993). We will use these foundations to discuss some ongoing nosologic controversies. We submit that an understanding of the limitations of classification is vital to clinicians and researchers interested in the neurobiology of anxiety disorders. Though we mentioned some of these issues in a previous chapter (Stein and Bienvenu, 2008), our emphasis is different here in that we focus more on individual diagnostic constructs and less on higher-order constructs and dimensions.

Before we begin, it is important to emphasize that we are all affected by our conceptualizations of psychopathology, and these conceptualizations *matter* in clinical practice and research. For example, in a group of studies conducted in the 1960s (the US/UK diagnostic project), researchers found that American psychiatrists had a relatively broad concept of schizophrenia compared with British psychiatrists, who more frequently diagnosed mood, "neurotic," and personality disorders (Cooper et al. 1969; Gurland et al. 1969; Kendell et al. 1971; Kramer 1969; Zubin 1969). Making note of such differences was important not just to clarify why so many Americans were diagnosed with schizophrenia, compared to Brits. That is, the illustration of these differences was important because reliable diagnoses are *essential* for studies of etiology, prognosis, and treatment effectiveness that inform clinical practice. Studies like these gave impetus to the development of explicit diagnostic criteria (Feigner et al. 1972; Spitzer et al. 1978) that prepared the way for DSM-III (APA 1980).

2 A Brief History of Anxiety Disorders Classification

Though anxiety itself has been discussed for millennia (McReynolds 1975), its appearance as a syndrome in the medical literature is a relatively recent phenomenon (Stone 2002). In the eighteenth century, physicians such as Boissier de Sauvages (1752), Battie (1758), Whytt (1765), and Cullen (1807) wrote of

"panophobia" (fear of everything), "praeternatural anxiety" (anxiety outside the normal range), "nervous disorders," and "neurosis," respectively (Stone 2002). Notably, by "neuroses" (nerve conditions) Cullen meant "those affections. . .which do not depend on topical affection of organs but upon general affection of the nervous system" (Knoff 1970); thus, the neuroses originally included a number of neurologic and psychiatric conditions, not just what we now call anxiety, depressive, somatoform, and dissociative disorders. Over time, the term "neurosis" has endured, though the group of phenomena it describes has narrowed, and the term has carried varying etiologic connotations (Stone 2002).

It is worth mentioning, in passing, that in the mid–late nineteenth century Beard coined the term "neurasthenia" (weakness of the nerves) (Beard 1880). This popular label was subsequently applied to what we now recognize as a broad range of anxiety and other syndromes (Stone 2002), though "neurosis" outlasted "neurasthenia" as a broad psychopathologic descriptive term [note that a much narrower definition of neurasthenia remains in ICD-10 (WHO 1992)]. Meanwhile, a number of other categories within neurosis were more or less delineated (Tyrer 1985), including agoraphobia (Westphal 1872), anxiety neurosis (Freud 1895/1962), specific and social phobias (Hartenberg 1901; Marks 1970; Ribot 1896), obsessive-compulsive neurosis (Janet 1908), milder depressive states (Lewis 1938), and panic disorder (Klein 1964). We say "more or less delineated" since most of the listed authors described relatively broad, overlapping syndromes. "Neurosis" was a broad diagnostic category in DSM until the third edition (APA 1980), and it remains a broad diagnostic category in ICD-10 (WHO 1992).

Table 1 summarizes the classification of neurotic and anxiety disorders from DSM-I through DSM-IV (APA 1952, 1968, 1980, 1987, 1994). Note that the authors of DSM-III moved somatoform and dissociative disorders to separate categories, eliminated "depressive neurosis," grouped the remaining former "neurotic" disorders as "anxiety disorders," and added posttraumatic stress disorder (PTSD) to this new category. By DSM-III, the term "neurosis" had long taken on psychoanalytic etiologic implications. DSM-III and its descendants were meant to provide atheoretical descriptions of mental disorders; this is why the term "neurosis" was phased out, despite its value and history as a descriptive term (Fava et al. 2008; Frances et al. 1993; Tyrer 1985). Table 2 summarizes the "Neurotic, stress-related, and somatoform disorders" in ICD-10 (WHO 1992).

By far, the most marked change in classification of the anxiety disorders occurred with DSM-III. All psychiatric diagnoses were defined more explicitly than before, with diagnostic criteria that facilitated clearer communication for clinical practice and research. In addition, the anxiety disorders were given substantially more attention (Frances et al. 1993), with the delineation of panic disorder and generalized anxiety disorder (GAD) from "anxiety neurosis" and the categorization of different kinds of phobias, including agoraphobia, social phobia, and simple (specific) phobia.

Notably, there are reasons to question the utility of carving neurosis into separate categories (Tyrer 1985). Long before explicit diagnostic criteria were developed, change over time and cross-sectional comorbidity among the various neurotic conditions was recognized as common (Slater and Slater 1944). This is no less

Table 1 Classification of neurotic/anxiety disorders in the American Psychiatric Association's Diagnostic and Statistical Manuals of Mental Disorders

DSM-I psychoneurotic disorders (1952)	DSM-II neuroses (1968)	DSM-III anxiety disorders (1980)	DSM-III-R anxiety disorders (1987)	DSM-IV anxiety disorders (1994)
Phobic reaction	Phobic neurosis	Agoraphobia with panic attacks	Agoraphobia without history of panic disorder	Agoraphobia without history of panic disorder
		Agoraphobia without panic attacks	Social phobia	Social phobia
		Social phobia	Simple phobia	Simple phobia
		Simple phobia		
Anxiety reaction	Anxiety neurosis	Panic disorder	Panic disorder with agoraphobia	Panic disorder with agoraphobia
		Generalized anxiety disorder	Panic disorder without agoraphobia	Panic disorder without agoraphobia
			Generalized anxiety disorder	Generalized anxiety disorder
Obsessive compulsive reaction	Obsessive compulsive neurosis	Obsessive-compulsive disorder	Obsessive-compulsive disorder	Obsessive-compulsive disorder
		Posttraumatic stress disorder	Posttraumatic stress disorder	Posttraumatic stress disorder
				Acute stress disorder
Depressive reaction	Depressive neurosis			
Conversion reaction	Hysterical neurosis			
Dissociative reaction	Neurasthenic neurosis			
	Hypochondriacal neurosis			
	Depersonalization neurosis			

Table 2 Neurotic, stress-related, and somatoform disorders in ICD-10 (1992)

Phobic anxiety disorders	Dissociative [conversion] disorders
– Agoraphobia	– Dissociative amnesia
– Social phobias	– Dissociative fugue
– Specific (isolated) phobias	– Dissociative stupor
	– Trance and possession disorders
Other anxiety disorders	– Dissociative motor disorders
– Panic disorder	– Dissociative convulsions
– Generalized anxiety disorder	– Dissociative anesthesia and sensory loss
– Mixed anxiety and depressive disorder	
	Somatoform disorders
Obsessive-compulsive disorder	– Somatization disorder
	– Hypochondriacal disorder
Reaction to severe stress, and adjustment disorders	– Somatoform autonomic dysfunction
– Acute stress reaction	– Persistent somatoform pain disorder
– Posttraumatic stress disorder	
– Adjustment disorders	Other neurotic disorders
	– Neurasthenia
	– Depersonalization-derealization syndrome

true since the introduction of explicit diagnostic criteria with DSM-III and its descendants (Andrews et al. 1990, 2002; Brown et al. 2001; Boyd et al. 1984; Creed and Barsky 2004; Freyberger and Spitzer 2005; Kessler 1995; Lieb et al. 2007; Merikangas et al. 1996). The extent of comorbidity among conditions formerly classified as neuroses has rightly turned attention toward what these conditions share, including personality correlates (Bienvenu et al. 2001; Kahn et al. 2005; Krueger et al. 2001). Factor analytic studies are consistent in finding that anxiety and depressive disorders are highly comorbid, though phobic, panic, and obsessive-compulsive disorders are particularly highly comorbid (forming a "fear factor"), and depressive, generalized anxiety, and posttraumatic stress disorders are also particularly highly comorbid (forming a "distress" or "anxious misery" factor) (Cox et al. 2002; Kendler et al. 2003; Kessler et al. 2005b; Krueger 1999; Krueger et al. 1998, 2003; Miller et al. 2008; Slade and Watson 2006; Vollebergh et al. 2001). There is also substantial evidence that anxiety and depressive disorders share a genetic basis that presumably underlies the phenotypic comorbidity patterns (Hettema 2008a; Kendler et al. 2003).

3 Controversy in Classification of Individual Anxiety Disorders

3.1 Agoraphobia Past and Present, in the United States and Elsewhere

Of all the anxiety disorders, agoraphobia's nosologic status is probably the most controversial. Westphal 1872 coined the term "agoraphobia" (fear of the

marketplace – open city squares) to describe a syndrome he encountered in his neurological/psychiatric practice. His patients had great difficulty crossing squares without extreme anxiety, though they also had difficulty in other situations – e.g., being alone on empty streets; using public transportation; being in situations like the theatre, concerts, or crowded rooms; or being in lectures and large meetings (Kuch and Swinson 1992). Westphal noted that the condition was somewhat inexplicable, though he recognized it as a condition of abnormal anxiety in particular situations with associated avoidance and resultant problems in everyday life (i.e., what we have, for many years, conceptualized as a phobia). The name "agoraphobia" has never been entirely satisfactory, as the feared situations clearly extend beyond "the marketplace"; Le Grand du Saulle 1878 preferred the term "fear of spaces" (Stone 2002). As noted by Marks 1970, the syndrome has had many other labels, including "phobic-anxiety-depersonalization syndrome," "phobic anxiety state," "locomotor anxiety," "topophobia" (fear of particular places), "kenophobia" (fear of empty spaces), and "platzangst" (place anxiety), though none of these have outlasted "agoraphobia."

As mentioned previously, categories of phobia (including agoraphobia) were not specified in the DSM until DSM-III (see Table 1). Interest in phobias appears to have increased, at least in part, due to interest in the use of behavioral therapy (Marks 1970). Also, with the delineation of panic disorder, the DSM gradually began to construe agoraphobia as strongly related to panic, even as a consequence of panic. That is, though panic and autonomic anxiety symptoms, including "fear of fear" (fear of incapacitating anxiety in certain situations), had long been noted in patients with agoraphobia (Freud 1895/1962; Westphal 1872), panic gradually became the predominant organizing symptom in the DSM (Frances et al. 1993), based largely on Klein's cogent argument that agoraphobia often developed after (and presumably as a result of) spontaneous panic attacks (Klein 1980). This influence was present in DSM-III and has increased since then (Frances et al. 1993); that is, a theory of the cause of agoraphobia has been built into the DSM definition of agoraphobia, despite the intention of DSM-III to produce an atheoretical descriptive document, and despite even Klein's observation that situational phobias (e.g., claustrophobia) often precede a first panic attack (Klein 1980). Thus, DSM-III included "agoraphobia with panic attacks" and "agoraphobia without panic attacks" (simple/specific and social phobias have never included such panic presence/absence specifiers) and stated that an agoraphobic "has marked fear of and thus avoids being alone or in public places from which escape might be difficult or help not available in case of sudden incapacitation." Restated, agoraphobia was not just fear and avoidance of specific typical situations; fear of incapacitation was built in to the definition. DSM-III-R went further, including "panic disorder with (or without) agoraphobia," as well as "agoraphobia without history of panic disorder." In "panic disorder with agoraphobia," agoraphobia was defined as "fear of being in public places or situations from which escape might be difficult (or embarrassing) or in which help might not be available in the event of a panic attack"; practioners were instructed to include "cases in which persistent avoidance behavior originated during an active phase of Panic Disorder, even if the

person does not attribute the avoidance behavior to fear of having a panic attack." Even the definition of "agoraphobia without a history of panic disorder" implied that what was feared was not the situation itself but "suddenly developing a symptom(s) that could be incapacitating or extremely embarrassing." DSM-IV went further still in the theme of agoraphobia as "fear of fear," stating that agoraphobia is "anxiety about being in places or situations in which escape might be difficult (or embarrassing) or in which help may not be available in the event of having an unexpected or situationally predisposed panic attack or panic-like symptoms." For these reasons, many modern psychiatric texts have no chapter on agoraphobia – agoraphobia is almost always discussed in chapters on panic disorder.

The DSM definition of agoraphobia stands in stark contrast to that of ICD-10. The ICD-10 definition embodies what is often referred to as the "European position," which considers agoraphobia a particularly severe phobia (Marks 1987, Fava et al. 2008), defined as a "fairly well-defined cluster of phobias embracing fears of leaving home, entering shops, crowds and public places, or travelling alone in trains, buses or planes" (WHO 1992). Thus, the focus in ICD-10 is on the fear and avoidance of the situations themselves, not on the fear of anxiety/panic in those situations. ICD-10 acknowledges the frequent accompaniment of panic, depressive, and obsessional symptoms but does not imply primacy of panic the way DSM does. ICD-10 was undoubtedly influenced by Marks 1987 and others arguments that agoraphobia should be a stand-alone diagnosis, often related to but not inextricably bound to panic. In support of this position, typical agoraphobic (situational) fears have long been noted to cluster together within individuals, separate from other types of fears (Arrindell et al. 2003, Marks 1987), and panic itself does not appear necessary for this natural clustering (Cox et al. 2003).

So, one may wonder, which definition, that in DSM or that in ICD-10, is "correct"? We submit that neither is correct in any absolute sense, since our diagnoses reflect concepts and conventions, not necessarily nature (though approximating nature as much as possible is presumably ideal). Nevertheless, it is reasonable to ask what evidence suggests panic is *causally* related to agoraphobia, as implied in DSM. In reviewing this evidence, it is important to note that definitional issues such as those outlined previously are crucial to understand and usually confusing, in part because these definitions have been a moving target. With this caveat in mind, we note several facts thoughtfully reviewed by Craske 1996 for the consideration of the DSM-IV anxiety disorders committee. [Note that, though the DSM-IV anxiety disorders committee appears to have given some consideration to the European position, they do not appear to have seriously called into question the DSM-III assumption that fear of incapacitation should define agoraphobia. We add additional context to the points reviewed by Craske]:

- Most DSM-III agoraphobics who presented to anxiety disorders clinics in 1980s American research studies did endorse panic attacks or other symptoms that could be incapacitating or embarrassing in those situations. [Since DSM-III

defined agoraphobia as fear of incapacitation, this should not be particularly surprising.]

- Early post-DSM-III epidemiologic studies, which used to measure more of an imperfect ICD-10 (fear/avoidance of situations) than DSM construct of agoraphobia, found that, though most persons with agoraphobia did not meet DSM-III panic disorder criteria, some of these respondents may have had fear of fear. [Note that this was not measured directly – i.e., the fact that respondents had autonomic anxiety symptoms, including panic attacks, in the phobic situation does not mean that respondents feared these symptoms themselves; such symptoms also occur in patients with other phobias when confronted with relevant stimuli. Also, the clinical reappraisal study by Horwath et al. 1993, which suggested epidemiologic "agoraphobia without panic" often reflected misclassification, used the DSM-III definition of agoraphobia.]
- In studies of patients with DSM panic and agoraphobia, patients often reported (retrospectively) that panic attacks preceded or began simultaneously with DSM agoraphobia. [Notably, such patients sometimes retrospectively report that agoraphobic symptoms preceded their first panic attack (e.g., Argyle and Roth 1989; Fava et al. 1988; Fava et al. 1992; Lelliott et al. 1989, Perugi et al. 1998). In addition, it is interesting that many patients with panic and agoraphobia had their first panic attack in a typical agoraphobic situation (Faravelli et al. 1992; Lelliott et al. 1989; Perugi et al. 1998)]. Craske noted that prospective longitudinal studies were nonexistent at the time of her review.
- In patients with DSM agoraphobia (usually panic disorder and agoraphobia), expectation of panic in particular situations (though not frequency, severity, or expected/cued nature of panic) predicted the degree of avoidance. Craske noted that this finding does not prove that panic is causally related to avoidance.

The authors of DSM-IV decided to continue with the "fear of fear" concept in defining agoraphobia, despite concerns raised by Craske and the definitional concerns we raise here, and despite the fact that this would conflict with the ICD-10 definition (Frances et al. 1993). Nevertheless, the authors of DSM-IV noted that the boundary between agoraphobia and situational (specific) phobia is indistinct (Craske et al. 1996). The specific feared situations overlap, and the age of onset is similar in these two putatively distinct groups.

More recent studies in community samples add credence to the notion that agoraphobia should not be defined as fear of panic (Fava et al. 2008; Hayward and Wilson 2008):

- Wittchen et al. (1998) found that many young Germans had agoraphobic syndromes (with multiple situational fears) but no history of fearful spells. These participants would meet the ICD-10 criteria for agoraphobia, though some would presumably not meet DSM criteria (e.g., persons who avoided traveling for fear of getting lost).
- Hayward et al. (2003) found that only 20% of adolescents with agoraphobic fears and avoidance had ever experienced a panic attack.

- Bienvenu et al. (2006) found that persons who had agoraphobic symptoms but no history of spontaneous fearful spells were at increased risk for a later onset of panic disorder during a 13-year follow-up period; i.e., in these persons, the DSM-implied one-way causal direction between panic and agoraphobia was backwards.

Though it is not clear how agoraphobia "should" be defined, we recognize that the DSM-IV definition has certain advantages. Specifically, compared to the definition in DSM-IV, the ICD-10 definition is relatively vague, even though it appears empirically valid. That is, in the DSM-IV definition, what is meant to be feared in typical agoraphobic situations is clear (i.e., embarrassing or incapacitating symptoms). Nevertheless, researchers, in particular, should be aware of this unresolved controversy and the lack of a clear demarcation between situational (specific) phobias and agoraphobia. In addition, researchers, in particular, should not assume that they "know" what persons with agoraphobic syndromes fear; conceptualizations do not obviate the need for a clear phenomenologic understanding.

3.2 Posttraumatic Phenomena

Though the diagnosis PTSD first appeared in DSM-III (see Table 1), the diagnosis "traumatic neurosis" (a.k.a. "shell shock," "combat fatigue," and "war neurosis") had been used for many years. Interestingly, DSM-I included a vaguely similar construct, "gross stress reaction," though this diagnosis was not carried forward into DSM-II (Andreasen 2004).

In DSM-III, PTSD was defined as a syndrome that occurred following a psychologically traumatic event that "would evoke significant symptoms of distress in almost everyone." The text noted that such events were "generally outside the range of human experience" (to differentiate this construct from common stressful experiences like simple bereavement, chronic illness, business losses, or marital conflict). In fact, this language was added to criterion A in DSM-III-R, along with examples of potential traumatic stressors (serious threats of death and injury, etc.). However, the field soon realized that many deleterious traumatic events were not outside the range of usual human experience – perhaps especially in the United States (Breslau et al. 1991; Wittchen et al. 2009), so that concept was dropped in DSM-IV. DSM-IV also expanded the definition of a potential PTSD-related trauma, including, for example, being diagnosed with a life-threatening illness. In addition, it was no longer necessary to experience the event first-hand. Thus, what was initially conceptualized as a relatively violent, immediate kind of stressor gradually changed to something substantially less well delineated. This "criterion creep" has been particularly controversial (Andreasen 2004; Breslau and Kessler 2001; McHugh and Treisman 2007; Rosen et al. 2008; Wittchen et al. 2009). Another controversial aspect of this diagnosis is that many within, and, particularly, outside the field have understood PTSD as being *caused* by the stressor in too simplistic a fashion; i.e., without consideration of

additional risk factors such as personality traits and family or personal history of psychopathology (McHugh and Treisman 2007; Rosen et al. 2008).

As noted above, the PTSD construct may be too broad and overinclusive. However, it is also arguable that the diagnostic criteria are under inclusive. For example, some studies have noted that the use of a strict PTSD definition fails to identify many persons with PTSD symptoms who have clinically significant distress and/or impairment (Marshall et al. 2001; Stein et al. 1997), though not all studies agree on the extent of impairment in subthreshold cases (Breslau et al. 2004). Notably, the DSM requirement that PTSD symptoms be spread across the three diagnostic clusters has little empirical support (Stein and Bienvenu 2008). Thus, it makes sense to consider alternate and, perhaps, easier-to-remember definitions that consider external construct validators like functional impairment (Norman et al. 2007).

The DSM-III definition of PTSD did not include a duration criterion, so patients could be diagnosed with the condition immediately after the trauma. However, depending on the nature of the trauma, many persons would be expected to have substantial symptoms immediately afterward. Thus, in DSM-III-R, a criterion was added specifying that the symptoms had to be present for at least 1 month. However, this left patients with substantial immediate PTSD symptoms and related distress and/or impairment without a diagnosis. In DSM-IV, a new diagnosis, acute stress disorder (ASD) was introduced. ASD, by definition, can only occur within 4 weeks of the trauma and can only last 4 weeks. This diagnosis emphasizes dissociative symptoms much more than PTSD does. The rationale for this emphasis was that several studies found that peritraumatic dissociative symptoms predicted later PTSD symptoms more than reexperiencing, avoidance/numbing, and hyperarousal symptoms did (Classen et al. 1998; Ehlers et al. 1998; Murray et al. 2002). Other studies, however, have not found that dissociative symptoms have unique prognostic significance (Brewin et al. 1999, 2003; Bryant et al. 2008; Harvey and Bryant 1999). In addition, many persons with PTSD apparently never met criteria for ASD, and it seems reasonable to argue that the presence of acute dissociative symptoms is simply one of several risk factors for PTSD (Marshall et al. 1999). Thus, it is unclear whether the addition of the ASD diagnosis has been particularly useful.

3.3 Generalized Anxiety Disorder

Chronic free-floating anxiety was noted by Freud 1895/1962 in his description of anxiety neurosis, and it was clear, by DSM-III, that not all patients from that category were well-characterized by the new diagnosis panic disorder. Since the introduction of GAD in DSM-III, nosologists have attempted to reliably and validly define it. Over time, the GAD construct has narrowed and may be more reliable, though many researchers have cast doubt on the validity of the narrower definitions. As noted by Hoehn-Saric et al. 2007, GAD does not appear to exist in nature as the sharply delineated condition implied in successive versions of the DSM.

In DSM-III, the duration criterion for GAD was 1 month. Similarly, in ICD-10, generalized anxiety must be present "most days for at least several weeks at a time." However, DSM-III-R (and DSM-IV) lengthened the duration requirement to 6 months, with little empirical basis (Breslau and Davis 1985). A number of community studies have used internal and external construct validators to examine whether or not the DSM duration criteria identify a unique group of individuals. These validators have included sociodemographic factors, age of onset, course, impairment, distress, severity, comorbidity, family history, and treatment for anxiety. In summary, there is little evidence that the 6-month criterion is valid in terms of describing a unique group of generalized anxiety sufferers (Angst et al. 2006; Bienvenu et al. 1998; Breslau and Davis 1985; Kessler et al. 2005a; Lee et al. 2008); shorter-duration syndromes (e.g., 1 month) appear quite similar. Importantly, generalized anxiety syndromes lasting 1 month appear as heritable as those lasting 6 months; this is inconsistent with the idea that shorter-duration generalized anxiety syndromes are purely environmentally-mediated transient stress reactions (Kendler et al. 1992a).

Since DSM-III-R, "excessive" worry "about two or more life circumstances" has been required for a GAD diagnosis, though this criterion was not present in DSM-III and is absent in ICD-10. As with the duration criterion, the nature-of-worry criteria have been examined using internal and external construct validators. Bienvenu et al. 1998 found that the DSM-III-R nature of worry criteria did not identify generally anxious persons with a particular demographic or comorbidity profile. Ruscio et al. 2005 examined the "excessive worry" criterion with regard to a broader range of construct validators and in a larger sample. The "excessiveness" criterion did identify persons with earlier-onset, more severe, and more comorbid generalized anxiety syndromes, though persons with substantial non"excessive" worry also had relatively high impairment, treatment-seeking, and comorbidity compared to persons without substantial generalized anxiety. To our knowledge, the other DSM-IV nature-of-worry criterion, "difficult to control," has not been examined using these methods.

Another DSM-IV decision is worthy of consideration here. In DSM-III and DSM-III-R, autonomic anxiety symptoms were counted as associated symptoms of GAD, and they remain so in ICD-10. However, in DSM-IV, autonomic symptoms were eliminated as associated symptoms of GAD. The rationale for excluding autonomic symptoms was that these were less frequent than hyperarousal symptoms in GAD patients in anxiety disorder specialty clinics (reviewed and examined in Marten et al. 1993). To our knowledge, this decision did not take into account the possibility that generally anxious patients presenting in primary care settings may have predominant physical symptoms, including autonomic symptoms (Rickels and Rynn 2001). To our knowledge, this issue remains unexplored.

A final controversy concerns whether GAD should be grouped with depressive disorders instead of the anxiety disorders, based on the comorbidity patterns mentioned previously (note that PTSD is also particularly highly comorbid with this group), as well as apparently overlapping genetic causes (e.g., Kendler 1996; Kendler et al. 1992b, 2006; Roy et al. 1995). Hettema (2008b) recently reviewed

the relevant literature focusing on multiple external validators of a GAD-major depression relationship, including genetics, childhood environment, demographics, personality traits and disorders, life events, biology, comorbidity, and pharmacology. He concluded that the evidence supported a close relationship between these conditions, but that this relationship was not particularly specific (i.e., other anxiety disorders are also strongly related to major depression).

3.4 OCD and Putatively Related Conditions

The most controversial issue in the nosology of OCD seems to be whether or not OCD should remain classified as an anxiety disorder, and whether putative OCD-related conditions should be classified as such in DSM-V (Hollander et al. 2008; Regier 2007). Notably, ICD-10 includes OCD in the "neurotic, stress-related, and somatoform disorders" but does not list OCD as an "anxiety disorder" (the same is true for PTSD, which is listed in the "reaction to severe stress and adjustment disorders" subsection). Additional arguments for removing OCD and grouping it with putatively related conditions include relatively distinctive phenomenology and neurocircuitry, though none of the arguments are free from controversy (Mataix-Cols et al. 2007; Stein 2008; Storch et al. 2008), and it is not clear that the remaining conditions classified as anxiety disorders are themselves homogenous in most respects. Notably, though some putative OCD-related conditions (e.g., body dysmorphic disorder) are highly comorbid in persons with OCD and appear to run in families of patients with OCD (e.g., Bienvenu et al. 2000), anxiety disorders like GAD are also common in persons with OCD and their family members (Nestadt et al. 2001).

Another controversial issue is whether compulsive hoarding (CH) should be classified within or outside of OCD. Though it has been common practice to refer to CH as an OCD symptom, some have argued that CH is a relatively discrete entity that occurs often, but not always, in the context of other OCD symptoms (Frost and Gross 1993; Steketee and Frost 2003; Wu and Watson 2005). In addition, neuropsychological and neuroimaging findings suggest some differences between those with CH and those with only other OCD symptoms (Saxena 2008). This area of research deserves further attention.

4 Summary

Anxiety disorders treatment and research depends on the adequate conceptualization and measurement of these conditions. We reviewed the history of the nosology of anxiety disorders and noted that divisions of "neurosis" have inadvertently taken attention away from what is shared among conditions now classified separately. We noted the changes in the definition of agoraphobia over time and the striking differences between DSM-IV and ICD-10 definitions. We mentioned ongoing

controversies in the diagnoses PTSD, ASD, and GAD. Finally, we discussed controversies surrounding the proper placement of OCD and putatively related conditions in future diagnostic classifications. We hope reviewing controversial aspects of diagnosis is useful to clinicians and researchers interested in the neurobiology of anxiety disorders.

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Epidemiology of Anxiety Disorders

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Abstract This chapter presents an overview of the descriptive epidemiology of anxiety disorders based on recently completed surveys of the general population. The overall prevalence of anxiety disorders is shown to be quite high, but with considerable variation from the most prevalent (specific phobias) to the least prevalent (agoraphobia without a history of panic disorder) disorders. Age-of-onset (AOO) of anxiety disorders is typically in childhood or adolescence and the course is often chronic-recurrent. Anxiety disorders are highly comorbid with each other and with other mental disorders. Because of their early AOO, they are often the temporally primary disorders in comorbid profiles, raising the question whether early interventions to treat anxiety disorders might have a positive effect on the onset, persistence, or severity of secondary disorders such as mood and substance use disorders. This possibility has not yet been extensively explored but warrants further study given the high societal costs of anxiety disorders.

Keywords Epidemiology · Anxiety disorders · Agoraphobia · Generalized anxiety disorder · Obsessive-compulsive disorder · Separation anxiety disorder · Comorbidity · Societal costs

1 Introduction

This chapter presents an overview of the descriptive epidemiology of anxiety disorders. We focus largely on evidence from general population epidemiological surveys about the prevalence, age-of-onset, course, comorbidity, and severity of mental disorders, although some findings are also reported from clinical epidemiological studies. In light of the fact that DSM criteria have been used much more widely than ICD criteria in epidemiological studies, most results involve disorders described according to the definitions and criteria of the DSM system, although a few important differences with ICD disorders are also highlighted.

The majority of contemporary epidemiological studies on the prevalence and correlates of mental disorders are based on a single diagnostic instrument, the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI) (Robins et al. 1988). The CIDI is a fully structured interview; that is, it is based on questions that for the most part have yes/no responses, making it possible for a trained lay interviewer to administer the interview. The initial version of the CIDI, which was developed in 1989, was based on extensive field trials carried out in close to two dozen countries throughout the world (Wittchen 1994). Results of CIDI surveys carried out over the next decade led to the expansion and refinement of the CIDI and to the creation of the WHO World Mental Health (WMH) Survey Initiative, an ongoing program aimed at carrying out and comparing the results of parallel CIDI surveys in many countries throughout the world (www.hcp.med.harvard.edu/wmh). Much of the research reviewed in this chapter comes from the WMH surveys (Kessler and Üstün 2008).

As the CIDI can be administered by lay interviewers, it is noteworthy that clinical reappraisal studies have been carried out to compare diagnoses based on the CIDI with diagnoses made in clinician-administered follow-up interviews. Although the results of the clinical reappraisal studies of the original CIDI were mixed (Brugha et al. 2001; Wittchen et al. 1995), concordance of CIDI-based diagnoses with diagnostic decisions based on independent clinician-administered interviews is generally quite good for the more recent version of the CIDI developed for the WMH surveys (Haro et al. 2006). However, no rigorous clinical reappraisal studies have yet been carried out in developing countries, where prevalence estimates in CIDI surveys have sometimes been implausibly low (Gureje et al. 2006). As a result, data on the epidemiology of anxiety disorders in less developed countries need to be interpreted with caution.

Another important consideration of data quality concerns analysis of lifetime prevalence and age-of-onset distributions in CIDI surveys. The term lifetime prevalence in the context of cross-sectional studies refers to the frequency at which respondents acknowledged having met the full criteria for a given disorder at some point in their life. In general population samples with a large age span that means that the period of risk to have or have had the disorder varies from one person to the other, depending on current age at the time of examination. In the case of early onset disorders, such as for many anxiety disorders, considerable recall bias may occur and the precision of the retrospective age of onset of information might lack precision. Most community epidemiological surveys find that lifetime anxiety disorder prevalence estimates are lower among older than among younger respondents (Kessler et al. 2007b). This pattern could be due to forgetting a disorder that was a problem long ago, earlier mortality of people with anxiety disorders than others in the population (Bruce et al. 1994), a genuine increase in the prevalence of anxiety disorders in recent cohorts, methodological factors of the diagnostic assessment instrument or a combination of both methodological and substantive processes. Although no definitive data exist to distinguish among these possibilities, it is noteworthy that they all imply that the lifetime prevalence estimates of anxiety disorders in community surveys in higher age ranges should be considered lower bounds on the true prevalence in recent cohorts.

2 Lifetime Prevalence and Course

2.1 Prevalence

A number of recent literature reviews have presented detailed summary tables of prevalence estimates for individual anxiety disorders across many epidemiological surveys (Fehm et al. 2005; Goodwin et al. 2005; Lépine 2002; Lieb 2005; Lieb et al. 2005; Wittchen and Jacobi 2005). We discuss the main patterns found in these reviews in this section of the chapter. Interested readers are referred to the reviews for more detailed estimates.

A clear pattern in the lifetime prevalence data is that anxiety disorders as a group are always found to be the most commonly occurring class of mental disorders. Specific phobia is consistently estimated to be the most common lifetime anxiety disorder, with prevalence estimates usually in the 6–12% range. In the interpretation of anxiety disorders overall and specific phobia in particular one should always consider what types of specific phobias have been covered in the survey. Social phobia is typically found to be the next most common anxiety disorder, with lifetime prevalence sometimes as high as 10%. There is considerable cross-national variation, though, with a higher prevalence of social phobia in North America than in Western Europe and a high prevalence accompanied by a distinctive symptom profile (fear of embarrassing other people rather than of embarrassing oneself) in Asia, (so-called taijin kyofusho, a culture-bound syndrome of social phobia). PTSD is the third commonly occurring anxiety disorder, although here again prevalence estimates vary quite widely across countries. This is due to the wide variation in exposure to traumatic events, especially events involving interpersonal violence (more common in the US than Western Europe, and more common yet in countries that have experienced sectarian violence), but also events involving accidental death or injury (more common in developed countries where automobile accidents are common). Because of these differences in exposure, lifetime prevalence of PTSD is estimated to be only 1–2% in Western Europe, 6–9% in North America, and over 10% in countries that have been exposed to long-term sectarian violence. The other anxiety disorders have much lower prevalence. Agoraphobia without a history of panic disorder is usually estimated to be about 2%, similar to obsessive-compulsive disorder (OCD; 2–3%) and childhood separation anxiety disorder (SAD; 2–3%). More variability exists for panic disorder (2–5%), and generalized anxiety disorder (GAD; 3–5%). As discussed below, some of these prevalence estimates could change as a result of changes in diagnostic conventions in future classificatory systems.

2.2 Age-of-onset

Although fewer published data exist on retrospectively reported AOO distributions than on prevalence of anxiety disorders, a review of this literature shows several noteworthy AOO patterns (Kessler et al. 2007a). First, anxiety disorders typically begin much earlier than other commonly occurring classes of mental disorders, including mood disorders, disruptive behavior disorders, nonaffective psychoses, and substance use disorders. Tic disorders and most specific phobia are always found to have a modal AOO in childhood, with the vast majority of lifetime cases having onsets by the age of 18. Social phobia and OCD are always found to have a modal AOO in adolescence or early adulthood, with the vast majority of cases beginning by their twenties. Panic disorder, agoraphobia, and GAD are always found to have later modal and more widely dispersed AOO distributions, with median AOO in the early-mid twenties and an interquartile range of up to two

decades. PTSD, finally, is generally found to have the latest and most variable AOO distribution, presumably reflecting the fact that trauma exposure can occur at any time in the course of life. Somewhat earlier AOO estimates are generally found in studies of younger age cohorts and prospective-longitudinal studies than in cross-sectional studies where AOO is assessed retrospectively (Wittchen et al. 1999; Beesdo et al. 2007; Schreier et al. 2008). Yet it is noteworthy that the overall pattern of the earlier versus the later onset of anxiety disorders is not different.

This early onset, coupled with the fact that significant associations exist between early-onset anxiety disorders and subsequently the first onset of other mental (Beesdo et al. 2007) and substance use disorders (e.g., Zimmermann et al. 2003), has led some commentators to suggest that aggressive treatment of child-adolescent anxiety disorders might be effective in preventing the onset of the secondary disorders that are found in the vast majority of people with serious mental illness (Wittchen et al. 2000a, b; Kendall and Kessler 2002). It is noteworthy in this regard that despite their generally early AOO, first treatment of anxiety disorders usually does not occur until adulthood, often more than a decade after the onset of the disorder (Christiana et al. 2000).

Reasons for the delay of treatment may be quite diverse and remain understudied. They range from system barriers, such as financial constraints and uncertainty over where to go for help (Olfson et al. 2000), to nonrecognition in primary care as the most frequent gatekeeper for treatment Wittchen et al. (2000a, b), to illness related factors, such as in the case of social phobia, where feelings of being ashamed or embarrassed because of their mental disorder were found to play a role (Patel et al. 2002).

2.3 Persistence

Progression of illness has received far less attention in epidemiological studies of anxiety disorders than either prevalence or AOO. However, estimates of recent prevalence (variously reported for the year, 6 months, or 1 month before interview) are often reported in parallel with estimates of lifetime prevalence, and indirect information about the persistence of the disorder can be obtained by computing recent-to-lifetime prevalence ratios. The 12-month to lifetime prevalence ratios for anxiety disorders are typically in the range 4–6, with the highest ratios usually found for specific phobia and the lowest for GAD (e.g., Kringlen et al. 2001). Ratios as high as these strongly imply that anxiety disorders are quite persistent throughout the life course. More detailed analyses of these ratios could be carried out by breaking them down separately for subsamples defined by age at the interview or by the time since the first onset, but we are unaware of any published research that has reported such analyses. Our own preliminary analyses of this sort in the WMH data suggest, as one might expect, that the 12-month to lifetime prevalence ratios decline with increasing age. The more striking result, though, is that this decline is fairly modest, suggesting that anxiety disorders are often persistent throughout the

entire life course. The few long-term longitudinal studies that exist in representative samples of people with anxiety disorders show that this persistence is usually due to a recurrent-intermittent course that often features waxing and waning of episodes of different comorbid anxiety disorders (Wittchen et al. 2000a, b; Bruce et al. 2005).

3 Special Issues Concerning Particular Anxiety Disorders

Uncertainties exist about diagnostic boundaries for a number of the anxiety disorders. Because of this, prevalence estimates could change considerably with future versions of the DSM or ICD systems depending on the revisions made to diagnostic criteria. This section of the chapter presents a brief overview of several anxiety disorders that are the focus of questions about appropriate diagnostic criteria: GAD, OCD, and SAD.

3.1 Agoraphobia Without a History of Panic Disorder

Agoraphobia is considered by many experts (especially in the US) to be a response to panic (Klein and Gorman 1987), which means that agoraphobia without panic disorder would only occur when the agoraphobia was caused by a fear of recurrence of panic-like symptoms rather than by a fear of recurrence of panic attacks. DSM-III-R was revised to embody this perspective, requiring fear of either panic attacks or panic-like symptoms as the precipitating factor for a diagnosis of agoraphobia. The ICD system, in contrast and in line with the traditional perspective that views agoraphobia as one of the core phobic disorders, along with social and specific phobias (Wittchen et al. 2008), allows for the possibility that agoraphobic fears and avoidance is caused by a broader range of fears, such as of open spaces, public transportation or about being trapped or about being safe when outside the home. Many experts outside the US subscribe to this broader view of agoraphobia. Consistent with the ICD perspective, community epidemiological surveys consistently find that agoraphobia without a history of prior panic attacks or panic-like symptoms is as common as, if not more common than, agoraphobia with a history of prior panic (Andrews and Slade 2002; Wittchen et al. 1998b).

Critics of this broader view argue that the people classified as having agoraphobia without a history of panic actually have a specific phobia or SAD rather than agoraphobia. However, investigation of this issue using a version of the CIDI designed to probe this diagnosis in depth found that the number of respondents who genuinely had agoraphobia without a history of panic was quite large (3.5% of the sample) even after clinical review of cases (Wittchen et al. 1998b). This estimate stands in sharp contrast to the 0.2% lifetime prevalence of agoraphobia without panic disorder reported in a very large, recent national survey of the US that operationalized the DSM-IV criteria rigorously (Grant et al. 2006). Whether the high prevalence of agoraphobia in the absence of prior panic-like symptoms holds

up in future epidemiological investigations is a matter of considerable importance, given the apparent severity of the syndrome in surveys where it has been rigorously evaluated (Andrews and Slade 2002; Wittchen et al. 1998b), but the number of such surveys are far too few to draw firm conclusions. This is a topic that is planned for analysis in the WMH surveys, which contained a special series of questions developed specifically to examine this issue.

3.2 Generalized Anxiety Disorder

Prevalence estimates of GAD have varied widely in community epidemiological surveys over the years due to the fact that the criteria for a diagnosis of GAD have changed dramatically in the various editions of the DSM: Most notable changes were the requirement that the anxious worrying as the core symptom had to persist for only 1 month or longer in the DSM-III but 6 months or longer in the DSM-III-R and DSM-IV and the considerable change of the mandatory associated psychic and somatic GAD symptoms. In an attempt to reduce the artificial overlap with other anxiety disorders, the latter were confined to a few "hypervigilance" items in DSM-IV instead of a longer list of fairly broad anxiety symptoms in DSM-III. Further complications have arisen from the fact that ICD-10 retained the longer list of symptoms of DSM-III and does not require the worry to be "excessive" to qualify for a diagnosis as DSM-IV does. Because of such variations in definition, lifetime prevalence estimates of GAD in recent epidemiological surveys have varied widely, from as low as 1% to as high as 66% (Kessler et al. 2005a) when the criteria are even further relaxed. Several large CIDI studies have examined whether episodes of GAD with durations less than 6 months might be less impairing than episodes lasting 6 months or longer (Ruscio et al. 2007). Shorter episodes (i.e., 1–5 months) were found to be nearly as impairing as longer episodes and quite similar in other characteristics such as comorbidity, course of the illness, and family history. Other CIDI research investigated the excessiveness requirement and found that, although GAD with excessive worry begins earlier in life and has a more severe and persistent course than GAD without excessive worry, the latter nonetheless is associated with substantial persistence and impairment compared to respondents without GAD (Ruscio et al. 2005). These results seem to argue for broadening the diagnostic criteria of GAD. If this broad definition is adopted, GAD will be one of the most common anxiety disorders.

3.3 Obsessive-Compulsive Disorder

As noted above, epidemiological surveys consistently find that OCD is one of the least prevalent anxiety disorders, with lifetime prevalence estimates consistently less than 3% (Ruscio et al. 2008). There is considerable interest, though, in the possibility

that a number of other disorders are part of an OCD spectrum that might be far more prevalent than OCD itself, including tic disorders, body dysmorphic disorder, trichotillomania and related self-harm disorders, and possibly even hypochondriasis (Goldsmith et al. 1998). The argument for the existence of this hypothesized spectrum is based on similarities across the different disorders in a subjective sense of compulsion, in difficulty inhibiting repetitive behaviors, in the age of the onset and the course of illness, in patterns of comorbidity, in family history, and in specificity of the response to the treatment (Neziroglu et al. 2006). Although some controversy exists about the notion of an OCD spectrum and the range of conditions that fall within this spectrum (Bienvenu et al. 2000; Richter et al. 2003), some experts have proposed that spectrum disorders should be reclassified in the ICD and DSM systems as subtypes of OCD (Yaryura-Tobias et al. 2000). Needless to say, if this happens, the estimated prevalence of OCD could increase substantially. As far as we know, no large-scale community epidemiological research yet exists on the prevalence of OCD spectrum disorders. Even if such data existed, they would not resolve the question whether OCD would still be appropriately grouped within the anxiety disorders.

3.4 Separation Anxiety Disorder

SAD is described in DSM-IV as a childhood disorder that seldom persists into adulthood. However, empirical studies in clinical samples suggest that adult SAD is more common than suggested by DSM-IV and that a substantial subset of those with the disorder have their first onsets in adulthood (Diener and Kim 2004). The WMH surveys are the only community epidemiological surveys of which we are aware that assessed adult SAD. Only US data have been reported so far (Shear et al. 2006). Lifetime prevalence estimates of childhood and adult SAD in this survey were 4.1% and 6.6%, respectively. Approximately one-third (36.1%) of respondents classified as childhood cases were found to persist into adulthood, while the majority (77.5%) of adult respondents with SAD at the time of interview reported first onsets in adulthood. In interpreting these results, it needs to be noted that adult SAD was found to be highly comorbid with other mental disorders, underscoring the need for further exploration of the boundaries between syndromal SAD, separation anxiety as an adjustment reaction, and normal response to the loss of an attachment figure in order to arrive at a clear and principled set of criteria for adult SAD in future editions of DSM and ICD.

4 Comorbidity Among the Anxiety Disorders

Comorbidity among anxiety disorders is quite common, with up to half of the people with a lifetime anxiety disorder in some surveys meeting criteria for two or more such disorders (Kessler 1995). Furthermore, there is some evidence that anxiety disorders are more highly comorbid than other mental disorders both with

each other and with other mental and physical disorders (Toft et al. 2005). Factor analytic studies of common mental disorders consistently document separate internalizing and externalizing factors in which anxiety and mood disorders have high factor loadings on the internalizing dimension while most disruptive behavior disorders and substance use disorders have high factor loadings on the externalizing dimension (Kendler et al. 2003). In some studies, the internalizing disorders divide further into secondary dimensions that distinguish between fear disorders (panic, phobia) and distress disorders (depression, dysthymia, GAD) (Watson 2005) The locations of OCD and PTSD in this two-dimensional space are less clear because of not being studied as extensively as the other disorders, with the former appearing to be more related to the fear dimension (Watson 2005) and the latter more related to the distress dimension (Cox et al. 2002), although neither is strongly indicated by either of these dimensions. Social phobia additionally appears to be somewhat more strongly related to the distress dimension than are the other phobias. SAD has not been included in these factor analytic studies to date.

These results have recently been used by Watson (2005) to call into question the codification of anxiety disorders as a distinct class of disorders in the DSM and ICD systems and to suggest that a more useful organizing scheme in the upcoming DSM-V and ICD-11 revisions would be one that distinguished between fear disorders and distress disorders, with the latter including not only GAD and possibly PTSD but also unipolar depression and dysthymia. The argument for a class of fear disorders has the stronger support of the two in neurobiological research based on extensive investigation of fear brain circuitry (Knight et al. 2005). The possibility also exists that future research might lead to OCD being classified apart from both fear and distress disorders as part of a spectrum of impulse-control disorders based on evidence of differential comorbidity and differences in brain circuitry (Whiteside et al. 2004).

Studies of multivariate disorder profiles confirm the complexity of the comorbidity that exists among anxiety disorders. The most comprehensive of these analyses was carried out in the US National Comorbidity Survey Replication (Kessler and Merikangas 2004) by examining the multivariate profiles among 19 separate DSM-IV disorders (Kessler et al. 2005b). Of the 524,288 (2^{19}) logically possible multivariate disorder profiles among these disorders, 433 were observed. Nearly 80% of these 433 involved highly comorbid cases (three or more disorders), accounting for 27.0% of all respondents with a disorder and 55.9% of all instances of these disorders.

Importantly, the distribution of comorbidity in this analysis was found to be significantly different from the distribution one would expect to find if the multivariate structure among the disorders was due entirely to the two-way associations that are the focus of factor analysis, suggesting that the more typical factor analytic studies of comorbidity fail to detect an important structure. Based on this result, latent class analysis (LCA) was used to study nonadditive comorbid profiles. A seven-class LCA model provided the best fit to the data, with four classes featuring anxiety disorders prominently.

5 The Societal Costs of Anxiety Disorders

We noted earlier in the chapter, but did not emphasize, that early-onset anxiety disorders are powerful predictors of the subsequent onset and persistence of other mental and substance use disorders. It is important to note that these predictive associations are part of a larger pattern of associations that have been documented between anxiety disorders and a much wider array of adverse life course outcomes that might be conceptualized as societal costs of these disorders, including reduced educational attainment, early marriage, marital instability, and low occupational and financial status (Lépine 2002). A considerable amount of research has been carried out to quantify the magnitude of the short-term societal costs of anxiety disorders in terms of healthcare expenditures, impaired functioning, and reduced longevity (Marciniak et al. 2004). The magnitude of the cost estimates in these studies is staggering. For example, Greenberg et al. (1999) estimated that the annual total societal costs of active anxiety disorders in the US over the decade of the 1990s exceeded $42 billion. This estimate excludes the indirect costs of early-onset anxiety disorders through adverse life course outcomes (e.g., the documented effects of child–adolescent anxiety disorders in predicting low educational attainment and consequent long-term effects on lower income) and through increased risk of other disorders (e.g., anxiety disorders predicting the subsequent onset of cardiovascular disorder). Similar evidence have recently become available from Europe (Andlin-Sobocki et al. 2005; Andlin-Sobocki and Wittchen 2005)

Based on results such as these, there is growing interest in evaluating the possibility that early and aggressive outreach and best-practices treatment of early-onset anxiety disorders might be cost-effective from a societal perspective in reducing a wide range of other adverse outcomes in the domains of health and social functioning (Kendall and Kessler 2002). There is also interest in the possibility that screening in the workplace and treatment of some anxiety disorders might have a positive return on investment (ROI) for employers by virtue of both increasing performance in the workplace and reducing healthcare costs associated with other disorders that are either partially caused or exacerbated by anxiety disorders. Large-scale workplace experiments have been carried out that document a substantially positive ROI of screening for, detecting, and providing best-practices treatment to depressed workers (Wang et al. 2007, 2008). Parallel workplace experiments are needed for anxiety disorders.

6 Conclusion

The results summarized here document that anxiety disorders commonly occur in the general population, often have an early age-of-onset, and are characterized by frequent comorbidity with each other as well as with other mental disorders. We

reviewed evidence to suggest that the current DSM and ICD definitions of anxiety disorders might substantially underestimate the proportion of the population with a clinically significant anxiety condition. It is noteworthy that research on comorbidity among anxiety disorders generally ignores the existence of anxiety spectrum conditions, a failing that should be rectified in future research.

Based on these results, along with results regarding the societal costs of anxiety disorders, we can safely conclude that anxiety disorders are common and consequential problems that are deeply interwoven with a wide range of other physical, mental, and broader personal difficulties in the general population. As early-onset conditions, anxiety disorders typically begin prior to the vast majority of the other problems with which they are subsequently associated. Yet, as noted earlier in the chapter, young people with early-onset anxiety disorders seldom receive treatment. This situation needs to be examined to determine if early intervention would help address the enormous public health burden created by anxiety disorders throughout the world. To do this will require a level of political will that has heretofore been lacking in even the most progressive countries in the world. One can but hope that future research focused on the long-term costs of illness and the impact of early effective treatment will correct this situation by documenting the cost-effectiveness of intervening as early as possible to detect and treat people suffering from these highly prevalent and impairing disorders.

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Comorbidity in Anxiety Disorders

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Abstract Ever since Feinstein coined the term "comorbidity", referring to the presence of any additional coexisting ailment in a patient with a particular index disease (J Chronic Dis 23:455–468, 1970), aspects of the phenomenon have been extensively studied. The aims of this chapter are: (1) to summarize the evidence of psychiatric comorbidity in anxiety disorders from adult population-based studies; (2) to present findings from the National Comorbidity Survey Replication (NCS-R); (3) to summarize evidence of psychiatric comorbidity in anxiety disorders from child and adolescent population-based samples; (4) to provide a summary of evidence on comorbidity from family and genetic studies; and (5) to examine patterns of comorbidity between anxiety disorders and medical conditions. Throughout each of these aims, implications of the comorbidity are explored, including whether these patterns reflect a need for redefining the disorders or rather an etiologic or even causal path.

Keywords Comorbidity · Anxiety disorders · NCS-R · Epidemiology · Family studies · Medical conditions

Abbreviations

NCS-R	National Comorbidity Survey Replication
NCS	National Comorbidity Survey
ESEMeD	European Study of Epidemiology of Mental Disorders
NESARC	National Epidemiologic Survey of Alcohol Related Conditions
NEMESIS	Netherlands Mental Health Survey and Incidence Study
OHS	Ontario Health Survey
ECA	Epidemiological Catchment Area
ZCS	Zurich Cohort Study
WMHI	World Mental Health Initiative
PTSD	Post-traumatic stress disorder
GAD	Generalized anxiety disorder
OCD	Obsessive compulsive disorder
ADHD	Attention-deficit/hyperactivity disorder
TBI	Traumatic brain injury

1 Introduction

Ever since Feinstein coined the term "comorbidity", referring to the presence of any additional coexisting ailment in a patient with a particular index disease (Feinstein 1970), aspects of the phenomenon have been extensively studied. A high magnitude of comorbidity between psychiatric disorders has been reported in large-scale

community surveys, prompting further research on the explanations and implications. Generally, failure to classify and analyze comorbid diseases can create misleading medical statistics and may cause spurious comparisons during the planning and evaluation of treatment for patients. Comorbidity can alter the clinical course of patients with the same diagnosis by affecting the time of detection, prognostic anticipations, therapeutic selection, and post-therapeutic outcome of an index diagnosis (Kaplan and Feinstein 1974). In addition, it can also affect the length of hospital stay, response to somatic treatment, and mortality (Buist-Bouwman et al. 2005).

Investigation of patterns of comorbidity is important for several reasons. First, identification of differential patterns of comorbidity may lead to the identification of subtypes of a particular index disorder for which the comorbid condition may indicate a different form or subtype, thereby enhancing the validity of their distinction in the classification system. Second, differential associations between particular pairs of diseases may yield clues regarding the pathogenesis of the index disease. If two conditions emanate from the same underlying etiologic factors, investigations of their etiology should focus on risk factors that are common to both conditions. Third, if the comorbid disorder is a consequence of the index disease, interventions can be developed to prevent the development of the secondary condition. Fourth, if comorbidity results in part from the lack of valid boundaries between discrete categories of disorders or the imposition of arbitrary thresholds on the components of disorders that are dimensional, future versions of the diagnostic nomenclature should consider reformulating these categories. In addition to these explanations, however, nonrandom co-occurrence of two conditions can be a methodological artifact in clinical settings, a bias commonly referred to as Berkson's Paradox (Berkson 1946); to avoid such biases, this chapter will focus primarily on population-based studies.

The aims of this chapter are: (1) to summarize the evidence of psychiatric comorbidity in anxiety disorders from adult population-based studies; (2) to present findings from the National Comorbidity Survey Replication (NCS-R; $n = 9{,}282$); (3) to summarize evidence of psychiatric comorbidity in anxiety disorders from child and adolescent population-based samples; (4) to provide a summary of evidence on comorbidity from family and genetic studies; and (5) to examine patterns of comorbidity between anxiety disorders and medical conditions.

2 Psychiatric Disorders Comorbidity in Population-Based Studies of Adults

Evidence from community samples reveals that anxiety disorders often co-occur with other psychiatric disorders, with the vast majority of anxiety disorder cases having at least one comorbid psychiatric disorder either concomitantly or across the lifetime (Kessler et al. 2005). This section will summarize evidence from recent population surveys on concomitant and lifetime comorbidity between anxiety

disorder and two major classes of disorders, mood and substance use. Because of the lack of consistency in the presentation of findings across surveys, we will provide summaries from recent research that addresses this issue. We will then present new data on patterns of comorbidity of anxiety disorders from the NCS-R ($n = 9{,}282$) in each section.

2.1 Concomitant Comorbidity with Mood Disorders

The concomitant association between anxiety and mood disorder has been documented extensively in numerous international population surveys (Wittchen et al. 1994; Kessler et al. 1996; Chen and Dilsaver 1995; Boyd et al. 1984). While the magnitude of the relationship varies between different samples, such trends are found across a multitude of studies. One of the most recent summaries of anxiety and mood disorder comorbidity presents data from the European Study of Epidemiology of Mental Disorders (ESEMeD; $n = 21{,}425$). Alonso et al. (2004) reported a strong degree of concurrence between major depression and specific anxiety disorders, with odds ratios (OR) ranging from 6.2 to 33.7 for agoraphobia, specific phobia, social phobia, generalized anxiety disorder (GAD), panic disorder, and post-traumatic stress disorder (PTSD). The National Epidemiologic Survey of Alcohol Related Conditions (NESARC; $n = 43{,}093$) was one of the few studies that investigated different subtypes of bipolar disorders. When examining bipolar I and bipolar II separately, bipolar I had a stronger relationship with GAD while bipolar II had a stronger relationship with specific phobia (Stinson et al. 2007; Grant et al. 2005b).

As seen in Table 1, results from the NCS-R confirm a relationship between 12-month major depression, dysthymia, bipolar disorder, and any mood disorder with all of the anxiety disorders including panic disorder, agoraphobia, specific phobia, social phobia, GAD, and PTSD. The OR range from 2.29 to 12.29 when adjusting for age, sex, race/ethnicity, education, marital status, and region. The strongest association between mood and anxiety disorders occurs for panic disorder, but the associations with social phobia and PTSD were consistently significantly elevated as well. Dysthymia was the mood disorder subtype that was most strongly comorbid with 12-month anxiety disorders.

2.2 Lifetime Comorbidity with Mood Disorders

Lifetime comorbidity between anxiety and mood disorders is even greater than that between the 12-month prevalence rates. Both the NESARC (Stinson et al. 2007) and the Netherlands Mental Health Survey and Incidence Study (NEMESIS; $n = 7{,}076$) (Depla et al. 2008) show a strong association between specific phobias and mood disorders (OR range 1.5–9.2), with an even stronger association between GAD and mood disorders (OR range 5.0–14.1) (Grant et al. 2005b).

Table 1 12-Month Comorbidity between Anxiety and Mood Disorders in the NCS-R

	Depression OR (95% CI)	Dysthymia OR (95% CI)	Bipolar OR (95% CI)	Any Mood Disorder OR (95% CI)
Panic Disorder	7.98 (5.63–11.32)	10.19 (6.75–15.38)	9.78 (6.65–14.40)	8.26 (5.85–11.67)
Agoraphobia	4.67 (2.62–8.31)	4.90 (1.98–12.14)	4.93 (2.23–10.90)	5.45 (2.91–10.20)
Specific Phobia	4.23 (3.35–5.35)	5.87 (4.01–8.59)	5.02 (3.77–6.68)	4.26 (3.44–5.27)
Social Phobia	5.89 (4.84–7.17)	8.22 (5.90–11.43)	6.26 (4.58–8.56)	5.88 (4.91–7.03)
Generalized Anxiety Disorder	5.74 (3.99–8.25)	2.29 (1.37–3.82)	5.29 (3.16–8.86)	6.26 (4.38–8.94)
Post-Traumatic Stress Disorder	6.54 (4.78–8.95)	8.12 (5.85–11.27)	6.28 (4.09–9.65)	6.28 (4.62–8.53)
Any Phobia	5.19 (4.35–6.19)	7.98 (5.66–11.25)	5.18 (3.92–6.84)	5.03 (4.23–5.99)
Any Anxiety Disorder	7.63 (6.48–8.98)	12.29 (8.92–16.93)	7.64 (5.54–10.52)	7.28 (6.23–8.52)

OR = odds ratio
CI = confidence interval
Controlling for age, sex, race/ethnicity, education, marital status, and region

With respect to specific mood disorder subtypes, major depression is more strongly associated with anxiety disorders than dysthymia. For example, in a review of the comorbidity of social phobia in population-based studies, Merikangas and Angst (1995) reported OR ranging from 2.2 to 18.1 for major depression, (Wittchen and Essau 1989; Schneier et al. 1992; Canino et al. 1987; Angst 1993; Davidson et al. 1993; Wacker et al. 1992) while dysthymia and social phobia had significant but smaller in magnitude OR ranging from 1.1 to 4.9 (Wittchen and Essau 1989; Schneier et al. 1992; Canino et al. 1987; Angst 1993; Wacker et al. 1992). In the first National Comorbidity Survey (NCS; $n = 8{,}098$), Kessler et al. (1999) showed the magnitude of these associations increased with the number of social fears.

Table 2 includes the adjusted OR between mood and anxiety disorders for lifetime occurrence in the NCS-R. The magnitudes of the associations are slightly lower than those for 12-month disorders, but nearly all were significantly elevated. Similar to the 12-month disorders, dysthymia was the mood disorder subtype with the greatest overall magnitude of comorbidity with anxiety disorders.

2.3 Concomitant Comorbidity with Substance Use Disorders

As with mood disorders, there is evidence that substance use disorders are highly comorbid with anxiety disorders. Further, there is a trend for dependence to have somewhat higher magnitudes of comorbidity than abuse, although not all studies

Table 2 Lifetime Comorbidity between Anxiety and Mood Disorders in the NCS-R

	Depression OR (95% CI)	Dysthymia OR (95% CI)	Bipolar OR (95% CI)	Any Mood Disorder OR (95% CI)
Panic Disorder	4.00 (3.14–5.10)	5.95 (4.39–8.08)	5.59 (4.18–7.47)	4.28 (3.37–5.43)
Agoraphobia	4.14 (2.60–6.58)	3.82 (2.04–7.13)	4.76 (2.68–8.47)	4.99 (3.03–8.24)
Specific Phobia	3.30 (2.92–3.74)	4.35 (3.44–5.50)	4.01 (3.15–5.10)	3.45 (2.95–4.05)
Social Phobia	4.55 (3.90–5.32)	6.01 (4.90–7.36)	4.54 (3.53–5.83)	4.78 (4.09–5.58)
Generalized Anxiety Disorder	5.08 (3.87–6.66)	3.34 (1.96–5.70)	3.95 (2.82–5.53)	5.83 (4.60–7.38)
Post-Traumatic Stress Disorder	5.14 (3.86–6.83)	6.37 (4.57–8.87)	4.79 (3.36–6.82)	5.46 (4.00–7.45)
Any Phobia	4.11 (3.63–4.67)	5.86 (4.52–7.60)	4.25 (3.30–5.47)	4.23 (3.69–4.85)
Any Anxiety Disorder	6.19 (5.34–7.17)	9.86 (7.27–13.39)	6.35 (4.60–8.76)	5.94 (5.03–7.00)

OR = odds ratio
CI = confidence interval
Controlling for age, sex, race/ethnicity, education, marital status, and region

find significant comorbidity for abuse. The trend that severity of the substance use is a mitigating factor in co-occurrence is seen in the NESARC, where specific phobia, social phobia, GAD, and panic disorder are all significantly related to alcohol dependence, but not alcohol abuse (Grant et al. 2004). Other studies have reported a significant relationship between alcohol abuse and specific disorders, including the ESEMeD which found agoraphobia, specific phobia, social phobia, GAD, and panic disorder to all be related to alcohol abuse; however, this sample again reveals an even stronger relationship between these disorders and alcohol dependence (Alonso et al. 2004). In the NESARC, both drug abuse and dependence were associated with specific phobia, social phobia, and GAD, with higher OR seen for dependence (Grant et al. 2004). There is some evidence that women with substance use disorders have different patterns of comorbidity than men, with some population-based studies reporting more comorbid alcohol dependence for women with PTSD, panic disorder, or phobias than men (Helzer et al. 1991).

Table 3 shows the OR for 12-month co-occurrence of specific anxiety and substance use disorders in data from the NCS-R. Similar to the findings of prior studies, the magnitude of comorbidity between substance dependence was far greater than that of substance abuse. Alcohol abuse was associated only with panic and PTSD, and none of the anxiety disorders except GAD were significantly associated with drug abuse. Interesting differences emerged for patterns of comorbidity with anxiety disorder subtypes with alcohol compared to drug dependence. Whereas alcohol dependence was strongly associated with all of the anxiety disorders except GAD, drug dependence comorbidity was most pronounced for phobic states, particularly social and specific phobias. By contrast, panic, agoraphobia, and GAD were not significantly associated with drug dependence.

Table 3 12-Month Comorbidity between Anxiety and Substance Use Disorders in the NCS-R

	Alcohol Abuse OR (95% CI)	Alcohol Dependence OR (95% CI)	Any Alcohol Use Disorder OR (95% CI)	Drug Abuse OR (95% CI)	Drug Dependence OR (95% CI)	Any Drug Use Disorder OR (95% CI)	Any Substance Use Disorder OR (95% CI)
Panic Disorder	3.56 (1.61–7.91)	4.03 (1.93–8.42)	4.09 (2.18–7.66)	1.20 (0.25–5.82)	4.12 (0.80–21.10)	2.34 (0.64–8.55)	3.31 (1.77–6.19)
Agoraphobia	NA	3.98 (1.41–11.24)	1.51 (0.54–4.23)	NA	3.47 (0.72–16.81)	1.12 (0.25–5.04)	1.23 (0.44–3.44)
Specific Phobia	1.30 (0.73–2.33)	2.96 (1.52–5.77)	2.08 (1.35–3.21)	0.88 (0.27–2.90)	4.82 (1.85–12.53)	2.05 (1.04–4.03)	1.97 (1.30–2.97)
Social Phobia	1.64 (0.94–2.86)	4.09 (2.23–7.50)	2.79 (1.82–4.27)	1.28 (0.48–3.42)	8.56 (3.99–18.38)	3.16 (1.60–6.28)	2.95 (1.99–4.37)
Generalized Anxiety Disorder	1.77 (0.62–5.04)	2.81 (0.99–8.01)	2.33 (1.08–5.06)	3.45 (1.12–10.68)	3.12 (0.85–11.43)	3.45 (1.40–8.46)	2.78 (1.39–5.53)
Post-Traumatic Stress Disorder	3.28 (1.48–7.26)	5.68 (3.39–9.53)	4.71 (3.12–7.14)	1.04 (0.27–4.01)	4.74 (1.85–12.15)	2.43 (1.20–4.91)	4.20 (2.78–6.36)
Any Phobia	1.32 (0.82–2.10)	4.06 (2.49–6.63)	2.44 (1.73–3.43)	1.10 (0.46–2.59)	6.94 (3.57–13.47)	2.45 (1.39–4.29)	2.53 (1.82–3.50)
Any Anxiety Disorder	1.75 (1.19–2.58)	5.31 (3.22–8.75)	3.11 (2.37–4.09)	1.77 (0.81–3.86)	9.51 (4.49–20.13)	3.29 (1.98–5.46)	3.15 (2.39–4.15)

OR = odds ratio

CI = confidence interval

Controlling for age, sex, race/ethnicity, education, marital status, and region

2.4 Lifetime Comorbidity with Substance Use Disorders

Patterns of lifetime comorbidity between substance use and anxiety disorders have also been studied in multiple population surveys (Grant et al. 2005b; Merikangas et al. 1998; Swendsen et al. 1998). There are important differences in patterns of comorbidity between specific subtypes of anxiety and substance use disorders. Analyzing data from both the NCS and the Ontario Health Survey (OHS; $n = 8{,}116$), Sareen et al. (2006) found that even illicit drug use (not necessarily abuse or dependence) was associated with anxiety disorders, with the strongest association being between panic disorder and hallucinogen use. In a review of four geographically-diverse population studies, Swendsen et al. (1998) found that alcohol use disorders were associated with "any phobia" and "any anxiety disorder" with at least two of the samples providing evidence for comorbidity between alcohol use disorders and agoraphobia, specific phobia, social phobia, and panic disorder alone. In another review of six population-based surveys, Merikangas et al. (1998) found anxiety disorders to be significantly comorbid with alcohol dependence in five of the populations, including two American, one German, one Dutch, and one Canadian sample but not in the Mexican sample; all six samples yield significant relationships between drug dependence and anxiety disorders.

Table 4 depicts the lifetime association between anxiety and substance use disorders in the NCS-R. In general, the lifetime associations were somewhat lower than those for concomitant comorbidity. Substance dependence was more strongly associated with anxiety disorders than was substance abuse, confirming the results of previous studies.

2.5 Comorbidity with Other Psychiatric Disorders

When focusing only on community-based samples, there is mixed evidence on comorbidity between anxiety disorders and disorders not in the mood or substance use classes. For example, the 12-month comorbidity OR of impulse disorders with anxiety disorders in the NCS-R is 4.59 (95% CI: 3.81–5.53). In a review of the relationship between anxiety and eating disorders, Swinbourne and Touyz (2007) cite several clinical and epidemiological studies supporting a relationship, but the findings are "strikingly inconsistent" in terms of the details. There is some evidence of personality and anxiety disorders overlapping in the NCS (Goodwin and Hamilton 2003) and NESARC (Grant et al. 2005a), but the large epidemiologic surveys conducted in the United States have tended to only include antisocial personality disorder and thus most studies in this area of research tend to have either clinical or small samples. Generally, the most robust relationships are those detailed earlier with mood and substance use disorders.

Table 4 Lifetime Comorbidity between Anxiety and Substance Use Disorders in the NCS-R

	Alcohol Abuse OR (95% CI)	Alcohol Dependence OR (95% CI)	Any Alcohol Use Disorder OR (95% CI)	Drug Abuse OR (95% CI)	Drug Dependence OR (95% CI)	Any Drug Use Disorder OR (95% CI)	Any Substance Use Disorder OR (95% CI)
Panic Disorder	1.87 (1.35–2.59)	3.66 (2.34–5.72)	3.03 (2.14–4.29)	2.04 (1.29–3.21)	3.21 (2.04–5.05)	2.77 (1.89–4.07)	2.80 (1.98–3.95)
Agoraphobia	1.46 (0.88–2.42)	3.17 (1.50–6.72)	2.41 (1.25–4.66)	1.50 (0.65–3.47)	3.29 (1.75–6.20)	2.37 (1.35–4.17)	2.63 (1.44–4.79)
Specific Phobia	1.67 (1.28–2.17)	3.15 (2.39–4.15)	2.52 (2.04–3.12)	1.39 (0.99–1.93)	3.42 (2.41–4.87)	2.25 (1.69–2.99)	2.47 (1.95–3.11)
Social Phobia	1.63 (1.34–1.98)	3.51 (2.80–4.41)	2.66 (2.24–3.16)	1.72 (1.29–2.31)	4.44 (3.27–6.03)	2.91 (2.37–3.57)	2.68 (2.28–3.16)
Generalized Anxiety Disorder	1.72 (1.25–2.35)	2.32 (1.59–3.38)	2.19 (1.72–2.79)	2.19 (1.56–3.06)	1.87 (1.09–3.21)	2.21 (1.57–3.12)	2.18 (1.73–2.75)
Post-Traumatic Stress Disorder	1.96 (1.34–2.85)	4.67 (3.40–6.42)	3.53 (2.76–4.53)	1.68 (1.14–2.50)	6.86 (4.40–10.71)	3.85 (2.96–5.01)	3.61 (2.92–4.47)
Any Phobia	1.66 (1.41–1.94)	3.97 (3.20–4.93)	2.78 (2.39–3.24)	1.59 (1.18–2.15)	4.75 (3.63–6.22)	2.73 (2.18–3.43)	2.78 (2.37–3.28)
Any Anxiety Disorder	1.91 (1.50–2.44)	4.86 (3.72–6.36)	3.24 (2.65–3.95)	2.02 (1.53–2.67)	5.42 (3.99–7.36)	3.20 (2.59–3.95)	3.28 (2.69–4.00)

OR = odds ratio

CI = confidence interval

Controlling for age, sex, race/ethnicity, education, marital status, and region

2.6 Findings from Prospective Studies

To better understand patterns of comorbidity, it is important to study people prospectively. Studies of adolescents and young adults are ideal for investigating the order of onset because this age range captures the typical onset of these psychiatric disorders; however, a few large adult studies have provided some evidence to this front as well. In research published from the Epidemiological Catchment Area (ECA), alcohol and drug abuse were found to be strong predictors of the onset of panic disorder and agoraphobia 12 months later, suggesting an etiologic or possibly causal path (Eaton and Keyl 1990; Keyl and Eaton 1990). However, a forthcoming paper utilizing the 10-year follow-up to a subsample of the NCS suggests the opposite direction of prediction, with PTSD, separation anxiety, and specific and social phobia all being predictive of the development of at least one form of substance dependence 10 years later (Swendsen et al. 2009). Assuming a causal model and not a shared etiologic path, it is estimated from this study that upwards of 4.5, 10.3, and 3.2% of nicotine, alcohol, and illicit drug dependence, respectively, may be reduced by early treatment or prevention of anxiety disorders. Looking instead at depression and anxiety patterns over time, in the Zurich Cohort Study (ZCS; $n = 591$), analyses of five assessment points over a 15-year period showed that individuals with anxiety states tended to develop either depression alone or a comorbid depression with their anxiety disorder, while subjects beginning with depression tended to have a more stable diagnosis (Merikangas et al. 2003a).

3 Psychiatric Disorders Comorbidity in Population-Based Studies of Children and Adolescents

As with adults, for children and adolescents "comorbidity is the rule rather than the exception" (Gotlib and Hammen 1992). In general, parallel patterns can be found between comorbidity in earlier life and comorbidity in adulthood. In fact, there is some suggestion that the comorbidity seen between anxiety disorders and other classes of disorders is more common in adolescents than in adults (Rohde et al. 1996; Lewinsohn et al. 1993).

3.1 Lifetime Comorbidity of Childhood and Adult Anxiety Disorders

Although most adults with a major anxiety disorder have a history of one of the childhood anxiety disorders, including separation anxiety, overanxious disorder, or phobic disorder, there does not seem to be much specificity in the extent to which

childhood anxiety disorders predict the development of specific anxiety disorders in adulthood. In an epidemiologic sample followed for 9 years bridging adolescence to young adulthood, Pine et al. (1998) found some specificity for specific and social phobia predicting adulthood disorders but less specificity for other anxiety disorders. Aschenbrand et al. (2003) found that separation anxiety in childhood was not specifically associated with agoraphobia.

3.2 Comorbidity with Mood Disorders

Parallel to the adult studies, mood disorders comprise the class of psychiatric disorders that are most frequently associated with anxiety disorders in youth. In a review of eight American community samples of children and adolescents, Angold and Costello (1993) reported approximately 30–75% of youth with depression had a concomitant anxiety disorder. Most major subtypes of anxiety disorder seem to be related to major depression, including panic, separation anxiety, overanxious disorders, social phobia, and specific phobia but not obsessive compulsive disorder (OCD) (Lewinsohn et al. 1997). There may also be a link between bipolar disorders and anxiety, with Geller and colleagues reporting rates of anxiety in clinical samples of youth with bipolar disorders at approximately 12–33% (Geller et al. 1995).

Importantly, studying comorbidity in prospective studies of children and adolescents provides information on order of onset since retrospective data collected in adults may be less accurate and onset has typically occurred by young adulthood. A handful of longitudinal studies found that anxiety tends to precede the onset of depression, with the majority of persons with concomitant expression of depression and anxiety developing depression after the onset of anxiety (Pine et al. 1998; Rohde et al. 1991; Reinherz et al. 1989; Orvaschel et al. 1995). Onset of specific phobia, separation anxiety, overanxious disorder, and social phobia all more frequently precede that of major depression while panic disorder and OCD onset tend to follow that of major depression (Wittchen et al. 1999). In a review of such comorbidity, Brady and Kendall (1992) suggested that anxiety and depression may be part of a developmental sequence in which anxiety is expressed earlier in life. On the other hand, Hayward et al. (2000) proposed there may be more of a bilateral temporal association, at least between panic attacks and depression.

3.3 Comorbidity with Substance Use Disorders

Anxiety disorders are associated with an increase in the risk of development of substance use and disorders, although the converse may be true as well. The potential mechanisms through which anxiety may be associated with smoking in adolescents were examined by Patton et al. (1998) who found that anxiety and

depression were associated with smoking initiation through increased susceptibility to peer influences. In a 4-year prospective community survey of adolescents and young adults, Zimmermann et al. (2003) found that baseline social phobia and panic attacks predicted subsequent hazardous use of alcohol while panic attacks also predicted onset of alcohol abuse; further, baseline panic attacks and panic disorder were strongly associated with the persistence of an alcohol use disorder (respective OR 18.7 and 13.2). Conversely, some research suggests that substance use may trigger anxiety disorders in susceptible youth. In one prospective study of a community sample, PTSD seemed to be triggered by substance abuse in approximately half of the cases (Giaconia et al. 2000). Similarly, Johnson et al. (2000) found that adolescent smoking predicted adult onset of panic attacks, panic disorder, and agoraphobia. Combined with the results mentioned previously from adult prospective samples (Eaton and Keyl 1990; Keyl and Eaton 1990; Swendsen et al. 2009), there is some suggestion of these comorbid patterns being bi-causal. The association between substance use and anxiety disorders may have preventive implications although further research will need to focus on the mechanisms for links between specific disorders.

3.4 Comorbidity with Other Psychiatric Disorders

As with adults, the most robust associations between anxiety disorders and another class of psychiatric disorders in minors are for mood and substance use; still, there is some evidence of other disorders being comorbid as well. In a review of comorbidity seen with attention-deficit/hyperactivity disorder (ADHD), Biederman et al. (1991) report upwards of 25% of children with ADHD having a comorbid anxiety disorder. A more recent review done by Spencer (2006) supports this claim. These reviews include primarily clinical samples with only two community-based studies cited finding this relationship (Bird et al. 1988; Anderson et al. 1987). Comorbidity between conduct and anxiety disorders in youth has also been found in some studies, including a large population-based survey of British youth that found 23, 24, 16, and 16% of children with GAD, separation anxiety, specific phobia, and social phobia, respectively, to have a conduct disorder; most of this overlap was with oppositional defiant disorder (Green et al. 2005).

4 Genetic Epidemiologic Approaches to Comorbidity

While the population-based studies are the optimal approach for measuring comorbidity, these studies cannot directly address the reason for the co-occurrence beyond gaining support against chance and sampling bias: do the disorders stem from a shared biological or environmental etiological path, is the comorbid state a third and independent disorder, are these different phases or alternative expressions

of the same disorder or is one disorder a risk factor for the other? Such questions can be approached through family and twin studies by examining patterns of coaggregation and discriminating between causal and shared etiologic models.

A recent review of family and twin studies that investigated the familial relationship of anxiety and depression evaluated mechanisms of comorbidity based on predictions that discriminate between common etiologic versus independent explanations for comorbidity (Middeldorp et al. 2005). They conclude that anxiety and depression are distinct disorders but that their comorbidity results from partially shared genetic risk factors. The conclusions from the twin data differ from those from the family study data that were included in their review. Whereas the twin data suggest shared genetic factors underlying anxiety and depression, the family study data support independent familial etiology of these conditions.

With respect to specific subtypes of anxiety disorders, family study data supports the independence of anxiety and depression in general as well as of many of the specific subtypes thereof (Merikangas and Angst 1995; Low et al. 2008; Coelho et al. 2007; Merikangas 1990). When taken together, the results suggest that there is greater specificity of familial transmission of anxiety disorders than of major depression, and that the phobic states are transmitted independently from panic and GAD despite the high magnitude of comorbidity between them. There was also an increase in the combined GAD and a generalized subtype of social phobia among relatives of comorbid GAD/generalized subtype of social phobia probands, as well as increases in either disorder alone. Finally, family studies also show that OCD is highly familial after controlling for comorbid mood and anxiety disorders (Nestadt et al. 2003). In terms of common risk factors, there is some evidence that suggests that GAD, panic and major depression have some shared familial risk as well as independent risk factors that influence their expression (Merikangas and Angst 1995; Merikangas et al. 1998; Klein et al. 2003; Maier et al. 1995).

Despite the pervasive comorbidity between mood and anxiety disorders with substance use disorders, family study data converge in demonstrating that substance use disorders are transmitted independently in families and do not result from the same familial risk factors as mood or anxiety disorders (Merikangas et al. 1998; Maier et al. 1994).

5 Comorbidity with Physical Disorders

Beyond comorbidity with other mental disorders, several studies have suggested an association between anxiety disorders and physical disorders. These findings are especially relevant for two reasons: (1) such comorbidity affects our understanding of impairment and role disability previously attributed solely to the physical conditions (Merikangas et al. 2007b) and (2) such comorbidity may be indicative of an underlying biological cause for both anxiety and the comorbid disorder.

Broadly, anxiety disorders are comorbid with having any physical disorder. In the NCS, Sareen et al. (2005) found a significantly elevated rate of having one or more of the physical disorders measured in this survey for subjects who had anxiety disorders compared to those who did not (OR = 1.88). This replicates earlier findings of overall higher prevalence of medical illness in patients with anxiety disorders (Wise and Taylor 1990; Rogers et al. 1994). Katon and Roy-Byrne (1989) found that panic disorder specifically was more often seen in the medically ill. As the following paragraphs detail, some classes of physical disorders are more comorbid with either anxiety disorders generally or specific anxiety disorder diagnoses.

5.1 Respiratory Conditions

Respiratory diseases may be associated with particular anxiety disorders. At least two studies have found an association between specific phobia and respiratory diseases (Sareen et al. 2005; Goodwin et al. 2003b). Meanwhile, many studies find respiratory diseases to be comorbid with panic attacks (Goodwin et al. 2003a; Goodwin and Eaton 2003; Goodwin and Pine 2002; Ortega et al. 2004), although this association was not found by Sareen et al. (2005). An association between PTSD and respiratory diseases was found in a large Canadian community sample (n = 36,984; Sareen et al. 2007). Asthma specifically was associated with GAD, panic disorder, social phobia, specific phobia, and PTSD but not agoraphobia in the NCS (Kessler et al. 2003); several other adult and child community samples find asthma to be associated with anxiety disorders (Goodwin et al. 2003b; Goodwin and Eaton 2003; Goodwin and Pine 2002; Hasler et al. 2005; Ortega et al. 2002). Looking longitudinally rather than concomitantly, respiratory ill health at age three was a predictor of panic disorder and agoraphobia more than 15 years later in a cohort study in New Zealand (Craske et al. 2001).

5.2 Gastrointestinal Conditions

Gastrointestinal conditions seem to be associated with anxiety. Some studies looking at anxiety broadly have seen significant comorbidity with gastrointestinal diseases (Sareen et al. 2005; Harter et al. 2003). More specifically, the study by Harter et al. (2003) showed a strong association between panic/GAD and gastrointestinal conditions (OR = 3.1). GAD has been linked to specific gastrointestinal conditions as well, including peptic ulcer disease (Goodwin and Stein 2002) and irritable bowel syndrome (Lydiard 2001). PTSD has also been linked to gastrointestinal illnesses (Sareen et al. 2007). In the NCS, Kessler et al. (2003) found ulcers to be associated with GAD, panic disorder, agoraphobia, social phobia, specific phobia, and PTSD.

5.3 Allergies and Atopic Disorders

The link between childhood allergies and eczema and behavioral inhibition was discussed by Kagan (1997), who proposed that the high levels of cortisol associated with anxiety may lead to immunologic sensitivity to environmental stimuli. Children with separation anxiety have increased rates of atopic disorders (Slattery et al. 2002). Kovalenko et al. (2001) found that the strongest association between allergy and psychiatric disorders was between allergy and panic disorder.

5.4 Head Injury

There is some evidence that anxiety symptom and anxiety disorder onset increases following a head injury. Luis and Mittenberg (2002) found increased rates of new onset anxiety disorders following brain injuries. Vasa et al. (2002) noted increases in anxiety symptoms following head injuries, with the most frequent symptoms being for OCD, separation anxiety, and specific phobia. More recently, comorbidity between PTSD and traumatic brain injury (TBI) has been noted in U.S. military personnel after serving in Iraq or Afghanistan (Stein and McAllister 2009). For example, one study of 2,200 veterans found that combat-incurred mild TBI doubled the risk of developing PTSD (Schneiderman et al. 2008). Since most of the recent research focuses on military samples it is less understood how this generalizes to other populations; one study of road traffic accident survivors found no significant difference in PTSD rates when comparing those with or without TBI 3 months after the accident (Jones et al. 2005). The circularity and paradoxes inherent in the diagnoses of TBI and PTSD further make it difficult to disentangle the causality and mechanisms of a measured association (Stein and McAllister 2009; Harvey et al. 2003; Bryant 2001; Sumpter and McMillan 2006).

5.5 Cardiovascular Conditions

There is substantial evidence supporting an association between cardiovascular diseases and anxiety disorders (Harter et al. 2003; Bonnet et al. 2005; Ballenger et al. 2001; Hansen 2003; Kubzansky et al. 2006). Cardiovascular disease is associated with anxiety symptoms (Kawachi et al. 1994; Coryell et al. 1986; Smoller et al. 2007), GAD (Goodwin et al. 2009; Barger and Sydeman 2005), panic disorder (Goodwin et al. 2009), PTSD (Sareen et al. 2007), and specific phobias (Goodwin et al. 2009). In terms of specific cardiovascular diseases, Haines et al. (2001) found an association between phobic anxiety and ischemic heart disease (particularly fatal ischemic events), Bovasso and Eaton (1999) found respiratory panic attacks were associated with subsequent risk of myocardial infarction, and Kubzansky et al. (2007) found coronary heart disease incidence

was associated with increased scores on a combat-related PTSD scale administered to a community sample of military men. In a prospective study of 3,369 postmenopausal women, Smoller et al. (2007) found that a history of panic attacks was an independent risk factor for both cardiovascular morbidity and mortality.

5.6 Migraine

One of the most robust associations between anxiety disorders and a physical disorder is with migraine (Ratcliffe et al. 2009; Merikangas et al. 1990; Breslau et al. 1991). In their study looking at comorbidity between various medical illnesses and anxiety, Harter et al. (2003) found migraine to have the strongest association overall. In a 26-year longitudinal study, Waldie and Poulton (2002) found migraine to be related to anxiety symptoms in childhood as well as anxiety disorders during adolescence. Although studies vary on whether they look at migraine specifically or a broader class of headache, associations have been found with GAD (Harter et al. 2003; Merikangas et al. 1990; Breslau et al. 1991; Kalaydjian and Merikangas 2008; Saunders et al. 2008), panic attacks or disorder (Ratcliffe et al. 2009; Merikangas et al. 1990; Breslau et al. 1991; Kalaydjian and Merikangas 2008; Saunders et al. 2008; Stewart et al. 1989; Swartz et al. 2000), PTSD (Saunders et al. 2008; Peterlin et al. 2008), any phobia (Merikangas et al. 1990; Breslau et al. 1991), agoraphobia (Ratcliffe et al. 2009), social phobia (Saunders et al. 2008), and specific phobia (Ratcliffe et al. 2009; Saunders et al. 2008).

5.7 Conclusion

It is well known that depression is highly comorbid with physical disorders (Moldin et al. 1993) and as detailed previously depression and anxiety are highly comorbid as well. Because of these known associations, it may be speculated that the relationship between anxiety and physical disorders is only through their joint relationships with depression. However, studies that specifically control for other mental disorders (depression or otherwise) tend to still find significant results. Even when controlling for depression, dysthymia, substance abuse, and substance dependence, Harter et al. (2003) found significant associations between anxiety disorders and cardiac disorders, hypertension, gastrointestinal problems, genitourinary disorders, and migraine. Utilizing compelling data from 17 countries in the World Mental Health Initiative (WMHI; $n = 42{,}249$), Scott et al. (2007) showed that depression alone, anxiety alone, and comorbid depression-anxiety all had significant comorbidity with many physical disorders. Specifically, anxiety without depression had significant OR for obesity, diabetes, asthma, hypertension, arthritis, ulcer, heart disease, back/neck problems, chronic headache, and multiple pains.

6 Future Directions and Conclusions

The adult and child epidemiologic studies reviewed in this chapter, along with the original results presented from the NCS-R, have highlighted the overlap between anxiety and other psychiatric disorders. While there is variation in the magnitude of the relationships estimated in each study, significant comorbidity with anxiety is consistently found with mood and substance use disorders. In addition, family and twin studies have shown that comorbidity between anxiety and mood disorders can be attributed in part to common underlying risk factors, whereas anxiety disorders and substance use disorders are transmitted independently in families, despite the high magnitude of comorbidity between them. Further, the observed comorbidity between anxiety disorders and various physical conditions provides leads for the underlying biological mechanisms and pathways that contribute to anxiety.

Comorbidity is certainly more than just the co-occurrence of disorders; it alters the clinical course of the anxiety disorder as well. For example, adolescents with both depression and anxiety have more severe anxiety symptoms (Strauss et al. 1988), more severe depressive symptoms (Kashani and Orvaschel 1990), more suicide attempts (Rohde et al. 1991), and impaired role function (Lewinsohn et al. 1998). Health economists would be interested in the societal costs: in the Health 2000 Study in Finland, 58% of individuals with comorbid depression and anxiety reported using health services versus only 28% with either disorder alone (Hamalainen et al. 2008). While the evidence presented in this chapter indicates notable psychiatric comorbidity with anxiety disorders, the family studies and medical comorbidity only begin to tease apart the mechanisms for these relationships.

This review of comorbidity of anxiety disorders with mood and substance use disorders suggests that there is now abundant evidence from cross-sectional population-based studies, and that future studies should focus more on identifying potential mechanisms for comorbidity through prospective research on specific subtypes of anxiety and other disorders. The growing evidence on comorbidity of physical disorders with anxiety disorders is a promising area for future research, particularly devoted to identifying common underlying biologic pathways. Finally, there is also a need for longer term prospective studies of comorbidity in children to identify common developmental factors that may influence the direction of disorders as they emerge in adulthood. Combining genetic epidemiologic approaches with population-based research will be particularly informative.

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Genetics

Katharina Domschke and Jürgen Deckert

Contents

Abstract The present state of knowledge on the genetics of anxiety disorders, in particular panic disorder, comprising clinical and molecular genetic studies, interaction analyses, as well as meta-analyses of single association studies will be presented in detail. A particular focus will be on the most robust findings in panic disorder to date in the serotonergic, noradrenergic, and dopaminergic system, such as the catechol-*O*-methyltransferase (COMT) gene. Additionally, findings on the adenosine receptor 2A (A2A) gene, which has been reported to be associated with panic disorder and also with anxiety levels after caffeine administration in a gene–environment interactional model, will be discussed. Furthermore, the first imaging genetic findings in panic disorder, social phobia, and anxiety-related traits using fMRI and PET techniques in combination with molecular genetic association

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analyses are reviewed, taking into account the present intermediate phenotype discussion in the investigation of complex genetic disorders. Finally, the first exemplary pharmacogenetic studies in panic disorder and generalized social phobia will be presented.

The pathomechanism of anxiety disorders and in particular panic disorder is considered to be multifactorial with converging evidence for a pivotal role of genetic factors in particular, which will be presented in detail in this chapter.

Keywords Clinical genetics · Molecular genetics · Linkage · Association · COMT · A2A · Imaging genetics endophenotype

1 Clinical Genetics

Clinical genetic methods comprise family studies, twin studies, adoption studies, and segregation studies and allow for an estimation of the contribution of genetic factors to the etiology of a particular disorder.

Family studies estimate the so-called "familiality" of a disease, which indicates the contribution of both genetic factors and environmental factors shared by a family. This is calculated from the prevalence of a particular disorder in first degree relatives of patients as compared to the prevalence in first degree relatives of a nonaffected control group. If the prevalence of the disease is higher in first degree relatives of patients, the disease is considered to be "familial." Panic disorder has been found to be highly familial with up to a 3-fold increased prevalence of the disorder in first degree relatives of patients with panic disorder (Maier et al. 1993). Also, generalized anxiety disorder and specific phobias were reported to have significant familial aggregation (Hettema et al. 2001).

Twin studies allow for an estimate of the actual contribution of genetic factors to the pathogenesis of the disorder ("heritability") by analyzing the concordance rate of a particular disorder in monozygotic versus dizygotic twins. In panic disorder, up to three times higher concordance rates were observed in monozygotic as compared to dizygotic twins. According to a comprehensive meta-analysis, the contribution of genetic factors has been calculated to be as high as up to 48%, with the remaining 52% being attributable to individual environmental factors. Generalized anxiety disorder has been estimated to have a heritability of about 32%, while the common heritability of phobias was reported to be about 30%, with highest estimates for agoraphobia (67%), blood-injection-phobia (59%), and social phobia (51%). The heritability of posttraumatic stress disorder was reported to be about 20–30% (Kendler et al. 1999; Hettema et al. 2001; Segman and Shalev 2003; see Table 1).

However, segregation analyses failed to identify a mode of inheritance according to Mendelian patterns, which points to a complex genetic inheritance with an interaction of multiple predisposing genes, each with only a minor individual influence, and environmental influences (Vieland et al. 1996).

Table 1 Heritability estimates for anxiety disorders

Diagnosis	Heritability (%)
Agoraphobia	61 (CI 24–63)
Blood-injection-phobia	59 (CI 43–78)
Social phobia	51 (CI 39–64)
Panic disorder	48 (CI 41–54)
Generalized anxiety disorder	32 (CI 24–39)
Posttraumatic stress disorder	30 (CI 28–32)

According to Kendler et al. (1999) and Hettema et al. (2001)

2 Molecular Genetics

Molecular genetic methods are helpful in dissecting complex genetic traits such as anxiety disorders, by allowing for the identification of particular single risk genes contributing to the overall genetic risk of the disease.

2.1 Linkage Studies

In linkage studies, coinheritance of particular genetic markers with the disease of interest is analyzed in affected families. If a marker significantly cosegregates with the disease, it is assumed that the region around this marker ("locus") contains genes conferring a disease risk. Linkage analysis methods can be applied to both monogenetic disorders (parametric linkage) and polygenic disorders such as panic disorder (model-free or nonparametric linkage). Evidence for linkage is most commonly expressed as a logarithm of the odds (LOD) score, which is a statistical measure based on the number of estimates of recombination frequency. A LOD score >3 meaning that the odds are a thousand to one in favor of genetic linkage is being considered "significant"; a LOD score >1.9, "suggestive" (Lander and Schork 1994). The advantage of linkage studies is that there is no need for an a priori hypothesis. However, detection sensitivity is rather low, particularly for genes with small effects in complex genetic disorders such as anxiety disorders.

In panic disorder, linkage studies have yielded a variety of potential risk loci on chromosomes 1p, 4q, 7p, 9q, 11p, 15q, and 20p, while in subgroups of patients with panic disorder comorbid with bipolar disorder or kidney/bladder dysfunction, respectively, risk loci on chromosomes 18 or 13 and 22, respectively, have been described (Crowe et al. 1987; Knowles et al. 1998; MacKinnon et al. 1998; Gelernter et al. 2001; Hamilton et al. 2003; Thorgeirsson et al. 2003; Fyer et al. 2006; Kaabi et al. 2006). In social or specific phobia, there was no evidence from linkage studies for a major influence of serotonin 2A (5-HT2A) receptor and serotonin transporter (5-HTT) loci, with however, potential risk loci on chromosomes 16q and 14p (Stein et al. 1998; Gelernter et al. 2003; Gelernter et al. 2004; and reviews by Arnold et al. 2004; Smoller et al. 2008a, b) (Fig. 1).

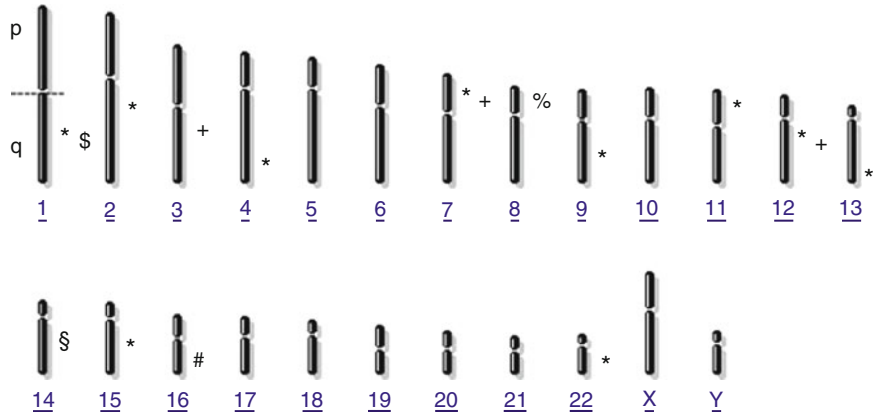

Fig. 1 Results of linkage studies of panic and phobic anxiety disorders. Linkage findings are indicated to the right of the respective chromosome: *asterisk* panic disorder/panic attacks/panic disorder syndrome; *plus* agoraphobia; *section symbol* simple phobia; *hash symbol* social phobia; *dollar* neuroticism; *percent* harm avoidance (based on review by Smoller et al. 2008a, b)

2.2 Cytogenetic Studies

In a subgroup of patients with panic disorder and joint laxity as well as mitral valve prolapse, a duplicated 17-Mb region on chromosome 15q24-26 (DUP25) containing about 60 genes has been reported to be highly significantly linked to the described phenotype (Gratacòs et al. 2001). The NTRK3 gene coding for the high-affinity receptor for neurotrophin 3 (NT-3) is located in this duplicated chromosomal region and has thus been considered as a candidate to participate in the pathophysiology of PD. A single nucleotide polymorphism in the 5' untranslated region (UTR) of the NTRK3 gene was found to be significantly associated with panic disorder (Armengol et al. 2002), which was interpreted as further support for a potential impact of DUP25 on the pathogenesis of panic disorder. However, no subsequent study so far was able to replicate the initial duplication finding in independent samples (Henrichsen et al. 2004; Zhu et al. 2004; Schumacher et al. 2003; Tabiner et al. 2003).

2.3 Association Studies/Interaction Analyses/Meta-Analyses

Association studies investigate the allelic frequency of a particular polymorphism in an a priori candidate gene in a patient sample as compared to a sample of healthy controls. If the allelic distribution of this polymorphism significantly differs between patients and controls, association of it or another closely located polymorphism ("linkage disequilibrium") can be assumed. The advantage of association studies is their high sensitivity to detect genetic variations with small overall

effects, which, however, renders association studies relatively prone to false positive results requiring replication in independent samples.

Most studies have investigated polymorphisms in classical candidate genes for panic disorder as suggested by animal models (e.g., knock-out mice), challenge experiments (e.g., cholecystokinin (CCK) challenge, caffeine challenge), or pharmacological observations (e.g., clinical efficacy of selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors). Association findings in at least two independent samples have been reported, e.g., for the cholezystokinin B (CCK-B) receptor gene (Kennedy et al. 1999; Hösing et al. 2004); the MAO-A gene, particularly in female patients (Deckert et al. 1999); the catechol-*O*-methyltransferase (COMT) gene, again particularly in female patients (Hamilton et al. 2002; Domschke et al. 2004); the serotonin receptor 1A (5-HT1A) gene (Rothe et al. 2004; Huang et al. 2004); the adenosine 2A receptor (A2A) gene (Deckert et al. 1998; Hamilton et al. 2004); and the RGS2 gene (Leygraf et al. 2006; Smoller et al. 2008a, b).

Interestingly, high risk genotypes of the serotonin receptor 1A and the COMT polymorphisms seem to interact in the modification of the risk for panic disorder conditional on the presence of the respective low-risk genotype of the second polymorphism. This might point to a ceiling effect at the molecular level; i.e., if one element of monoaminergic transmission is dysfunctional, dysfunction of other elements does not further increase the risk for panic disorder (Freitag et al. 2006).

In social phobia and generalized anxiety disorder, variants in the dopamine transporter (DAT1) have been proposed to contribute to the disease risk (Rowe et al. 1998), while dopamine D2 receptor (DRD2) variants have been reported to play a role in the pathogenesis of posttraumatic stress disorder (Segman and Shalev 2003; Kennedy et al. 2001). Also, the beta-1 adrenergic receptor has been found to explain some of the population variance in extraversion and related personality traits, which might be a relevant finding with respect to the study of disorders marked by low extraversion, namely social phobia and other phobic disorders (Stein et al. 2004).

However, since most positive association studies either did not withstand replication in independent samples or still warrant replication or even in case of positive replications also were followed by negative replications, these results have to be considered preliminary (see Domschke and Deckert 2007). Reasons for this relatively high inconsistency of association findings across different studies (nonreplications, "flip-flop phenomenon," where a previously reported disease-marker association is replicated but with the risk allele reversed from the previous report, which can occur when the investigated variant is correlated, through interactive effects or linkage disequilibrium, with a causal variant at another locus (Lin et al. 2007), etc.) might comprise low power of the individual studies to detect a small effect, linkage disequilibrium with a causal polymorphism at another locus, interactions with other relevant polymorphisms, etiological heterogeneity, or random error in the absence of a true effect. Besides replication studies in independent samples and interaction analyses, meta-analyses of genetic association studies have been performed in order to clarify the influence of gene variation on the pathogenesis of anxiety disorders and panic disorder in particular. Two meta-analyses using

original genotype data from six available case–control studies (see above) with respect to the role of the COMT val158met polymorphism in panic disorder provided tentative support for the COMT val158met polymorphism as a possible risk factor for panic disorder, with differential effects in Caucasian and Asian populations, and suggest a female-specific effect (Domschke et al. 2007; Zintzaras and Sakelaridis 2007). Another meta-analysis of genetic association studies in panic disorder published so far focuses on the functional serotonin transporter promoter polymorphism 5-HTTLPR. Analyzing 10 individual studies, no statistically significant association between 5-HTTLPR and panic disorder was found (Blaya et al. 2007), although a recent study reported association with another polymorphism in the gene (Strug et al. 2008). The latter study stressing the importance of haplotype studies may point to a solution of the paradox that the functional serotonin transporter promoter polymorphism 5-HTTLPR has repeatedly been found as predisposing to the anxiety-related personality dimension neuroticism (Lesch et al. 1996, for meta-analysis see Munafò et al. 2005).

2.4 Gene–Environment Interaction Analyses

As suggested by segregation studies, the etiology of anxiety disorders is to be considered to be complex with an interaction of multiple risk genes and environmental influences (Vieland et al. 1996). Thus, a further crucial step in the dissection of the etiology of anxiety disorders is the investigation of the interplay of genetic factors with environmental factors. In this respect, an A2A gene polymorphism, which has previously been found to be associated with panic disorder as a categorical diagnosis, was additionally reported to significantly influence anxiety levels after caffeine administration (Alsene et al. 2003). This initial report was supported by another study showing a significant association between caffeine-induced anxiety and the A2A polymorphism as well as DRD2 polymorphism (Childs et al. 2008). This finding was interpreted as a genetic interactive effect on anxiety paralleling the well known colocalization and functional interaction of adenosine and dopamine receptors on the cellular level. Interestingly, the A2A polymorphism mediated the effects of caffeine only at medium, but not at low or high caffeine doses (Childs et al. 2008) highlighting the context-dependency of minor genetic effects.

3 Analysis of Intermediate Phenotypes

To date, most association studies of particular genetic variants have been performed in samples of patients with the categorical diagnosis of anxiety disorders according to the diagnostic criteria of the International Classification of Diseases (ICD-10) or Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), respectively.

However, disease entities as described in these diagnostic manuals are composed of a variety of clinical characteristics regarding onset, severity, and duration of the illness with diverse and overlapping neuropsychological and probably also neurobiological features yielding a rather heterogenous nosological picture. As risk genes probably influence neurobiological or neuropsychological traits rather than categorical clinical phenotypes, association or linkage studies investigating the overall categorical disease phenotype in variable composition of the individual neurobiological or neuropsychological features might not have the power to detect single genetic risk markers. In order to target this major limitation of association studies, the concept of "endophenotypes" or "intermediate phenotypes" has been proposed. Endophenotypes comprise neurobiological or neuropsychological traits that are linked to the disease of interest and that are heritable, and however, that are dimensional, thus more precisely defined and considered to be closer to the underlying genotype than the overall disease phenotype (Gottesman and Gould 2003).

Smoller and Tsuang (1998) suggested behavioral inhibition (BI) to the unfamiliar, a heritable temperamental profile involving an avoidant response to novel situations, to serve as a promising endophenotype of anxiety and anxiety disorders. Association findings between variants in the corticotropin releasing hormone (CRH) gene and BI in children as well as among offsprings of parents with panic disorder (Smoller et al. 2003, 2005) provide support for an important role of abnormalities of the CRH system in the pathogenesis of intermediate traits predisposing to anxiety and panic disorder, in particular.

As another interesting endophenotype of mental disorders, neuronal activation correlates of emotional processing as captured by functional imaging techniques such as functional magnetic resonance imaging (fMRI) have been proposed (Hariri et al. 2002).

In panic disorder, two recent studies investigated neuronal activation patterns in response to emotional stimuli dependent on COMT and 5-HT1A polymorphisms in 20 panic patients. Particular attention was paid to amygdala activation, since amygdala activation is considered to be crucial for emotional perception and processing in the brain fear circuit (e.g., Sakai et al. 2005; Javanmard et al. 1999; Pfleiderer et al. 2007). In response to fearful faces versus background, increased activation of the right amygdala and the right gyrus fusiformis was observed in patients with panic disorder carrying at least one COMT 158val allele (Domschke et al. 2008). These pilot data suggest that the functional COMT val158met polymorphism might influence neuronal processing of anxiety-related emotional stimuli in patients with panic disorder, which is in line with previous association studies in Caucasian populations with the categorical diagnosis of panic disorder (Hamilton et al. 2002; Domschke et al. 2004; Rothe et al. 2006). Thus, the COMT val158met variant might increase the vulnerability to panic disorder by modulating the dopaminergic or noradrenergic tonus in anxiety-relevant brain regions, particularly the amygdala, and thereby altering neuronal processing of anxiety-relevant stimuli. With respect to the 5-HT1A-1019C/G SNP, panic disorder patients homozygous for the 5-HT1A-1019G risk allele showed a significantly decreased activation of the

ventromedial prefrontal cortex, the orbitofrontal cortex, and the anterior cingulum in response to fearful faces versus neutral faces, without, however, any effect on the amygdala. This finding might be interpreted as indicative of a functional deficit in the prefrontal cortex in patients with the 5-HT1A-1019G risk allele reducing its inhibitory effect on the amygdala and thus causing excessive amygdala activation to emotional stimuli (Domschke et al. 2006).

Despite no support from linkage studies for a major influence of the serotonin transporter (5-HTT) locus in social phobia (see Sect. 2.1), a functional polymorphism in the promoter region of the human serotonin transporter (5-HTT) gene was studied with respect to amygdala activation during social anxiety provocation in relation to affective ratings using [H2(15)O] positron emission tomography in social phobics, since there is converging evidence for a pivotal role of 5-HTT variants in the modulation of amygdala reactivity to emotional stimuli (e.g., Hariri et al. 2002). The less active, shorter allele of this 5-HTT genetic variation was found to be associated with symptom severity and amygdala excitability in social phobia (Furmark et al. 2004) suggesting 5-HTT driven amygdala activation to constitute a potential intermediate phenotype of social phobia.

Markers spanning RGS2 reported to be associated with childhood BI, a temperamental precursor of social anxiety disorder, and introversion as a core personality trait in social anxiety disorder were also found to influence limbic activation (insular cortex and amygdala) during emotion processing (brain phenotypes correlated with social anxiety, Smoller et al. 2008a, b).

In summary, these first imaging genetics findings in panic disorder, social phobia, and anxiety-related traits may indicate that – depending on variants of the *COMT*, serotonin transporter, serotonin receptor 1A, and RGS2 genes – patients with anxiety disorders are prone to impaired cerebral processing of anxiety-related stimuli in cortical regions known to play a crucial role in the evaluation of emotional stimuli and determination of salient events (Bishop et al. 2004; Northoff et al. 2004).

4 Pharmacogenetics

Recently, advances in molecular genetic techniques have allowed for the detailed dissection of the genetically influenced heterogeneity of psychotropic drug response. This study of genetically controlled variation in drug response is commonly termed "pharmacogenetics." While there is converging support for a considerable genetic influence on the individual antidepressant treatment response via pharmacokinetic as well as pharmacodynamic processes in major depression, only few studies have looked at pharmacogenetic processes in anxiety disorders so far. Two exemplary studies have reported genetic variation in the serotonin transporter gene to predict response to treatment with SSRIs in panic disorder and generalized social anxiety disorder (Perna et al. 2005; Stein et al. 2006).

5 Discussion and Perspectives

There is converging evidence that anxiety disorders and panic disorder in particular are complex genetic disorders with an etiological influence of biological as well as environmental or psychological factors, respectively. The contribution of genetic factors has been proposed to be considerable with estimated heritabilities of up to 67%, with multiple genes each of a small individual effect interacting with environmental factors. In panic disorder, several chromosomal risk loci and risk variants in candidate genes have been identified by linkage and association studies with suggestive evidence for a role of variants in genes coding for the COMT, the MAO-A, the serotonin receptor 1A (5-HT1A), the CCK-B receptor and the adenosine A2A receptor (A2A), with a potential interactional effect of the 5-HT1A and COMT polymorphisms. Since the effects of genetic variation in the COMT and the MAO-A gene seem to be restricted to the female subgroup, gender-specific genetic mechanisms may be acting in panic disorder. Gene–environment analyses revealed a significant impact of A2A receptor gene variation on anxiety levels after caffeine consumption, while COMT, 5-HTT, 5-HT1A, and RGS2 variants appear to increase the vulnerability to panic disorder, social phobia, and anxiety-related traits by alteration of neuronal processing of anxiety-related emotional cues as shown using an imaging genetics approach. First pharmacogenetic studies in panic disorder and generalized social phobia provided evidence for a significant influence of 5-HTT variants on treatment with SSRIs.

Despite this converging evidence for a genetically conferred risk of anxiety disorders, it remains to be elucidated how exactly these genes exert their effect on a functional level and how they interact with each other or with nongenetic risk factors, respectively, to increase the disease risk. Investigating endophenotypes of anxiety disorders will, therefore, have to be intensified with the search for novel anxiety-related neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological endophenotypes and their analysis with respect to their genetic basis.

So far, most probably only a small number of the assumed number of risk genes (>50) contributing to the development of anxiety disorders has been identified. Therefore, the identified genetic risk factors are of no diagnostic or predictive value. However, the identification of genetic risk factors tremendously helps in better understanding the pathophysiology of anxiety disorders pointing to among others serotonergic, noradrenergic, dopaminergic, cholocystokinergic and adenosinergic pathways being involved. They may thus contribute to the development of innovative psychopharmacotherapeutic substances in the treatment of anxiety disorders, preferably in an individually, genotype-based tailored manner on the basis of pharmacogenetic studies.

Future research will have to aim at unraveling the complex genetic mechanisms of anxiety disorders in a more detailed way including more intensive, systematic study of linkage candidate regions and most importantly genome-wide association (GWA) studies and thus providing predisposing genes beyond the classical neurotransmitter systems. Another promising future approach will be to perform large-scale studies of

gene–environment interactions as reviewed by Poulton et al. (2008). An exemplary gene–environment study with respect to anxiety traits has recently been published by Stein et al. (2008), who observed a significant interaction between levels of childhood emotional (or physical) maltreatment and a serotonin transporter variant on anxiety sensitivity. Similarly, in posttraumatic stress disorder, a disorder with a marked environmental impact, FKBP5 polymorphisms and childhood abuse have been proposed to interact to increase the disease risk in adulthood (Binder et al. 2008).

Studies like these will enhance the integration of psychopharmacological and psychotherapeutic approaches to anxiety on the way to multidimensional therapies of these multidimensional disorders.

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Neuroanatomy of Anxiety

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Contents

Abstract The evolutionary approach to human anxiety is based on the defensive responses that nonhuman animals show to fear-provoking stimuli. Studies performed mostly on rodents have related areas such as the medial prefrontal cortex, amygdaloid

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and hypothalamic nuclei, hipoccampal formation, and midbrain central gray to these responses. It is clear, however, that animals show different and sometimes opposite responses according to the threatening stimulus. These responses include immediate reactions such as freezing or flight, behavioral inhibition or avoidance, which are organized by at least partially distinct brain systems. As discussed in this chapter, several pieces of evidence indicate that these brain systems are similar in rodents and primates. In addition, recent neuroimaging studies also suggest dysfunctions in these systems are probably related to anxiety disorders in humans.

Keywords Amygdala · Dorsal premammilary nucleus · Generalized anxiety disorder · Hippocampus · Hypothalamus · Medial prefrontal cortex · Obsessive-compulsive disorder · Panic disorder · Periaqueductal gray · Post-traumatic stress disorder

Abbreviations

ACg	Anterior cingulate
AID	Dorsal agranular insular cortex
AIV	Ventral agranular insular cortex
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
CA1	Ammon's horn 1
CeA	Central nucleus of the amygdala
DLPFC	Lateral/dorsolateral prefrontal cortex
DMH	Dorsomedial hypothalamic nucleus
HAP	Hypothalamus–pituitary–adrenal
IL	Infralimbic cortex
LH	Lateral hypothalamus
MO	Medial orbital cortex
mPFC	Medial prefrontal cortex
OCD	Obsessive-compulsive disorder
OMPFC	Orbito-medial prefrontal cortex
PAG	Periaqueductal gray
PFC	Prefrontal cortex
PL	Prelimbic cortex
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
SSDR	Species-specific defensive reaction
VLO	Ventral lateral orbital cortex
vmPFC	Ventromedial prefrontal cortex
VO	Ventral orbital cortex

1 Introduction

A significant work in the study of emotion was Charles Darwin's book *The expression of the emotions in man and animals* (1872). From there came the key concept that the biological origin of human anxiety is based on the defensive responses that nonhumans show to threatening stimuli. These responses, therefore, can be seen as normal emotional states with adaptive function aimed at promoting survival across species (Rauch et al. 2006). Anxiety disorders, on the other hand, can be characterized as extreme manifestations of aspects of normal anxiety (Gray and McNaughton 2000).

For obvious technical and ethical issues, the study of the neural basis of anxiety has been made to a great extent in nonhumans. Therefore, in this chapter we will discuss data originated mostly from studies carried out in rodents. It is written, however, on grounds that a translational approach that correlates these data with the neural substrate responsible for anxiety in humans is possible. As will be briefly discussed at the end of the chapter, this conviction has received great support from recent neuroimaging studies in humans. Together they confirm that human anxiety is accompanied by changes in neural systems that coordinate distinct defensive responses to threats. They include areas such as the medial prefrontal cortex (mPFC), amygdaloid and hypothalamic nuclei, hipoccampal formation, and midbrain central gray.

It is generally accepted that animals have a set of several, genetically determined, prepackaged behaviors to solve certain functional problems. This idea has been used in the species-specific defensive reaction (SSDR) theory, suggesting that the same innately determined defensive behavior (such as freezing and flight) should occur during the response to either a natural predator or an artificial harmful stimulus (Bolles 1970). This seemingly limited way of reacting to a dangerous stimulus has led to a misleading assumption that animals process predators' cues in the same way they do an artificial threat. However, animals are naturally selected to protect themselves from dangers associated with predators differently from how they do in relation to an artificial harm, in a way that the predator presence alone can evoke a sensation of fear and associated behavioral responses.

With this caveat in mind, we shall discuss two main experimental approaches that have been used to investigate the question of the brain systems underlying fear and anxiety (i.e., Pavlovian fear conditioning to a context or to a neutral stimulus, and exposure to a real predator or its odor).

2 Pavlovian Fear Conditioning Studies

Pavlovian conditioning is by far the most commonly used approach. The phenomenon of Pavlovian fear conditioning is highly reproducible and generates clearly measurable responses. Moreover, the main responses measured in this

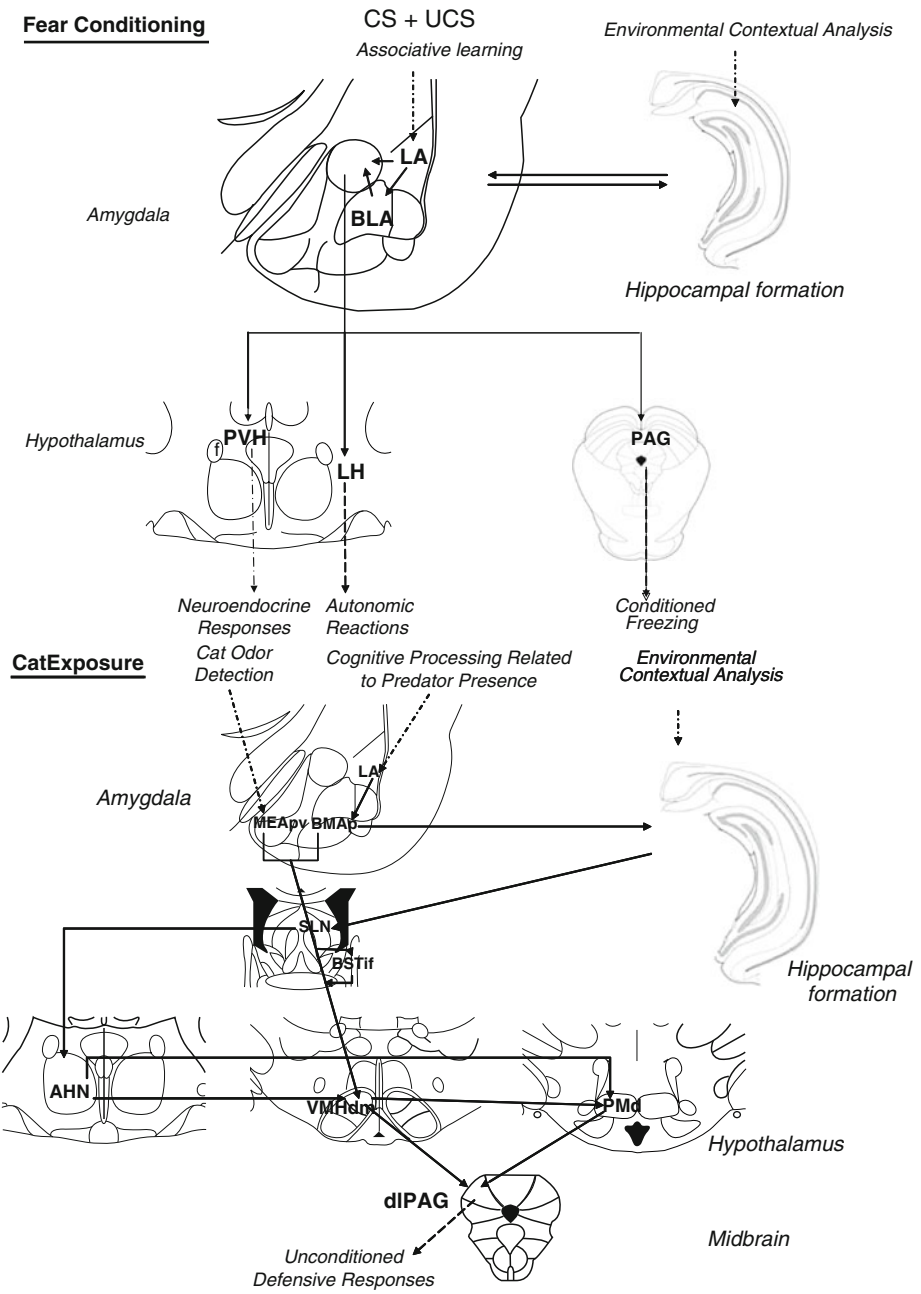

Fig. 1 *Upper part*. Neural substrate proposed to be involved in Pavlovian conditioned fear. Associative learning between the conditioned and unconditioned stimuli is likely to occur in the lateral nucleus of the amygdala (LA), which, in turn, projects to the central nucleus (CeA) both directly and indirectly via projections to the basolateral nucleus (BLA). The central nucleus, via projections to the hypothalamus and brainstem, is critical for the expression of fear conditioning.

context – freezing and startle – are both seen in the repertory of animals confronting unconditioned threats, and have been thought to provide a good validation to both sets of models (Fanselow 1994). Studies on the neural basis of Pavlovian conditioned fear indicate the amygdala as a major player in learning, storage, and expression of fear conditioning. Among the amygdalar regions, three nuclei have been particularly focused on the fear conditioning research, namely, the lateral nucleus, the central nucleus, and the basolateral nucleus (LeDoux 2000). As shown in Fig. 1, associative learning between the conditioned and unconditioned stimuli is likely to occur in the lateral nucleus. In fact, both acquisition and retention of fear conditioning occur in the lateral nucleus, where electrolytic and excitotoxic lesions, as well as pharmacological blockade, prevent acquisition and expression of fear conditioning (Campeau and Davis 1995; Muller et al. 1997; LeDoux 2000; Gale et al. 2004). The lateral nucleus presents clear synapse plasticity during fear conditioning, and changes the way a conditioned stimulus is processed after the shock pairing. The lateral nucleus, in turn, projects to the central nucleus both directly and indirectly via projections to the basolateral nucleus (Pitkänen et al. 1997; Pare and Smith 1998). The central nucleus, via projections to the hypothalamus and brainstem (Fig. 1), is critical for the expression of fear conditioning. In fact, lesions of the central nucleus disrupt freezing, along with the autonomic reactions observed during fear conditioning (LeDoux 2000). More recent studies, however, have also suggested a role for the central nucleus in learning and storage of fear conditioning (Wilensky et al. 2006). The role of the basolateral nucleus in fear conditioning is controversial, and only post-training, but not pre-training, lesions appear to interfere with the expression of conditioned fear (Angladа-Figueroa and Quirk 2005).

This well-developed neural circuitry model for the processing of fear conditioning provides an excellent framework for comparison to the neural processing for innate fear responses, such as those that occur during exposure to a predator. Some differences are immediately apparent, as lesions of the central nucleus, a key region for the expression of fear conditioning, produce only marginal deficits, at best, on defensive responses to a predator (De Oca and Fanselow 2004) or its odor (Li et al. 2004).

Fig. 1 (continued) *Lower part*. Neural substrate involved in odor-elicited antipredator defensive responses. Predator odors seem to be processed in the accessory olfactory bulb, which projects principally to the medial amygdala. The amygdalar sites related to predator detection project either directly or indirectly, via the transverse nucleus of the BST, mostly to the dorsomedial part of the ventromedial nucleus of the hypothalamus (VMHdm). Together with the anterior and the dorsal premammillary hypothalamic nuclei, the VMHdm is part of a medial hypothalamic defensive system. The main brainstem target of this system is the periaqueductal gray (PAG). The septo-hipoccampal system, via its direct connections to the medial hypothalamic defensive system, is in a position to control antipredatory defensive responses by providing contextual analysis. *AHN* anterior hypothalamic nucleus; *BMAp* posterior basomedial amygdala; *BSTif* transverse nucleus of the bed nucleus of the stria terminalis; *dl*PAG dorsolateral periaqueductal gray; *LH* lateral hypothalamus; *MEApv* posteroventral medial amygdala; *PMd* dorsal premammillary hypothalamic nucleus; *PVH* paraventricular nucleus of the hypothalamus; *SLN* lateral septal nucleus (Drawings based on Paxinos and Watson 2005)

Also, latent Toxoplasma infection resulting in entry of parasites into rodent brains that presumably affect the circuits involved in response to predator threats, inducing loss of innate defensive responses towards cat odor, has recently been reported to have no effect on fear responses to a conditioned stimulus previously paired to a shock (Berdoy et al. 1995; Vyas et al. 2007). Taken together, the evidence suggests that, at least in rats, innate defensive behavior to a predator or its odor is likely to be processed by neural circuits, somewhat different from those for fear conditioning.

3 Antipredatory Defensive Systems

3.1 Amygdalar Systems Involved in Predator Detection

Over the last years, a great deal has been learned about the neural system involved in processing innate defensive behaviors to a predator or its odor. As discussed in the case of fear conditioning, the amygdala also occupies a central role in integrating the sensory clues related to the predator. A recent finding is that predator odors may in fact be processed by prey species in the accessory olfactory bulb, rather than the main olfactory bulb (McGregor et al. 2004). This suggests that cat odor is processed by rats as a pheromone rather than a conventional odor, and the authors suggested that cat odor may be an example of a "kairomone" – a semiochemical released by one species that has a favorable adaptive effect on a different "receiving" species (McGregor et al. 2004). The accessory olfactory bulb projects principally to the medial amygdala, and rats exposed to cat odor also show substantial activation in this nucleus, particularly in its posteroventral part (Dielenberg et al. 2001; McGregor et al. 2004). In line with this view, rats with cytotoxic lesions in the medial nucleus, but not in the central nucleus, exhibited a significant reduction in unconditioned fear responses to cat odor (Li et al. 2004). During exposure to a live predator, in addition to activation of the posteroventral part of the medial amygdalar nucleus, we have observed a distinct Fos increase in two other amygdalar sites, namely, the posterior basomedial amygdalar nucleus and caudal levels of the lateral amygdalar nucleus (Canteras et al. 2001). Importantly, these amygdalar nuclei receive inputs from visual and auditory association areas, and are likely to integrate predator-derived sensory clues, other than olfactory ones (McDonald 1988). As shown in Fig. 1, the amygdalar sites related to predator detection project either directly or indirectly, via the transverse nucleus of the bed nucleus of the stria terminalis (BST), to the ventromedial nucleus of the hypothalamus, where its dorsomedial part receives most of the direct projections from the amygdala and is particularly mobilized during exposure to a live predator or its odor. Therefore, studies using rats exposed to a live cat or its fur odor suggest an amygdalar-BST-hypothalamic path to detect a live predator or its cues (Canteras et al. 2001). Recent work testing mice exposed to rats substantiate the idea of this particular amygdalar-hypothalamic path for predator detection in other prey species (Martinez et al. 2008).

3.2 The Hipoccampal Formation and the Contextual Analysis for Predatory Environment

Apart from the well-known hipoccampal functions related to mnemonic processing, spatial learning, and navigation, the hippocampus also works as a context analyzer. In this regard, it is relevant to point out that the hipoccampal formation receives inputs from the amygdalar sites involved in detecting predator-related cues and, therefore, are likely to be involved in associating predator threats to a given environment (Petrovich et al. 2001).

The septo-hipoccampal system is directly related to the medial hypothalamic defensive system, and, therefore, is in a position to control antipredatory defensive responses. In fact, recent findings suggest that the ventral hippocampus is related to anxiety functions (Bannerman et al. 2004; Bertoglio et al. 2006; Pentkowski et al. 2006; Nascimento Häckl and Carobrez 2007). This match was first pointed out by Gray (1970), based on data confined mainly to barbiturates, and later proven to be valid for other classical (benzodiazepines) and novel (buspirone) clinically effective, centrally acting, anxiolytic drugs. In line with this view, the ventral half of field CA1 and subiculum targets distinct septal regions that project to the anterior hypothalamic nucleus, which is part of the medial hypothalamic defensive system (Risold and Swanson 1996, 1997).

3.3 The Medial Hypothalamic Defensive System

Exposure to a predator or its odor induces in the medial hypothalamic zone a distinct Fos up-regulation in the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, and the dorsal premammillary nucleus (Canteras et al. 1997; Dielenberg et al. 2001; Martinez et al. 2008).

Both the anterior hypothalamic nucleus and the dorsomedial part of the ventromedial nucleus project to the dorsal premammillary nucleus, which, by far, represents the most sensitive brain region responding to a predator or its clues, and where lesions have been most effective in reducing antipredator defensive responses (Canteras et al. 1997; Blanchard et al. 2003). In fact, the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, and the dorsal premammillary nucleus are particularly interconnected, forming a partially segregated circuit in the medial zone of the hypothalamus, the so-called medial hypothalamic defensive circuit (Canteras 2002). Notably, the dorsal premammilary nucleus appears to work as an amplifier for the neural processing in the medial hypothalamic defensive circuit. This would explain why this region is so responsive to predator threats, and why lesions therein are able to reduce defensive responses so drastically (Canteras et al. 1997; Blanchard et al. 2003, 2005; Markham et al. 2004).

The hypothalamic systems are well known for integrating a number of behaviors critical for the survival of the individual or the species (Swanson 1987), and it

comes as no surprise that the hypothalamus, and not the amygdala (as previously suggested by fear conditioning studies), should occupy this central role in integrating antipredator defensive responses. In fact, fear responses to predators are comparable to other forms of goal-oriented behavior, such as drinking, feeding, and mating, in a way that they are engaged by strong motivational factors leading to behavioral actions critical for the maintenance of the species. The hypothalamus is classically known to have a central role in the organization of a number of goal-oriented behaviors (Swanson 1987).

3.4 The Periaqueductal Gray and the Integration of Antipredatory Responses

The periaqueductal gray (PAG) represents the main brainstem targets of the medial hypothalamic defensive system, and is critical for the expression of defensive responses. Of special relevance, the pattern of projection from the medial hypothalamic defensive system to the PAG largely overlaps the pattern of PAG activation in animals exposed to a predator or its odor, where Fos expression was mostly seen in the rostral two thirds of the PAG in the dorsomedial and dorsolateral regions, whereas in the caudal PAG, a less intense, but more widespread, activation was observed (Canteras and Goto 1999; Dielenberg et al. 2001). A similar pattern of PAG activation was also described after administration of drugs known to induce panic in humans (Singewald and Sharp 2000). In fact, the dorsolateral PAG particularly appears to play a critical role in integrating forebrain limbic information related to "psychological stressors" such as the presence of a natural predator (Canteras and Goto 1999; Dielenberg et al. 2001). Fear conditioning-induced freezing is also known to depend on the PAG. However, in contrast to what was found in animals exposed to a predator or its odor, the ventrolateral PAG, but not the dorsolateral PAG, is mostly mobilized during fear conditioning to a context previously associated with footshocks (Carrive et al. 1997).

4 The Septum–Hippocampus and the Behavioral Inhibition System

In addition to its role in context analysis in threatening situations, the septo-hipoccampal system is also proposed to play a larger role in anxiety. This suggestion was originally based on the observation that the behavioral effects of anxiolytics parallel those of either permanent or temporary lesions to the septum and/or the hippocampus (Gray and McNaughton 2000; Degroot and Treit 2004). According to this view, the septo-hippocampus system generates anxiety in response to conflict situations by interrupting ongoing behavior, and increasing the level of arousal and attention to enhance gathering information (Fig. 2). These conflict situations occur

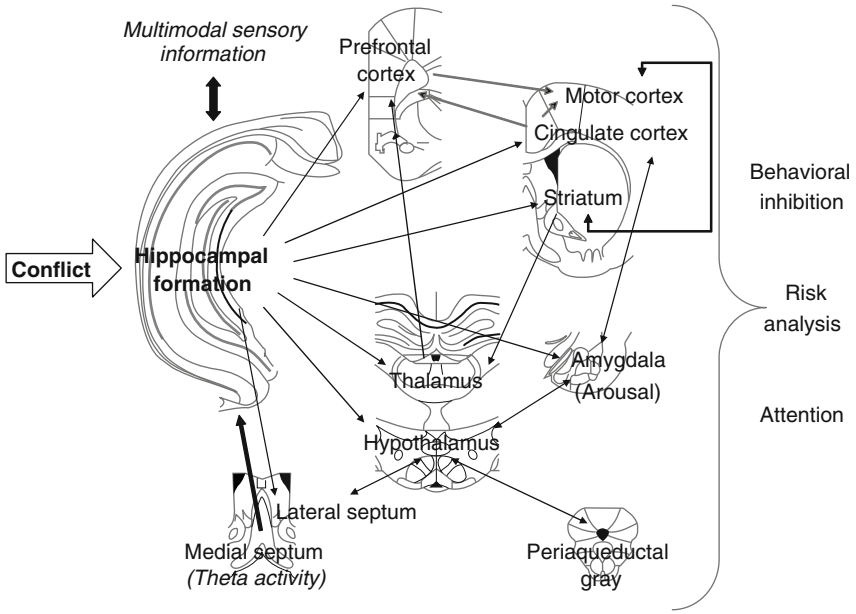

Fig. 2 Outline of the septo-hipoccampal circuitry subserving the behavioral inhibition, risk analysis, and attentional outputs triggered by conflict. The arousal output of the behavioral inhibition system is held to be mediated independently by the amygdala and has been omitted. Gray and McNaughton (2000) see these modules as mainly concerned with the organization of goal-directed action. The hipoccampal formation comprises the entorhinal cortex, the dentate gyrus, the subicular area, and the posterior cingulate cortex (Drawings based on Paxinos and Watson 2005)

when the animal has to choose between competing available goals. For instance, novelty is a stimulus that can elicit approach and avoidance tendencies, creating conflict. In turn, this conflict will generate an immediate inhibition of motor programs (apparently at the level of its planning) that are in the course of execution (Gray and McNaughton 2000). A reduction in the capacity of behavioral inhibition of pre-threat behaviors after either septal or hipoccampal damage is consistent with this proposition. In fact, as noted above, the hippocampus, working as a context analyzer, can modulate adaptive motor activity in response to particular sensory events in the environment (Oddie and Bland 1998). Animals with hipoccampal or fimbria-fornix lesions, but not with entorhinal cortex lesions, present increased motor activity, which can be blocked by lesions of the nucleus accumbens. This suggests that a subiculo-accumbal pathway is particularly important for hipoccampal formation control of locomotor behavior (Oddie and Bland 1998; Coutureau et al. 2000). Actually, it has been shown that hyperpolarization of subicular neurons is associated with an increase in accumbal dopamine transmission (Mitchell et al. 2000).

It is noteworthy that the mediodorsal thalamic nucleus is thought to represent a key target for the neural processing in the accumbal circuit, and known to play a

critical role in modulating locomotor activity (Swanson and Kalivas 2000). Lesions of the mediodorsal nucleus blunt psychomotor stimulant drug-induced behavioral activity (Kalivas et al. 1999), and microinjections of drugs mimicking GABAergic neurotransmission into the mediodorsal nucleus produce dose-dependent increases in locomotion (Churchill et al. 1996). Notably, the mediodorsal nucleus is known to project to the prefrontal cortex (PFC), which at the top of the perception–action cycle, plays a critical role in mediation of contingencies of action across time, thus influencing the temporal organization of behavior (Fuster 2000a, b).

5 The Prefrontal Cortex

The PFC comprises the anterior pole of the mammalian brain which predominantly receives projections from the mediodorsal thalamic nucleus (Rose and Woolsey 1948). A major problem in studying the functional role of the PFC is the controversy regarding the equivalence between regions in different species (Dalley et al. 2004). In primates the PFC consists of three major divisions: orbital, medial, and lateral parts (Vertes 2006). The lateral/dorsolateral PFC (DLPFC) receives information from cortical sensory processing and sends projections to somatomotor structures of the cortex, striatum, and brainstem. The DLPFC has been related to executive functions, defined as a set of cognitive control processes necessary for optimal scheduling of complex sequences of behavior (Dalley et al. 2004). It receives few direct inputs from limbic structures such as the amygdala and hippocampus but has access to emotional information through significant connections with the orbito-medial prefrontal cortex (OMPFC). These inputs are probably important for an integrated response to environmental events (see Vertes 2006 for review). The OMPFC includes the orbital and medial networks. The orbital network consists of most of the areas on the orbital surface while the medial network includes areas on the medial wall and two areas in the posterolateral orbital cortex. Some areas are represented in both networks. The orbital network receives sensorial inputs from different modalities and connects with multisensorial areas in the ventrolateral prefrontal cortex and perirhinal cortex. The medial network is reciprocally connected to limbic structures and it also connects to a cortical circuit that includes the rostral part of the superior temporal gyrus and dorsal bank of the superior temporal sulcus, the cingulate and retrosplenial cortex, the enthorhinal and posterior parahippocampal cortex, and the dorsomedial prefrontal cortex (Price 2007).

The rat PFC has been divided into three distinct regions, medial (mPFC), lateral and ventral, based on functional and hodological criteria. The mPFC constitutes the major portion of the medial wall of the hemisphere anterior and dorsal to the genu of the corpus callosum (Leonard 1972; Groenewegen 1988).This area can be further divided into a dorsal region that includes the precentral area, anterior cingulate (ACg) and dorsal part of the prelimbic (PL) area, and a ventral region (vmPFC) comprised of the ventral part of the PL, infralimbic (IL) and medial orbital (MO)

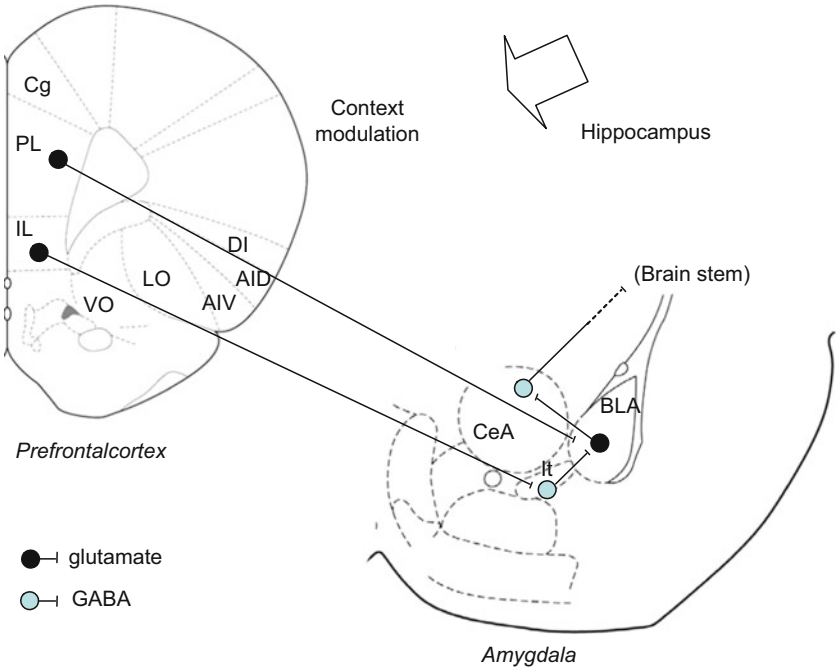

Fig. 3 Basic circuits describing a proposed interplay between the mPFC, amygdala and hippocampus in fear conditioning extinction. IL glutamatergic neurons project to GABAergic intercalated cells that inhibit CeA output neurons to promote extinction of aversively conditioned responses. On the other hand, PL neurons project to the BLA which, in turn, exerts excitatory effects on the CeA to enhance fear. The hippocampus, meanwhile, is thought to provide contextual modulation of the process. *Cg* cingulate cortex; *PL* prelimbic cortex; *IL* infralimbic cortex; *VO* ventral orbital cortex; *LO* lateral orbital cortex; *AIV* ventral agranular insular cortex; *AID* dorsal agranular insular cortex; *DI* dysgranular insular cortex; *CeA* central amygdaloid nuclei; *BLA* basolateral amygdala; *It* intercalated nucleus (Dalley et al. 2004; Quirk and Mueller 2008; Bishop 2007; Drawings based on Paxinos and Watson 2005)

cortices (Heidbreder and Groenewegen 2003). The lateral region includes the dorsal and ventral agranular insular (AID, AIV, respectively) and lateral orbital (LO) cortices. In addition, there is also a ventral region that includes the ventral orbital (VO) and ventral lateral orbital (VLO) cortices (Fig. 3, Dalley et al. 2004).

Although the PFC is much smaller in rodents and there are considerable variations across species, it is becoming clear, based on similarities of position and connections, that the caudal OMPFC is relatively comparable across species (Price 2007). For example, the IL, PL, and ACg areas of the rat PFC seem to correspond to areas 25, 32, and 24 of primates, respectively (Price 2007). As in primates, a growing body of evidence indicates the presence of a functional heterogeneity in different regions of the rat PFC (Heidbreder and Groenewegen 2003).

Several findings indicate that the OMPFC, particularly its medial part (mPFC), is involved in the coordination of the behavioral responses related to fear and anxiety (Bishop 2007). For example, exposure to different stimuli such as a predator, elevated plus-maze, novel environment, or swim and immobilization stress increases c-fos expression in the PL (Sewards and Sewards 2003). Activation of the mPFC in normal volunteers is changed during exposure to fearful stimuli, and abnormal mPFC activity has been associated with a number of anxiety disorders. Corroborating these findings, the mPFC has direct connections with subcortical, diencephalic, and brainstem structures closely related to anxiety, such as the amygdala, hypothalamus, solitary nucleus, PAG, dorsal raphe, and ventral tegmental area (Gabbott et al. 2005; Vertes 2006).

5.1 mPFC and Conditioned Fear

As discussed above, a large body of evidence from rodent studies indicates that the amygdala plays a critical role in the acquisition and expression of conditioned fear.

The mPFC, on the other hand, has been particularly related to extinction, an important form of emotional regulation characterized by a decrease in conditioned response to a stimulus when the reinforcer is omitted (for review, see Quirk and Mueller 2008). The vmPFC is proposed to have a dual role in fear extinction, retaining information that fearful events are no longer fearful (retention of fear extinction), and acting on visceral structures to reduce fear (expression of fear extinction). Several pieces of evidence indicate that projections from the mPFC to the amygdala facilitate extinction of conditioned fear responses (Milad et al. 2006), for example, lesions of the vmPFC that have no effect on acquisition impaired extinction of freezing responses to an aversively conditioned stimulus (Milad et al. 2006). This observation was reminiscent of the perseverative behavior seen in monkeys with prefrontal lesions and was described as “emotional perseveration.” Paralleling these lesion findings, single neurons in the IL exhibited no response to tones during the conditioning or extinction phases of training, but showed robust response to tones the following day, when rats were recalling the extinction process. These findings support the hypothesis of extinction as new learning and are consistent with recent observations that extinction involves protein synthesis and gene expression in the mPFC.

The mechanism of PFC interference on extinction seems to involve projections form the vmPFC to the amygdala. Neurons from the IL project strongly to the capsular division of the central nucleus of the amygdala (CeA), which contains GABAergic intercalated cells that inhibit CeA output neurons (Fig. 3). IL stimulation, therefore, will prevent CeA neurons from being activated by inputs from the basolateral amygdala (BLA). The IL can also modulate the expression of conditioned fear via direct projections to the hypothalamus, PAG, and other brain stem regions critical for generating conditioned responses (see Fig. 1). In addition to the IL, the medial, dorsomedial, and DLPFC areas have also been recently

implicated in extinction. Finally, there is also evidence that the IL and PL play a distinct role in fear conditioning by producing opposing effects in the amygdala. While conditioned stimuli (tones) paired with IL stimulation decrease fear (strengthened fear extinction), those paired with PL stimulation increased fear (impaired fear extinction). These opposite effects can be mediated by distinct neural pathways. Instead of projecting to GABAergic intercalated neurons in the amygdala, as does the IL (see above), the PL projects to the BLA which, in turn, exerts excitatory effects on the CeA to enhance fear (Fig. 3).

5.2 *mPFC and Innate Fear*

Although several studies indicate that lesions or inactivation of the mPFC affect anxiety responses in animals submitted to animal models based on innate fear, the results are still controversial, with reports of increased, decreased, or unchanged anxiety (Burns et al. 1996; Maaswinkel et al. 1996; Jinks and McGregor 1997; Lacroix et al. 1998; Heidbreder and Groenewegen 2003; Shah and Treit 2003; Wall et al. 2004). As discussed above, functional heterogeneity between subregions of the PFC could be partially responsible for these contradictory results (Jinks and McGregor 1997).

5.3 *mPFC and Visceral Reactions to Threat*

Stimulation of the vmPFC induces changes in physiological correlates of anxiety, such as respiration, heart rate, piloerection, and blood pressure. The vmPFC controls the increases in mean arterial pressure and heart rate associated with fear conditioning (Resstel and Corrêa 2006; Resstel et al. 2006) and stimulation of this region inhibits cardiovascular responses evoked by electrical stimulation of the hypothalamus or CeA. Although these effects were originally thought to be mediated by indirect projections (Verberne and Owens 1998), recent results indicate that the vmPFC influences these autonomic responses by means of its extensive connections with hypothalamic areas such as the region immediately ventral to the paraventricular nucleus (sub-PVN), the dorsomedial hypothalamic nucleus (DMH), and the lateral hypothalamus (LH) (Van Eden and Buijs 2000). The mPFC can also participate in endocrine responses to aversive stimuli, modulating hypothalamus–pituitary–adrenal (HPA) activity through its connections to the bed nucleus of the amygdala, stria terminalis (BNST), or the peri-PVN region. Finally, in the midbrain, the mPFC projects to the PAG in a topological manner where it can modulate the behavioral and autonomic responses to emotional inputs integrated by this structure (Van Eden and Buijs 2000).

5.4 PFC Responses to Stress

Stress exposure has been related to the development of several anxiety disorders, particularly post-traumatic stress disorder (PTSD). Recent evidence indicates that the mPFC could be involved in this effect. Brief exposure to uncontrollable stress impairs fear extinction and induces retraction of terminal branches of apical dendrites of IL neurons (Izquierdo et al. 2006). Similarly, chronic restraint stress for 20 days decreases dendritic branching in the PFC (PL region) and hippocampus. However, it increases dendritic branching in the BLA (see Quirck and Mueller 2008, for review).

6 Clinical Findings and Anxiety Disorders

Although methodological problems still persist, difficulties of disclosing the human neural network involved in anxiety responses have been partially overcome with the use of recent neuroimaging techniques. These studies have implicated most of the neural substrates unveiled by animal studies. For example, there is increased activity in the amygdala of healthy volunteers during acquisition and extinction of the conditioned stimulus. As predicted by animal studies, during the latter process there is also enhanced activation of the mPFC and hippocampus (for review see Bishop 2007). In addition, success in interpreting negative stimuli as less threatening correlates with an increase in PFC and a decrease in amygdala activity (Bishop 2007, see Fig. 3). It seems, therefore, that the prefrontal-amygdala circuitry mediates basic mechanisms involved in human anxiety such as selective attention to threat, interpretation of stimuli, and acquisition and extinction of conditioned fear. An increased and decreased activity in the amygdala and PFC, respectively, would lead to increased representation of negative stimuli and failure to activate alternate nonthreatening related representations (Bishop 2007).

The role of these structures, however, is probably very complex. For example, stimulation of area 32 of the OMPFC (suggested to be analogous to the PL cortex of rodents) or the rostral cingulum below the ACg cortex in humans caused reports of fear and anxiety (for review see Sewards and Sewards 2003). In addition, a recent study by Butler et al. (2007) found increased activity of the amygdala only at the earlier portion of an experimentally induced state of conscious fear. Interestingly, regions usually associated with motor behavior show robust responses in the same paradigm, with increased activation of the dorsal basal ganglia and deactivation of the primary motor cortex. Other regions with sustained activity were the insula, thalamus, and brain stem. The amygdala, therefore, was proposed to work as a threat and novelty detector whose initial, brief, and phasic activity is accompanied by sustained tonic activity of other brain regions responsible for maintaining the behavioral, anatomic, and metabolic responses for danger stimuli (Butler et al. 2007).

In a recent neuroimaging study Mobbs et al. (2007) showed that subjects faced with an active avoidance paradigm where they are pursued through a maze by a virtual predator presented changes in brain activity according to the proximity of the threat stimulus. When the predator was remote there was increased activity in the vmPFC and lateral amygdala. As the virtual predator grew closer there was a shift in activity from these areas to the PAG and central amygdala. Moreover, PAG activation was directly correlated with the degree of dread and inversely correlated with confidence to escape (Mobbs et al. 2007). These results suggest that vmPFC and lateral amygdala coordinate avoidance behavior triggered by distal threat whereas the central amygdala and PAG coordinate behavioral responses when threat is imminent (Maren 2007). They also agree with the proposal that activation of forebrain areas such as the PFC and amygdala by distal or potential threats upholds anxiety whereas activation of the PAG by proximal aversive stimuli promotes panic (Deakin and Graeff 1991; Maren 2007).

These neural mechanisms, involved in human normal anxiety, are probably more akin to the defensive responses in rodents discussed so far. The study of the neuroanatomical basis of pathological anxiety in animals, on the other hand, is complicated by the well documented cognitive bias towards cues signaling danger shown by patients with anxiety disorders. This difficult to mimic the capacity to worry excessively about the future, a key feature in most anxiety disorders, is a limitation of animal models (Bishop 2007). Since this capacity has been linked to the greater development of the PFC in primates, this brain region has been proposed to play a more important role in human anxiety (Berkowitz et al. 2007). A hypoactive OMPFC has been related to a failure to inhibit inappropriate fear or anxiety responses whereas conditions such as obsessive-compulsive disorder (OCD) would involve hyperactivity of the lateral orbital PFC (Milad and Rauch 2007). In agreement with this possibility, several neuroimaging studies have shown abnormalities in the PFC in patients with anxiety disorders, with decreased neuronal activity in disorders characterized by intense fear, such as panic disorder (PD), PTSD, and phobias, and increased activity in disorders involving worry and rumination (PTSD and OCD) (Milad and Rauch 2007). OCD is proposed to arise from dysfunctions in a cortico-striato-thalamo-cortical circuitry, in a way that changes in the striatum lead to ineffective gating in the thalamus (Milad and Rauch 2007) and hyperactivity of the orbital PFC and ACg cortex. Successful pharmacological or behavior therapy has been able to decrease activity in these areas (see Milad and Rauch 2007, for review).

PTSD, on the other hand, has also been related to changes in a circuit that involves the OMPFC plus the hippocampus and amygdala. Patients with PTSD have smaller vmPFC and hippocampal volumes, and increased activity in the amygdala (Quirk and Mueller 2008). These changes would translate in a failure to consolidate and retrieve extinction of aversive events (see Fig. 3).

Another human brain structure that shows hyperactivity in anxiety disorders such as PTSD, social anxiety disorder, and specific phobias, is the insular cortex. It is activated by negative emotions and regulates the autonomic nervous system activity. The insula has been proposed, together with the amygdala, hypothalamus,

periaquedutal gray, parabrachial nucleus and nucleus tractus solitarius, to be part of an internal regulation system that controls the visceromotor, neuroendocrine, and pulmonary system as well as pain sensations (Nagai et al. 2007, for review see Etkin and Wager 2007). It has been implicated in the recognition and experience of disgust, sadness, and fear (for review see Morris 2002).

With regard to PD, neuroimaging studies in patients or volunteers submitted to panic symptoms-inducing drugs have shown increased activation of parahippocampal gyrus, the superior temporal lobe, the ACg, cerebellar vermis, insula, temporal poles and the hypothalamus, and PAG (Javanmard et al. 1999; Boshuisen et al. 2002). Both before and after a drug-induced panic attack, however, PD patients showed hypoactivity in the precentral gyrus, OMPFC, the right amygdala, and the anterior insula (Boshuisen et al. 2002). PD patients who improve after cognitive-behavior therapy showed decreased activation of the right hippocampus, left ACg, left cerebellum and pons, and increased activity in the OMPFC. Further strengthening the proposal of PAG involvement, a significant correlation between activity in the midbrain and the number of panic attacks has been described (Sakai et al. 2006).

7 Conclusions

Similar to other complex functions, anxiety does not appear to depend on specific areas performing unique functions but should be rather seen as an emerging property of interacting brain regions (Morgane et al. 2005). Even so, it is clear that animals show different and sometimes opposite behavioral responses to specific stimuli such as distal or proximal threats and that these responses are organized by at least partially distinct brain systems (Deakin and Graeff 1991; McNaughton and Corr 2004; Maren 2007).

The several pieces of evidence reviewed here indicate that these brain systems are similar in rodents and humans. They would include a defense system, aimed at making immediate responses to threatening stimuli, and a behavioral inhibition system, responsible for the suppression of behaviors that could enhance danger. Other partially distinct networks could be responsible for responses such as avoidance. These systems would work in concert and engage distinct hierarchic circuits depending on increasing demand for cognitive processing (McNaughton and Corr 2004; Sandford et al. 2000). Dysfunctions in these circuits are likely to generate chronic anxiety disorders (Maren 2007). In addition, these circuits are under modulatory influence of several other neural systems such as serotonergic, noradrenergic, and dopaminergic inputs from the raphe nuclei, locus coeruleus, and ventral tegmental area, respectively. A complete review of these modulatory circuits would be beyond the aims of this chapter. They are however, particularly important for the anxiolytic effects of drugs such as antidepressants and benzodiazepines and will be reviewed elsewhere in this book.

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Stress and the Neuroendocrinology of Anxiety Disorders

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Contents

Abstract Stress is a risk factor for depressive and anxiety disorders. Changes in lifestyle patterns that are associated with increased stress therefore place a greater burden on mental health. Stress challenges the organism's homeostatic mechanisms, triggering a cascade of events that should, normally, maintain or allow a return to equilibrium. Stressful events are perceived by sensory systems in the brain, facilitating evaluation and comparison of the existing and previous stimuli as well as the activation of hormones responsible for energy mobilization. The limbic system coordinates the release of corticosteroids, the primary stress hormones, by modulating activation of the hypothalamic paraventricular nucleus (PVN). The amygdala, a limbic structure related to emotional behavior, has a putative role in the evaluation of emotional events and formation of fearful memories; it is also a

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target of the neurochemical and hormonal mediators of stress. Clinical and experimental data have correlated changes in the structure/function of the amygdala with emotional disorders such as anxiety. In this chapter we review the neuroendocrinology of the stress response, focusing on the role of the limbic system in its establishment and supplementing that information with new experimental data that demonstrates the relationship between stress and anxiety disorders; we also discuss the structural changes that occur in the amygdala after stress.

Keywords Anxiety · Bed Nucleus of the Stria Terminalis · Rat · Stress · Animal Models · Amygdala · Corticosteroids

1 Stress and Emotional Behavior

Stress is implicated in the etiology of several emotional disorders, including depression (McEwen 2004; Hammen 2005), phobia (Risbrough and Stein 2006) and anxiety (Charney et al. 1993). Stress-related disorders affect a substantial portion of the work force and therefore represent an important burden on health providers, caregivers and society at large. World Health Organization statistics indicate emotional/mood disorders as a leading cause of disability worldwide and predict that by 2020 these disorders will be the second leading global burden of illness, only surpassed by cardiovascular disease.

Stress may be defined as a challenge to homeostasis, imposed by physical or psychological events. Exposure to stress triggers a cascade of hormonal and behavioral changes that, under normal conditions, facilitate the organism's adjustment to, and control over, the threatening events. Adaptation to stress involves activation of neural, neuroendocrine and neuroimmune mechanisms. Effective coping implies that a stress response is triggered when needed and terminated thereafter. However, if the stress response is exaggerated or protracted, a series of pathophysiological changes occur in the brain and immune system (and viscera) that may ultimately result in disease (Mayer and Fanselow 2003; Musselman and Nemeroff 2000; Sorrells and Sapolsky 2007).

The hypothalamo-pituitary-adrenal (HPA) axis plays a central role in the stress response. It is activated early after the arrival of stressful stimuli, resulting in the secretion of the adrenocorticotrophic hormone (ACTH) secretagogues, corticotrophin-releasing factor (CRF) and arginine-vasopressin (AVP), by neurosecretory neurons in the parvocellular component of the PVN; in turn ACTH stimulates the synthesis and secretion of corticosteroids by the adrenal glands. Therefore, by integrating the output of different stress-sensitive brain circuitries (Herman and Cullinan 1997), the PVN plays a key role in regulating the HPA stress response (Whitnall 1993).

The PVN is subject to differential activation by distinct neuronal pathways, depending on the quality and/or immediacy of the demand for an appropriate

response (Herman et al. 1997, 2003): stressors such as hemorrhage, respiratory distress or systemic inflammation, which represent an immediate threat to homeostasis, directly activate the PVN, bypassing cortical and limbic areas, by activation of somatic, visceral or circumventricular sensory pathways (Chan et al. 1993; Cole and Sawchenko 2002). More precisely, excitatory ascending pathways originating in the brainstem nuclei that convey noradrenergic inputs from the nucleus of tractus solitarius (Abercrombie and Jacobs 1987; Cullinan et al. 1995; Gann et al. 1977; Smith et al. 1991), serotonergic inputs from the raphe nuclei (Feldman et al. 1987; Sawchenko et al. 1983) or inputs from adjacent hypothalamic nuclei (Herman et al. 2003) are well positioned to receive visceral and autonomic inputs so as to evoke rapid neuroendocrine responses. Other stressors result in the central generation of responses, allowing mobilization of energy and immune reserves in anticipation of homeostatic disruption. The activation of these complex pathways seems to occur by contextual, conditioned (memory) or species-specific cues that predict adversity. The mnemonic aspects of this response are important determinants of the magnitude of the HPA response as, per definition, stimuli are only stressful by comparison with previous experience(s). The limbic system plays a central role in the coordination of this "anticipatory" stress response (Herman et al. 2005) and characterizes stress-induced anxiety behavior, as will be addressed later.

1.1 Mediators of the Stress Response

Circulating corticosteroids play a central role in the adaptative response to stress, primarily by influencing energy metabolism and dampening the immune and inflammatory responses; thus, corticosteroids act in the short term to prevent overshooting of the innate response (de Kloet et al. 2005; Sapolsky et al. 2000). However, prolonged exposure to elevated levels of corticosteroids leads to immune dysfunction (Sapolsky 2000; Sorrells and Sapolsky 2007), endocrine dysregulation (Sapolsky 2000; Sousa et al. 2008) and, ultimately, to behavioral and neuropathology (Cerqueira et al. 2008; Sapolsky 1999; Sousa and Almeida 2002; Sousa et al. 2007).

Corticosteroids actions are mediated by two biochemically distinct receptors which bind the same ligand (cortisol in humans, corticosterone in rodents), albeit with differing affinities. While glucocorticoid (GR) receptors are ubiquitously distributed, mineralocorticoid receptors (MR) are more discretely distributed; however, both receptors are expressed at particularly high levels in limbic areas that are responsible for the modulation of the stress response (Reul and de Kloet 1986). As compared to GR, MR have a higher affinity for cortisol/corticosterone and are, therefore, highly occupied even under basal (stress-free) conditions (Reul and de Kloet 1985). In contrast, GR become increasingly occupied as circulating corticosteroid levels rise, e.g., during stress. MR have been implicated in the appraisal process and onset of the stress response, while GR have been ascribed a role in the mobilization of energy substrates and most stress-induced changes in behavior.

The latter includes anxious-like behavior and facilitated learning and memory (in particular, consolidation of memories). On the other hand, long-term GR activation is associated with deleterious effects on several cognitive functions (Cerqueira et al. 2005a, 2007a, b; McEwen 2005; Sapolsky et al. 1986; Sousa et al. 2000, 2007). Interestingly, these effects have been correlated with neuroarchitectural changes in several brain regions, including the hippocampal formation, prefrontal cortex (PFC) and amygdala (McEwen 2007; Sousa et al. 2007). It is worth noting here that the hippocampal formation and PFC are involved in mediating corticosteroid negative feedback on the HPA axis and in curtailing the endocrine response to stress (Mizoguchi et al. 2003. Not surprisingly, these areas also undergo structural and functional remodeling by stress. These changes, thought to have a putative role in the perpetuation of the hypercortisolism state that characterizes chronic stress, are thought to make a pivotal contribution to the maladaptation by some individuals to stress (Cerqueira et al. 2008; Sousa et al. 2007).

While elevated corticosteroid levels characterize stress, it is important to note that high levels of corticosteroids do not mimic stress, but rather only one aspect of it. A plethora of other processes are altered by stressors, among them, the previously mentioned hypothalamic neuropeptide CRF (Lau et al. 1983) and the related peptides: Urocortin (Ucn), UcnII and UcnIII. The Unc family of peptides act through the G-protein-coupled CRF receptors, $CRFR_1$ and $CRFR_2$. While CRF and Ucn bind both $CRFR_1$ and $CRFR_2$ the more recently discovered UcnII and UcnIII show high selectivity for $CRFR_2$. In the brain, there is a variable expression and distribution of CRF related peptides (Dautzenberg and Hauger 2002; Reul and Holsboer 2002). CRF is expressed in neurons of the PVN, cerebral cortex, cerebellum, amygdala and hippocampus; Ucn expression is limited to the Edinger-Westphal, lateral olivary and supraoptic nuclei; UcnII has a distinct subcortical expression in regions related to stress response, such as the PVN, locus coeruleus, hypothalamic supraoptic and arcuate nuclei, and several motor nuclei of the brainstem and spinal cord; UcnIII is expressed in the rostral perifornical area, posterior part of the bed nucleus of the stria terminalis (BNST), medial nucleus of the amygdala and lateral septum.

Each of the CRF receptors displays a different expression pattern and has distinct physiological functions (Chalmers et al. 1995; Chen et al. 1993; Dautzenberg and Hauger 2002; Lovenberg et al. 1995; Reul and Holsboer 2002; Steckler and Holsboer 1999). The $CRFR_1$, which is mainly expressed in the anterior pituitary, cerebral cortex, cerebellum, amygdala, hippocampus and olfactory bulbs (Dautzenberg and Hauger 2002; Reul and Holsboer 2002), has been consistently implicated in stress-related behavior (Heinrichs and Koob 2004; Heinrichs et al. 1997; Liebsch et al. 1995, 1999; Skutella et al. 1998), and in particular the mediation of anxiety-like responses in various test paradigms such as the elevated-plus maze (File et al. 1988), social interaction test (Dunn and File 1987) and acoustic startle response test (Swerdlow et al. 1986). Additionally this receptor plays a role in hormonal (Dunn 1987) and autonomic activation (Nakamori et al. 1993) by stress. While the $CRFR_1$ appears to be crucial to the initiation of the stress response, it does not seem to be responsible for baseline drive of the HPA axis

(Reul Holsboes 2002). $CRFR_2$ are mainly expressed in subcortical areas, including the PVN, lateral septum, amygdala, hippocampus and BNST. A specific role has still to be ascribed to $CRFR_2$: while some studies implicate it in anxiogenesis (Radulovic et al. 1999), others suggest that it may mediate anxiolytic effects (Valdez et al. 2003; Zhao et al. 2007); these opposing reports may reflect differences in regional activation of the receptor or treatment paradigms (e.g., duration and intensity of stress). Importantly however, there is consensus that $CRFR_2$ are critical for the extinction of HPA activation (Bale et al. 2000; Coste et al. 2000).

2 Anxiety Disorders and Stress

Anxiety and fear are two closely related aspects of emotional behavior. However, although they have "similar" phenotypic expressions, each is governed by specific brain circuitries that are activated by distinct types of stimuli These differences will be given special consideration in order to clarify the characteristics that typify anxiety.

Anxiety disorders are highly prevalent (Alonso and Lepine 2007; Kessler et al. 2007b; Merikangas and Kalaydjian 2007; Moussavi et al. 2007) and represent a heterogeneous group of disorders that range from phobias to generalized anxiety disorders (American Psychiatric Association 1994). Anxiety is characterized by a number of mental and physical symptoms with no apparent explanation. Mental symptoms include a general sensation of discomfort and apprehension in response to unconditioned diffuse cues (Koch 1999). Healthy individuals commonly experience anxiety upon experience of unspecific frightening cues which, nevertheless, do not have clear threatening outcomes (e.g., loud noises, fast approaching objects, etc.). One might assume that the state of anxiety/fear has a biological advantage insofar that it helps the individual prepare a defense against potentially harmful encounters; however, apart from keeping the individual attentive for recurrences of the adverse situation, persistence of anxiety/fear after the "flight or fight" response has achieved its goals is likely to be disadvantageous. The symptomatic response to anxiogenic stimuli includes increased heart rate, muscle tension, dizziness, light headedness, nausea, and chest/abdominal pain, eventually focusing the individual's attention to determine the source of threat, which translates into persistent activation of the autonomic nervous system. Frequently, the latter outlasts exposure to the anxiogenic stimulus, indicating long-term activation of the neural substrates and processes that regulate anxiety (Lee and Davis 1997a, b; Lee et al. 1994).

As already noted, anxiety disorders develop when symptoms occur without any recognizable stimulus or when the stimulus does not warrant such a reaction. In contrast, fear involves the conditional learning that a specific cue, or a more complex context, predicts imminent adversity (Brown et al. 1951; Davis 1986, 1992). Fear is also found in lower organisms (Davies et al. 2002; Eisenberg and Dudai 2004; Portavella et al. 2004), suggesting that it may have advantages related to the survival of the individual and continuity of the species. By learning which

situations are threatening, the organism can actively avoid those specific objects or contexts in the future. The fear response is characterized by a set of symptoms relayed by neuroendocrine and autonomic centers that comprise largely the same pathways activated in the anxiety response (Walker et al. 2003). However, these common pathways are activated in a particular set of conditions that result in the activation of distinct initiating centers. Fear responses are elicited by explicit, adversity-predicting, short-lasting cues, in which the period of danger is finite. In general, fear responses are self-limiting in time and magnitude, the symptoms resulting from its activation being abolished upon termination of the adverse stimulus. In summary, the closely related states of fear and anxiety are mediated by common pathways that are activated, however, over distinctly different time-frames and in response to particular conditioning stimuli.

Animal (Anisman and Matheson 2005; Arborelius et al. 1999; File 1996; Pêgo et al. 2008; Vyas et al. 2002) and clinical (Chrousos and Kino 2007; Dranovsky and Hen 2006; Greaves-Lord et al. 2007) studies have consistently shown a correlation between chronic stress and altered emotional behavior. Most importantly, anxiety disorders have been correlated with abnormalities in the HPA axis although the nature of these changes are different from those observed in mood disorders such as depression (Mathew et al. 2008; Risbrough and Stein 2006). For example, while many depressed patients display signs of a hyperactive HPA axis (Shelton 2007), patients with anxiety disorders show a wide spectrum of patterns of HPA activity, possibly because such disorders represent a very heterogeneous group of pathologies. Although particular subgroups of anxiety disorders (Mathew et al. 2008; Risbrough and Stein 2006), best exemplified by patients suffering from post-traumatic stress disorder (PTSD), show signs of increased CRF production (Baker et al. 1999; Bremner et al. 1997), they are characterized by abnormalities of the HPA which are more subtle and less consistent than those found in depression. In fact, PTSD patients tend to display an exaggerated inhibition of cortisol secretion after the administration of exogenous glucocorticoids such as dexamethasone (Shekhar et al. 2001). This suggests that the HPA axis of PTSD patients is sensitized, rather than blunted (as in depression); these findings in humans have been reproduced in rodent models of PTSD (Liberzon et al. 1999).

Independently of the triggering mechanisms, the impact of the hormonal mediators of stress mediators on anxiety behavior is notable (Arborelius et al. 1999). Glucocorticoids have been shown to enhance the excitability of amygdalar neurons and to influence the acquisition of fear behavior (Duvarci and Pare 2007; Skorzewska et al. 2006; Yang et al. 2007) and stress has been repeatedly shown to induce anxiety-like behavior (McEwen 2003, 2004; Pêgo et al. 2008; Vyas and Chattarji 2004; Vyas et al. 2002) while a role for GR in the modulation of anxiety-associated behavior has also been demonstrated (Boyle et al. 2006). The amygdala and extended amygdala (including the BNST), both of which are richly populated with corticosteroid and CRF receptors that are activated during stress (Cullinan et al. 1995; Figueiredo et al. 2003; Ju et al. 1989b; Mathew et al. 2008), have a putative role in the integration of polysensory information that culminates in the

expression of emotional behavior. Therefore, these brain areas have been targets of recent research in the field of stress-related anxiety behavior.

3 Animal Models of Stress and Anxiety

Animal models of emotional disorders attempt to reproduce various characteristics of human psychiatric disorders, from behavioral and physiological changes associated with a particular emotional state (face validity) to the etiology (construct validity) and responses to therapeutic interventions (predictive validity). These models have become invaluable tools in the analysis of various causes – genetic, environmental (e.g., stress) or pharmacological – of these disorders. Additionally, they have served as screening tools for identifying potential anxiolytic drugs as well as the neuroanatomical circuits responsible for mediating these behaviors. Despite evident flaws in the reproduction of certain aspects of human behavior, these models have been an essential first step towards understanding the neurobiology of psychiatric diseases (Bourin et al. 2007; Fuchs and Fliugge 2006; Shekhar et al. 2001).

Several animal models of anxiety have been used since the 1960s. Most of these models were validated using behavioral tests that involved challenging the animal with a stressful/painful stimulus before assessment of the changes in response to by benzodiazepines (BZD) – at that time, the only approved class of drug with anxiolytic properties (Shekhar et al. 2001). However, results showing that new non-BZD anxiolytics were inactive in the classical laboratory tests of anxiety, led to the realization that anxiety disorders represent a heterogeneous group of disorders in which a variety of neurochemical systems may be involved and which have distinct etiological origins (Bourin et al. 2007). Those findings also highlighted the importance of using more than one test to examine the different aspects of anxiety behavior (Sousa et al. 2006).

It should also be mentioned that it is inappropriate to assume that a single animal model will necessarily display all aspects of a behavior as complex as anxiety. It is now consensual that animal models may represent "normal" or "state" anxiety on the one hand, and "pathological" or "trait" anxiety on the other hand (see Lister 1990). In fact, most experimental procedures involve exposure to external (e.g., pain, a predator) or internal (e.g., drugs) stimuli that induce anxiety. Behavioral responses in these paradigms may correlate to the level of stress induced by the stimuli (and method of presentation) but, nevertheless, only represent a normal response – "state" anxiety. In contrast, "trait" anxiety does not vary between occasions and is considered to be an enduring feature of an individual and to have a distinct neurobiological basis (Lister 1990).

Anxiety tests in rodents are usually based on the assessment of fear/anxiety behavior by creating a conflict between the animal's innate exploratory activity and an aversive condition. In most instances, animals are submitted to an acute stressful or traumatic event and their response to these experiences is expressed as an index

of exploratory/avoidance behavior. Although these approaches are very useful to test behavioral responses in acute settings and how they are modulated by pharmacological agents they do not necessarily reflect the etiological aspects of anxiety disorders. Anxiety like many other psychiatric disorders is thought to result from the interaction between constitutive factors and exposure to disruptive environmental events, often in a chronic fashion. Human studies (Hettema et al. 2001, 2005) assessing the role of the family inheritance in the genesis of anxiety disorders have shown a strong influence of the genetic background. Both specific phobias and unspecific anxiety disorders showed a strong familial aggregation which clustered into families. Although a strong genetic contribution was identified part of the variability could be explained by family and nonshared environmental factors. These observations highlight the relevance of recognizing predisposing factors for anxiety disorders and understand their interaction with individual constitution in order to identify potential therapeutic targets. Recently, an effort has been made to develop models which emulate predisposing/causative environmental events and which take genetic background, gender and age into account (Shekhar et al. 2001).

Genetic models have used either knock-out mice that exhibit phenotypic behaviors considered to be suggestive of anxiety with the aim of identifying the involvement of specific genes in such behavior, or the selective breeding of rodent lines that exhibit high and low levels of emotionality. Mutant mice have shown involvement of the serotoninergic system in the genesis of anxiety behavior (Sibille et al. 2000), consistent with the therapeutic efficiency of antidepressants and non-BZD anxiolytics (e.g., budpirone) that interact with serotonin receptors in the treatment of specific types of anxiety. In fact, serotonin receptor (1A) receptor was shown to be important to the anxiolytic effect of BZD and that the lack of 5-HT_{1A} receptor elicits the down regulation of BZD-sensitive $GABA_A$ receptors predominantly in the amygdala and to a lesser extent in the hippocampus and cortex. The authors postulate that the expression of certain $GABA_A$ subunits are under serotoninergic control exerted by 5-HT_{1A} receptors in areas of the brain that are relevant to the expression of anxious-like behavior. Strains of rats (Liebsch et al. 1998b) and mice (Kessler et al. 2007a) exhibiting *high-anxiety related behavior* (HAB) have pointed to the contribution of genetic background and associated disruption of the HPA axis to anxiety-like behaviors (Liebsch et al. 1998a; Wigger et al. 2001; Neumann et al. 1998). Actually, cross-fostering did not influence the expression of behavioral phenotype after establishment of the behavioral trait of the strain (Wigger et al. 2001), reinforcing the major contribution of the genetic background. Moreover, although on basal conditions HAB rats show little differences in the activation of the HPA axis, there is increased secretion of either ACTH or corticosterone in response to anxiogenic stimuli and during pregnancy (Liebsch et al. 1998a; Wigger et al. 2001; Neumann et al. 1998). This latter finding raises the concern that the inheritance of anxious-like behavior may reflect a hormonal imprinting of the HPA axis during prenatal/early life development. Additionally, other studies (Hermann et al. 2000; Keck et al. 2005; Lancel et al. 2002) have also shown that these behavioral traits can be reversed with traditional anxiolytic drugs, much like the observations in humans disorders.

Early life events are thought to constitute a developmental risk factor for the expression of anxiety/fear behavior in adulthood; i.e., adverse events occurring early in life seem to program behavior in adulthood. This relationship is particularly well established in anxiety disorders both in humans and animal studies. Longitudinal studies have shown that anxiety disorders associated to adverse events occurring during early childhood (Carpenter et al. 2007; Espejo et al. 2007; Moffitt et al. 2007) are correlated with changes in function of the HPA axis. On the other hand, animal models have shown that early life-stress, like *prenatal stress* (Weinstock 2001) or the *maternal separation stress* (Mesquita et al. 2007) paradigm result in profound dysregulation of the HPA axis in tandem with an increased incidence of anxiety-like behavior. Importantly, these changes appear to be established during restricted windows of susceptibility at a time when the HPA axis seems to be particularly vulnerable to stress. Such observations accord with clinical studies showing that children exposed to traumatic events or emotional stressors are at a greater risk of developing psychopathologies (Shekhar et al. 2001).

Surprisingly, few animal studies have addressed gender and age as constitutionally relevant risk factors (Bessa et al. 2005; Imhof et al. 1993; Pego et al. 2006; Shekhar et al. 2001). This observation contrasts with clinical experience showing that women have a greater incidence of anxiety disorders, as compared to men (Bekker and van Mens-Verhulst 2007) and that anxiety disorders are highly prevalent among aged human subjects (Gum and Cheavens 2008). When compared to men women show an almost 2 times higher incidence of anxiety disorders (Vesga-López et al. 2008) and had significantly higher rates of co morbid mood disorders (except bipolar disorder) and anxiety disorders (except social anxiety disorder). Most importantly, copying strategies were different among genders, men showing lower rates for treatment seeking. Interestingly, animal studies report sex differences in exploratory and defensive responses to threatening stimuli (e.g., Shekhar et al. 2001), reflecting different copying strategies between males and females. These findings are apparently the result of exposure to sex-specific gonadal steroids that also exert an important influence on anxiety levels (Mitev et al. 2003). Anxiety disorders are highly prevalent in elderly persons, and they are associated with functional impairment, poorer quality of life, and adverse long-term consequences such as cognitive decline (Lenze and Wetherell 2009). Although these observations emphasize the importance of daily-life stressors in the shaping of anxiety disorders little is known about specific aspects of unhealthy ageing. This fact is paralleled by the paucity of morphological changes in animal models of aging that display an anxious phenotype (Pêgo et al. 2006).

Stress models have been widely used for studying anxiety and testing the efficacy of potential anxiolytic substances. The various models used differ in two important respects, namely duration of exposure to stress (acute vs. chronic) and the nature (quality) of stress imposed. The pitfall of the many anxiety tests in which acute stressors have been applied to induce an anxiety "state" is that the deleterious effects of stress on anxiety-related behavior are only really expressed when stress is applied chronically. Moreover, the suitability of such models has been questioned since testing anxiety responses in otherwise "normal" animals does not reproduce

the indolent course of the onset, or the nature of, anxiety disorders. In fact, the inadequate response to stressful challenges that characterizes chronic stress is the end-result of the exhaustion of several immediate stress-responsive systems and is time-dependent (Cerqueira et al. 2007a; McLaughlin et al. 2007). Our experimental observations (Cerqueira et al. 2005b; Pego et al. 2008) have shown that most biological markers associated with chronic stress (e.g., poor coat quality, occurrence of gastric ulcers, impaired weight gain, etc.) only develop overtly after 2–3 weeks of exposure to stress; a similar time-lag is observed with respect to the manifestation of the stress-induced anxious phenotype. Thus, other authors (e.g., Vyas et al. 2002) who used stress paradigms similar to those used by us (Cerqueira et al. 2005b; Pego et al. 2008), albeit over shorter periods, failed to observe behavioral traits. Interestingly, clinical cases of anxiety disorders do not appear until well after the presumed causal stressor, being dependent on a prolonged period of sensitization (e.g., generalized anxiety disorder) (American Psychiatric Association 1994). Also, PTSD and several specific phobias often have a particularly late onset (American Psychiatric Association 1994).

With respect to the nature of the stressor(s) applied, one should keep in mind that stressors can be physical (e.g., exposure to cold or hot temperatures, food deprivation, etc.) or psychological (e.g., restraint, overcrowding, social dominance, etc.), and certain paradigms may combine elements of both; further, the stressors may be given in a predictable (order of multiple stressors, time of day) or unpredictable fashion. Psychological stressors, in particular social stressors, recapitulate "real life" situations and are thought to more closely mimic the situation in clinical cases. The (un)predictable presentation of stressful insults is considered a useful amplification factor, i.e., reinforces disruption of stress-coping mechanisms; it is known that predictable events, even if unavoidable and inescapable are more aversive both, in terms of behavior and incidence of gastric ulcers (Gliner 1972). The anticipation of the arrival of random and unpredictable stimuli induces a state of permanent alertness and preparedness to mount a prompt and appropriate behavioral and physiological response. The latter responses are perpetuated by the persistently active HPA axis which, as mentioned before, results in deleterious effects on the physical and behavioral well-being of the individual. To once again draw parallels with the clinical setting: in PTSD and obsessive-compulsive disorders, where anxiety prevails as an underlying condition, patients report feelings of anxiety in response to ordinary daily life events, suggesting a diminished threshold of alertness.

4 The Neurocircuitry of Stress – Implications for Anxiety

Stimuli such as stress and fear conditioning that require the assembly and integration of information from multiple sensory input-modalities are processed by limbic centers that regulate emotional behavior (amygdala and extended amygdala related to fear and anxiety) (LeDoux 2000, 2007), memory and learning (hippocampus)

and executive and cognitive functions (PFC) (Maier et al. 2006). Acting in a coordinated way, the intricate neuronal circuitries that characterize these networks establish a temporal and contextual framework of interpretation that determines the aversive/rewarding value of the particular stimulus. Characteristically, the modulatory action that limbic structures exert on the HPA is not conveyed by direct excitatory pathways; rather, information from the different limbic inputs are integrated through the modulatory action of neurons located in the hypothalamus or in the BNST (Herman et al. 1994, 2003); the latter, in turn, convey signals to the neurosecretory neurons of the PVN. More specifically, GABAergic neurons projecting from the BNST exert an inhibitory tone over the PVN, under the control of excitatory glutamatergic input originating in the PFC and hippocampus (Cullinan et al. 1993) and of an inhibitory GABAergic/CRF input from the central and medial nuclei of the amygdala (Herman et al. 2005; Prewitt and Herman 1998).

4.1 Structural and Functional Organization of the Amygdala

The amygdala (Mandelkern or almond-shaped nucleus; Burdach 1819–1822), a deep temporal structure with rich interconnections with the hippocampus and PFC, has long been known to be involved in emotional behavior (Goddard 1964; Robinson 1963; Weiskrantz 1956). Lesions in the amygdala induce disturbances in emotional and social behavior, attention, and memory consolidation/extinction (Bucy and Kluver 1955; Weiskrantz 1956). The lesions in the original studies were later recognized to include the BNST, and recent work using more selective lesion and pharmacological approaches have pointed to the differential role of the amygdala and BNST in the control of emotional behavior (Davis 1992, 1998, 2006; Davis et al. 1997; Lee and Davis 2007a).

The early view that the amygdala is a relatively homogeneous structure has been challenged by more recent studies (De Olmos et al. 2004; Swanson and Petrovich 1998), that have proposed a new subdivision that takes into consideration connectional, neurochemical and detailed anatomical data. Briefly, the following subdivisions can be recognized (Swanson and Petrovich 1998): (1) the caudal olfactory system (nucleus of the lateral olfactory tract, cortical nucleus and postpiriform and piriform-amygdalar areas), which displays the typical laminar arrangement of cortical areas and forms the caudal part of the piriform lobe and receive projections that originate in the main and accessory olfactory lobes (Alheid and Heimer 1988); (2) the frontotemporal system (lateral, basal and posterior nuclei), a ventral extension of the claustrum that forms part of the deepest cortical layers of the temporal, endopiriform and frontal lobes (Swanson and Petrovich 1998), that receives privileged information from several sensory modalities (somato-sensorial, auditory, visual) (LeDoux 2007), and is intricately connected to the hippocampus and the PFC (Akirav and Maroun 2007; Canteras and Swanson 1992; Petrovich et al. 2001) establishing a reciprocal modulatory influence over executive and mnesic (Bishop 2007; McEwen 2007)

functions and emotional behavior (Akirav and Maroun 2007; Phillips and LeDoux 1992); iii) a specialized ventromedial expansion of the striatum (central [CeA) and medial [MeA] amygdaloid nuclei, and anterior amygdaloid area) that exerts an inhibitory output modulating basal activity of several brainstem and basal forebrain nuclei through the CeA nucleus, the main output of the amygdala, relaying processed information from the amygdaloid nuclei.

4.2 Structural and Functional Organization of the BNST

Johnston (1923) initially described the BNST as a ventral extension of the pallidum, that forms a continuum extending from the olfactory tubercule and nucleus accumbens anteriorly and the amygdala posteriorly. It receives massive projections from adjacent areas including the amygdala through a bundle of fibers that forms the stria terminalis. In fact, the lateral and medial parts of the BNST form two corridors of sublenticular neurons that are contiguous with the CeA and MeA and have, therefore, led to their being designated as the central and medial extended amygdala (Alheid 2003). Extensive studies by Swanson and collaborators (Dong et al. 2001; Ju and Swanson, 1989a; Ju et al. 1989b; Swanson 1998) have resulted in different proposals about the anatomical organization of the BNST which is still under debate. The BNST are parceled into the major anterior and posterior divisions (relative to stria terminalis main fiber bundles); the former can be further parceled into medial and lateral groups. The medial division, which includes the anterodorsal and anteroventral areas (Dong and Swanson 2006a, b, c), is characterized by dense projections to hypothalamic regions that are implicated in neuroendocrine regulation; the lateral group of the anterior division (which includes the anterolateral area) is characterized by projections to hypothalamic areas concerned with autonomic and energy homeostasis as well as feeding behavior (Dong and Swanson, 2004).

The importance of the BNST in the activation of the HPA axis (Choi et al. 2007; Herman et al. 2003) may be appreciated when its anatomical connections with the neuroendocrine hypothalamus (Dong and Swanson 2006a; Dunn 1987) as well as with other brain regions such as the brainstem and ventral striatopallidal – all areas that regulate defensive, sexual, ingestive, and exploratory behaviors – are considered. Additionally, the BNST, along with the nucleus of tractus solitarius, preoptic area and dorsal hypothalamus, is one of the relay stations where inputs from stress-sensitive areas of the cortex and limbic systems are conveyed and integrated to elicit adequate activation of the HPA axis (Herman et al. 1994, 2003, 2005; Herman et al., 1997).

The role of the BNST in emotional behavior has been extensively explored by Davis and collaborators (Davis 1986, 1992, 1998; Davis et al. 1997; Lee and Davis 2007a). Although the phenotypic expressions of BNST and amygdala activities resemble each other closely, differences that confer distinct roles to each of these structures in the control of emotional behavior can be recognized. Involvement of the BNST is evident in paradigms in which behavior is influenced by long-duration

stimuli (e.g., CRF- or light-enhanced startle) and in paradigms that assess the persistent behavioral effects of even a brief unconditioned stressor (e.g., long-term shock-dependent increases in baseline startle, conditioned defeat, the effects of inescapable shock in the learned helplessness model or on subsequent eye blink conditioning) (Davis et al. 1997; Walker et al. 2003). Despite behavioral outcomes that are often similar in form (i.e., increased startle), the BNST does not appear to mediate behaviors elicited by specific short-lasting threats in which the period of endangerment is finite (i.e., fear-potentiated startle or freezing to a discrete conditioned fear stimulus) (Walker et al. 2003). These characteristics of BNST-dependent behavior suggest its special role in anxiety, as opposed to fear, insofar that anxiety, unlike fear, is typically viewed as a sustained state of apprehension that is unrelated to immediate environmental threats (Walker et al. 2003).

In summary, whereas the amygdala is transiently activated in fear conditioning (an emotional state elicited by explicit neutral clues), anxiety (a similar emotional state thought to be elicited by diffuse contextual clues) seems to result from persistent activation of the BNST (Davis et al. 1997).

4.3 Structural Remodeling of the Amygdala and BNST – Implications for Anxiety and the Stress Response

Mounting evidence indicates that the amygdala and BNST are sensitive to the chemical/humoral mediators of stress (Pego et al. 2008; Rubinow et al. 2007; Vyas et al. 2002, 2003). Different models of stress which increase the level of anxiety have been found to induce structural reorganization of neurons in the basolateral nucleus of the amygdala and in the anteromedial division of the BNST (Pego et al. 2008; Vyas et al. 2002, 2003). One of the most surprising observations in our own studies was the relatively good preservation of the morphology of the amygdala after exposure to chronic unpredictable stress, contrasting with a significant level of plasticity in the BNST. The absence of structural changes in the amygdala after chronic stress is consistent with our observation that chronic stress does not produce changes in fear-acquisition (Pego et al. 2008). Although such observations may seem to be at odds with previous reports (Vyas et al. 2002), they highlight the stimulus-dependent specificity of the neuronal circuitry responsible for generating anxiety (Dagnino-Subiabre et al. 2005; Gewirtz et al. 1998; Miracle et al. 2006; Rosen et al. 1998). The inescapability associated with the chronic immobilization stress paradigm used by Vyas et al. (2002) may trigger an emotional phenotype that results in the expression of fear responses rather than anxiety, explaining the stress-induced morphological changes in the amygdala observed by those authors; in fact, the same laboratory failed to observe anxiety when a chronic unpredictable stress paradigm was applied (Mitra et al. 2005; Vyas et al. 2002).

In fact, by playing a key role in the learning and long-term storage of fearful memories, the amygdala appears to be essential for the establishment of strong associations between overtly threatening cues (or contexts) and an aversive condition that must be avoided. This process represents a specific form of learning that provides biological advantage, inasmuch as it diminishes the probability of encounters with harmful/threatening events. Importantly, however, if this process were to be triggered by unspecific stressors experienced in daily life (the so-called "allostatic load" – see McEwen, 2003), survival would be jeopardized by an overactive "all-or-nothing" system. Thus, the refractoriness of the amygdala to certain stressful situations makes evolutionary sense. The BNST, on the other hand, is known to be involved in specific aspects of anxiety behavior (Walker et al. 2003). Consequently, the structural changes in the BNST observed after chronic stress correlate with the behavioral responses to stress.

5 Final Remarks

We have reviewed the organization and structure of areas of the brain involved in the coordination of the endocrine response to stress. Stressful events are perceived by limbic structures which generate a contextual image and integrate polysensory information. The amygdala and BNST play important roles in the assessment of emotional values and in the formation of fearful memories. Supporting this, recent data suggest that the structural changes that occur in these areas after exposure to stress hormones are probably correlated with anxiety behavior. Future research should focus on identifying the molecular processes and mechanisms that occur in amygdala and BNST, therefore extending our understanding of the neurobiological basis of anxiety and improving the therapeutic options for anxiety disorders.

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Animal Models of Anxiety and Anxiolytic Drug Action

Dallas Treit, Elif Engin, and Kris McEown

Contents

Abstract Animal models of anxiety attempt to represent some aspect of the etiology, symptomatology, or treatment of human anxiety disorders, in order to facilitate their scientific study. Within this context, animal models of anxiolytic drug action can be viewed as treatment models relevant to the pharmacological control of human anxiety. A major purpose of these models is to identify novel anxiolytic compounds and to study the mechanisms whereby these compounds produce their anxiolytic effects. After a critical analysis of "face," "construct," and "predictive" validity, the biological context in which animal models of anxiety are to be evaluated is specified. We then review the models in terms of their general pharmacological profiles, with particular attention to their sensitivity to 5-HT_{1A} agonists and antidepressant compounds. Although there are important exceptions, most of these models are sensitive to one or perhaps two classes of anxiolytic compounds, limiting their pharmacological generality somewhat, but allowing in depth analysis of individual mechanisms of anxiolytic drug action (e.g., $GABA_A$

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agonism). We end with a discussion of possible sources of variability between models in response to 5-HT_{1A} agonists and antidepressant drugs.

Keywords Anxiety · Animal models · Face validity · Construct validity · Biological validity · Anxiolytic · Antidepressant · Drug receptors

1 Animal Models of Anxiety and Anxiolytic Drug Action: Introduction

The validation and development of animal models of anxiety (e.g., the elevated plus-maze) has relied mainly on the putative models' sensitivity to well-established anxiolytic drugs, such as the benzodiazepines (e.g., diazepam). It is important to emphasize, however, that the purpose of this work was to establish the predictive validity of the animal model with respect to known anxiolytic compounds, and *not* to establish the clinical utility of indirect $GABA_A$ receptor agonists such as diazepam. A pharmacologically valid model also had to discriminate between "anxiolytic" compounds (e.g., diazepam) and "nonanxiolytic" compounds, such as imipramine, an antidepressant drug. For example, from the late 1960s to the late 1980s, if an antidepressant drug registered the same as diazepam in an animal model of anxiety, it was designated a "false positive." The more "false positives" a putative model accumulated, the less "valid" it became in terms of predictive validity, and the less useful as a potential "screening test" for finding novel anxiolytic compounds (Treit 1985a, b).

Unfortunately, for animal models of anxiety the pharmacological treatment of human anxiety disorders began to change, almost imperceptibly, after an early report of the successful treatment of panic disorder with imipramine (Klein 1964). The treatment of anxiety disorders such as panic with antidepressant drugs steadily increased through the 1990s (e.g., Tyrer and Tyrer 1994), and by the new millennium they had emerged as the frontline pharmacological treatment for all human anxiety disorders (e.g., Hoffman and Mathew 2008; Borsini et al. 2002). Animal models of anxiety had been validated mainly on their differential sensitivity to benzodiazepine anxiolytics, and were, not surprisingly, insensitive to the anxiolytic effects of antidepressant drugs. More often than not, these animal models completely failed to detect antidepressant drugs, or registered them as "anxiogenic" (Treit et al. 2003; Borsini et al. 2002).

This deficiency, and the unpredictable effects in these models of 5-HT_{1A} anxiolytics such as buspirone before it, has raised serious questions about the "validity" of so-called animal models of anxiety (e.g., Treit et al. 2003). Apart from the general failure of researchers to study the chronic effects of these compounds, which are clinically more relevant than their acute effects, we do not believe that the problem is specific to antidepressant drugs, for the following reason. Imagine for a moment that the history of therapeutic drug discovery had been reversed,

because the focus of clinical interest at the time had differed slightly. We know, for example, that clinicians in the early 1950s had noticed that the anti-tuberculosis drug iproniazid had a peculiar side-effect in patients: it elevated the patient's mood, sometimes to the point of euphoria (Pletscher 1991). Imagine now that what impressed these clinicians even more was another "side-effect" that gradually emerged after chronic treatment with iproniazid: patients became progressively less anxious about their condition, and more tractable than patients treated with other anti-tuberculosis drugs. If the second (perhaps fictitious) observation had attracted more clinical attention than "mood elevation," iproniazid and its congeners might have been studied in psychiatric patients suffering from anxiety, and eventually have become the first, clinically effective "anxiolytic" drugs. Other drugs would have been assessed for their "anxiolytic" potential, and some, such as imipramine, would emerge serendipitously. In any case, a clinical precedent would have been set. In addition, the careful study of the effects of these drugs on brain function would lead to the following generalization: compounds that possessed "anxiolytic" effects in patients invariably elevated brain monoamines. After repeated demonstrations of this common effect, the "monoamine hypothesis of anxiety" would emerge and become dominant. As this "monoamine hypothesis" was tested further, at both behavioral and neural levels, the importance of reliable "animal models of anxiety" would be reinforced. Their "predictive validity" would depend on their relative sensitivity to the effects of chronic iproniazid and other "standard" anxiolytic drugs (e.g., imipramine), and their relative *insensitivity* to "nonanxiolytic" drugs. At some point along this not entirely fanciful path to drug discovery, the predictive validity of these early animal models of anxiety would be challenged by a little known, indirect $GABA_A$ receptor agonist called diazepam, which registered as a "false positive" in the models, but was eventually shown to be anxiolytic in clinical populations.

The point of this modest revision of history, of course, is not to disparage our inability to see into the future, but to illustrate the fundamental weakness of the pharmacological "validation" of animal models of anxiety: it is always constrained by historical accident, and is completely post hoc. If and only if the test drug has been validated clinically can it be used to evaluate the "predictive" validity of a particular animal model of anxiety. A *novel* compound that has anxiolytic-like effects in the model (e.g., diazepam in the above scenario) would initially be viewed as a "false positive" and would detract from the predictive validity of the model – at least, until overwhelming clinical evidence suggested otherwise.

In summary, what counts as a "false positive" for an animal model of anxiety is time-sensitive and subject to clinical revision. "False negatives" are not subject to these historical vagaries, but in their case, attempts must be made to show that the model can, with appropriate tweaking, be made sensitive to the previously unrecognized classes of anxiolytic drug (e.g., antidepressants). In an absolute sense, then, no animal model of "anxiety" can be summarily invalidated simply on the basis of a "false positive" or a "false negative."

As the notion of pharmacological or "predictive" validity becomes more ambiguous, other criteria such as "face" validity and "construct" validity become more

attractive. At the very least, these aspects of validity may offer some distraction from the uncomfortable circularity of predictive validity (for original guidelines, definitions, and applications of "face" and "construct" validity in the social sciences, see Cronbach and Meehl 1955; for recent quantitative approaches see Westen and Rosenthal 2003). "Face" validity, however, means only that the test or model *appears* to measure anxiety, not that it actually measures anxiety. Face validity, in this light, is far from an unambiguous substitute for predictive validity. Predictive validity is at least based on the quantifiable behavior of experimental animals under the influence of a chemically defined set of molecules, not just the opinion of a set of observers. It seems curious, then, that "face validity," as used in the experimental literature, often implies something more concrete than a common "understanding" among observers, expert, or otherwise (e.g., Bourin et al. 2007).

If face validity is a weak criterion, "construct validity" is a strong criterion (Cronbach and Meehl 1955), mainly because the latter is central to all other "validity" issues (e.g., Westen and Rosenthal 2003). Construct validity specifically addresses the question: "does the test or model *actually* measure or represent what it was intended to measure or represent?" This question rarely can be answered by experimental data alone, and must be evaluated in the context of a scientific *theory* of the construct in question. For animal models of anxiety, however, this amounts to an almost insurmountable task for three reasons. First, there is no widely accepted or established scientific theory of "anxiety" in humans. Second, there is no unambiguous translation of such a theory – if it existed – to animal models of anxiety.[1] Third, even if a tentative translation of this hypothetical theory existed, our empirical understanding of anxiety does not yet have the precision needed for a scientific theory, at least in comparison with other areas of science in which the term "theory" is used (e.g., cell theory).

Returning to the issue of "correlational" validity and the sensitivity of animal models of anxiety to one particular subclass of anxiolytic compounds (e.g., benzodiazepines)—pharmacological validity can be bolstered by documenting behavioral correlates such as increased vocalization or thigmotaxis (e.g., Treit and Fundytus 1988), and physiological correlates such as increased serum corticosteroids or brain norepinephrine release (e.g., Pellow et al. 1985; Bondi et al. 2007; De Boer et al. 1990). Furthermore, selective sensitivity to only one class of anxiolytic compounds (e.g., benzodiazepines) may in fact be advantageous for exploring the molecular mechanisms that underlie the anxiolytic effects of a particular drug class. If there are significant variations in drug response within these "one drug-class" animal models, they may reflect the degree to which the model engages particular receptors underlying the drugs' effects. For example, there may be differences in drug response that reflect differences in the expression of particular receptor subunits (α β γ δ), or differences in the specific subunit combinations of a given receptor

[1]From an evolutionary perspective, one could argue that this is putting the cart before the horse: a scientific understanding of anxiety in humans first requires a detailed understanding of its distal and proximal causes in lower animals.

(e.g., Kaufmann et al. 2003). Conversely, receptors and their subunits can be directly manipulated using genetic (e.g., "knock-out" or "knock-in") techniques, and the behavioral effects measured in animal models of anxiety (e.g., Finn et al. 2003; Low et al. 2000; McKernan et al. 2000; Marowsky et al. 2004; Kaufmann et al. 2003; Cryan and Holmes 2005). Working in parallel, biochemists can rapidly synthesize hundreds of structural analogues of known anxiolytic compounds[2], based on their relative affinity for particular receptor subtypes or receptor subunits (e.g., Caliendo et al. 2005). With these points in mind, our general approach here is to provisionally accept the utility of *pharmacologically* valid animal "screening" tests of anxiolytic drugs (i.e., purely correlational models), whether or not they are sensitive to antidepressant drugs. However, the overall relevance of pharmacologically valid models to "anxiety" must be assessed in the light of three biological imperatives, which together form a heuristic framework for evaluating their specific "validity" as animal models of anxiety.

First, there should be some formal correspondence between the behavioral expressions of fear in the animal model (e.g., avoidance), the physiological correlates of these behaviors (e.g., increased corticosterone), and their expressions in humans (i.e., isomorphism). Second, in the absence of clear isomorphism, there should be some continuity of biological *function* between behavioral responses in the animal model and fear responses in humans (e.g., defense). Third, and specifically at the neural level, there should be considerable conservation of the underlying brain mechanisms of fear and anxiety across mammalian species (either by homology or analogy), and these should be engaged during specific fear reactions. Together, these biological constraints are dictated by the fundamental role that fears or anxieties normally play in the survival of organisms in their natural habitats. There is no reason to believe that a model that does not conform to any of these biological imperatives has any relationship to anxiety. For example, PTZ-induced convulsions in mice are exquisitely sensitive to suppression by benzodiazepine anxiolytics, but the convulsive response itself has little or no isomorphism with fear or anxiety, is functionally unrelated to defense, and is unlikely to reflect the same brain mechanisms as those evolutionarily conserved for survival. As such, the PTZ test is not a plausible "animal model of anxiety," despite its pharmacological validity as a "test" or predictor of anxiolytic drug action (Treit 1985a, b). At the same time, there is no reason why the PTZ "test" could not serve as a valuable "model" of epileptic convulsions, their neurochemical mechanisms and pharmacological treatments (e.g., Luszczki 2008). In some sense, then, "tests" and "models" can only be distinguished by their use in specific experimental contexts (Kalueff and Murphy 2007).

In summary, it is from this overarching biological perspective that we now review the validity of current animal models of anxiety and anxiolytic drug action.

[2]For readers interested in investigational drugs, their anxiolytic properties and mechanisms of action, see Chapters Metabotropic Glutamate Receptors (W. Spooren), Neuropeptides (T. Steckler), and Cannabinoids (C. Wotjak) in the present volume.

1.1 Conflict Tests

In the "Geller" and the "Vogel" conflict tests, a food or water deprived rat is punished with electric shock if it makes a response (e.g., bar-pressing, licking) that other wise results in reward (e.g., food, water). Punishment inhibits rewarded responding, and standard anxiolytic drugs such as diazepam selectively disinhibit punished responding. In so far as conflict between opposing drives may be indicated in human anxiety, "conflict" models may also have some degree of isomorphism and homology (Treit 1985a, b).

There are a number of procedural variations that add to the complexity of conflict tests, and thus affect the ease with which anxiolytic compounds can be assessed (Treit 1985a, b). For example, most versions of the standard Geller conflict test take weeks of training before drug testing can begin, whereas the Vogel conflict procedures take little or no pretraining. The advantage of the more complex procedures, however, is that control measures for nonspecific drug effects are embedded into the test (e.g., periods of nonpunished responding). Use of less complex conflict procedures, however, has gradually become more prevalent over the past 20 years. For example, our ISI Web of Science search of research papers published between 1988 and 2008 showed that the Vogel test is cited nearly five times more than the Geller test. This trend has prevailed mainly because the Vogel test (1) is procedurally more practical, (2) produces results that differ little from the Geller test, and (3) allows immediate and rapid testing of target compounds. Since there is little to distinguish the two tests other than experimental expedience, the results from each are combined below.

Anxiolytic drugs, especially the benzodiazepines, increase punished responding, while having little or no effect on other responses (Table 1). So called nonanxiolytic compounds (e.g., neuroleptics, analgesics, stimulants) generally have no effect, or suppress punished responding further. In addition to standard benzodiazepine anxiolytics, alprazolam, which has both anxiolytic and antidepressant properties, produced reliable, dose-dependent anticonflict effects in rats across a wide range of doses (Ellis et al. 1990; Giusti et al. 1991; Hascoet and Bourin 1997). The anti-conflict effect of alprazolam was apparent after both acute and chronic administration. Similarly, a number of standard antidepressant drugs such as the TCAs imipramine, desipramine, and amitriptyline all produced significant anticonflict effects after chronic or subchronic administration, but not after acute administration (Commissaris and Hill 1995; Fontana and Commissaris 1988; Fontana et al. 1989). Chronic bupropion, mianserin, and trazodone also produced significant anticonflict effects (Commissaris et al. 1990), as did the MAOIs phenelzine and pargyline (Commissaris et al. 1995; Fontana et al. 1989). These findings suggest that conflict tests may be broadly sensitive to the anxiolytic effects of benzodiazepines given acutely, and antidepressant drugs given chronically, although standard SSRIs such as fluoxetine have yet to be assessed.

In contrast to their reliable sensitivity to the anxiolytic effects of benzodiazepines and antidepressant drugs, the sensitivity of the conflict tests to the anxiolytic

Table 1 Summary of findings with Vogel-type and Geller-type conflict tests following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Ellis et al. (1990), Giusti et al. (1991), Hascoet and Bourin (1997), Kennett et al. (1998), Rudzik et al. (1973), Soderpalm et al. (1989), Kapus et al. (2008), Mathiasen et al. (2008), Gleason and Witkin (2007), Wesolowska and Nikiforuk (2007), Popik et al. (2006), Moreira et al. (2006), Tatarczynska et al. (2004), Ballard et al. (2005), Busse et al. (2004)	Dekeyne et al. (2000), Deren-Wesolek et al. (1998), McCloskey et al. (1987), Meneses and Hong (1993), Pich and Samanin (1986), Sanger (1992), Schefke et al. (1989), Weissman et al. (1984), Yamashita et al. (1995), Young et al. (1987), Stefanski et al. (1992), Bojarski et al. (2006), Vaidya et al. (2005), Jurczyki et al. (2004), Liao et al. (2003)	Chaki et al. (2005)	Commissaris and Hill (1995), Fontana and Commissaris (1988), Fontana et al. (1989), Viglieccа et al. (2007)	Fontana et al. (1989), Commissaris et al. (1995)
(−)		Costello et al. (1991)			
(0)		Costello et al. (1991), Gardner (1986), Kennett et al. (1998), Sanger (1990), Witkin and Perez (1990)			

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

effects of 5-HT_{1A} drugs such as buspirone has been erratic (Howard and Pollard 1990). Furthermore, when anxiolytic effects of 5-HT_{1A} drugs are detected, they are often small, and occur over a much narrower dose range (e.g., Dekeyne et al. 2000; Deren-Wesolek et al. 1998; Sanger 1992; Schefke et al. 1989). Howard and Pollard (1990) studied the effects of buspirone in the conflict test under a wide variety of experimental conditions and failed to find a robust anxiolytic effect under any condition. Other researchers have reported no significant effects of buspirone in the conflict tests, or even dose-related *decreases* in punished responses (e.g., Brocco et al. 1990; Costello et al. 1991; Gardner 1986; Vaidya et al. 2005; Witkin and Perez 1990; but see Bojarski et al. 2006).

Despite their questionable sensitivity to 5-HT_{1A} compounds, conflict tests have broad utility for detecting the anxiolytic effects of benzodiazepine and antidepressant drugs. The exact conditions under which reliable anxiolytic effects of 5-HT_{1A} compounds can be detected in these tests remain to be determined.

1.2 Fear Potentiated Startle

The magnitude of rats' naturally-occurring startle reflex to an acoustic stimulus is "potentiated" when the acoustic stimulus is presented together with a cue (e.g., light) that has previously been paired with shock (Brown et al. 1951; Chi 1965; Davis 1986a, b; Richardson et al. 1999). The fact that the startle response has a nonzero baseline makes it possible to distinguish the effects of a drug on base-line startle (acoustic stimulus alone) from its effects on "potentiated startle" (acoustic stimulus plus conditioned fear cue). Insofar as human fears and phobias can be acquired through experiences analogous to "fear conditioning" in rats, and insofar as conditioned fear stimuli (e.g., light) can "potentiate" unconditioned responses such as startle, the model appears to have some degree of isomorphism and homology with human anxiety. It should be noted, however, that Seligman (1970) and others have made persuasive arguments that conditioned fears to artificial stimuli such as lights and tones may have little relevance to the etiology of human fears and phobias.

As seen in Table 2, fear-potentiated startle is reliably suppressed by benzodiazepine anxiolytics, and also by 5-HT_{1A}-type anxiolytics such as buspirone. The anxiolytic effects of 5-HT_{1A}-type anxiolytics occur across a wide range of doses, and the magnitude of these effects nearly matches that of benzodiazepine anxiolytics (Davis et al. 1988; Kehne et al. 1988; Mansbach and Geyer 1988; Nevins and Anthony 1994). The behavioral profile of 5-HT_{1A}-type anxiolytics in the fear-potentiated startle test is particularly impressive when compared to their weak and inconsistent profile in the conflict tests (see above). While fear-potentiated startle was unaffected by "nonanxiolytic" compounds such as naloxone, nicotine, *d*-amphetamine, and cinanserin (Hijzen et al. 1995; Casella and Davis 1985; Davis and Casella 1988), "false positives" have been reported to morphine and haloperidol (Davis 1986b; Hijzen et al. 1995; Winslow et al. 2007).

Table 2 Summary of findings with the fear-potentiated startle test following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Berg and Davis (1984), Brodkin et al. (2002), Davis (1979), Hijzen and Slangen (1989), Joordens et al. (1996), Martin et al. (2002), Pietraszek et al. (2005), Risbrough et al. (2003), Santos et al. (2005), Schulz et al. (2001), Serradeil-Le Gal et al. (2005), Tizzano et al. (2002), Winslow et al. (2007)	Davis et al. (1985), Joordens et al. (1998), Mansbach and Geyer (1988), Nevins and Anthony (1994), Paschall and Davis (2002), Risbrough et al. (2003)			
(−)					
(0)		Brodkin et al. (2002), Winslow et al. (2007)	Joordens et al. (1996)	Casella and Davis (1985), Hijzen et al. (1995)	

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

Antidepressant compounds such as TCAs and SSRIs, however, have been ineffective in the fear-potentiated startle test (Casella and Davis 1985; Hijzen et al. 1995; Joordens et al. 1996). Of even more concern is the finding that yohimbine and FG-7142, drugs that have been shown to be *anxiogenic* in humans, also suppress "fear" potentiated startle (Risbrough and Geyer 2005). Until this paradoxical result can be resolved, the pharmacological validity of this model is questionable. Nevertheless, fear-potentiated startle, and fear conditioning paradigms in general have been extremely valuable behavioral models for dissecting the role of amygdala in fear and anxiety (e.g., Lang et al. 2000; LeDoux 2000).

On the other hand, the fear-conditioning models have difficulty differentiating drug or lesion effects on learning and memory from specific effects on anxiety responses (see Engin and Treit 2008 for a discussion). A variant of the fear-potentiated startle model called the light-enhanced startle has been developed that appears to overcome this deficiency (Walker and Davis 1997). In this version of the model, rats show a potentiated startle response in a brightly illuminated environment, compared to a dark environment, and this "unconditioned" potentiation is used as a measure of fear. Light-enhanced startle was found to be sensitive to the effects of benzodiazepines and 5-HT_{1A} agonists (de Jongh et al. 2002; Walker and Davis 2002), but SSRIs were ineffective, as in the standard fear-potentiated startle model (de Jongh et al. 2002).

Commissaris et al. (2004) have argued that another problem with the original fear-potentiated startle model is that the testing occurs in the extinction phase (i.e., the light is no longer paired with the shock). It follows that the model is not suitable for repeated testing of a drug, as the fear response itself is diminishing as a function of extinction test trials. Consequently, they have proposed a "startle-potentiated" startle paradigm, where the shock is removed from the classical model and noise-only versus *light+noise* groups are subjected to the test repeatedly. Although the two groups did not differ in startle amplitude initially, repeated trials led to an increase in the startle amplitude of the light+noise group, supposedly through the anticipation of the noise stimulus. The startle amplitude for the light +noise group in subsequent testing was reduced by buspirone or mixed anxiolytic-antidepressant alprazolam. However, it should be noted that these drugs seemed to influence the baseline (i.e., noise only) startle in this version of the test. Thus, further pharmacological characterization of the "startle-potentiated" startle test is required before it can be employed as a model of anxiolytic drug action.

Overall, the startle models are broadly sensitive to benzodiazepine and 5-HT_{1A}-type anxiolytics, and have the advantage of providing a baseline acoustic reflex to which the fear-potentiated reflex can be compared. This simplifies the problem of distinguishing drug effects on anxiety from nonspecific drug effects on the baseline startle reflex itself. However, antidepressants, which have clinically-proven anti-anxiety effects, fail to register in any version of the model, thus restricting its generality as a test of anxiolytic compounds.

1.3 Defensive Burying

The defensive burying model is based on a species-typical response of rodents confronted with a nociceptive or predator-related stimulus (for reviews see Treit 1985a, b; De Boer and Koolhaas 2003; Treit and Pinel 2005; Lapiz-Bluhm et al. 2008). Rodents spray substrate materials (e.g., sand) toward the threat stimulus (e.g., snake) with rapid, alternating movements of the forepaws, while avoiding direct contact (i.e., "burying behavior"; Pinel and Treit 1978). Burying can be induced in the laboratory with an electrified shock-probe, which protrudes through one of the walls of a Plexiglas chamber. Rats shocked once from the stationary probe stereotypically spray bedding material on the floor of the chamber toward and over the probe, while avoiding further contact with the probe (e.g., Pinel and Treit 1978; Treit 1985a, b; Treit et al. 1994; Echevarria et al. 2005; Bondi et al. 2007; Engin and Treit 2008). The amount of time spent burying the shock probe is taken as the primary index of anxiety, and the number of contacts with the probe is a secondary index (Treit 1990; Treit et al. 1981). A genuine "antianxiety" effect in this model is indicated by decreased burying behavior and/or increased probe contacts, independent of changes in pain sensitivity or general activity levels (Treit et al. 1990).

Plasma levels of corticosteroid and catecholamines are increased during the burying test, and these increases can be suppressed by standard anxiolytic drugs (de Boer et al. 1990). Concomitant changes in these "stress" hormones in response to shock and anxiolytic drugs add to the correlational validity of the model. More recent studies have corroborated these findings, documenting increases in heart rate, blood pressure, catecholamines, prolactin, ACTH, and corticosteroids in response to the shock-probe (Lapiz-Bluhm et al. 2008). Interestingly, burying behavior is also sensitive to the delayed anxiogenic effects of repeated, unpredictable stress, similar to the delayed effects of stress in human anxiety disorders (e.g., post-traumatic stress disorder; Matuszewich et al. 2007). In addition to these stress correlates, burying behavior also seems to have biological validity insofar as it represents an evolved adaptation of rodents to natural threat stimuli (homology). Although it has limited isomorphism with human anxiety responses – or to avoidance responses in general – the burying response may have functional relevance insofar as it can block or neutralize dangerous or threatening stimuli. Finally, many of the defining features of anxiety in humans (sweating, dizziness, tachycardia, trembling) may only emerge full-blown when *approach* is required to a threatening stimulus or situation (McNaughton and Corr 2004).

As seen in Table 3, shock-probe burying is quite sensitive to benzodiazepine anxiolytics, as well as 5-HT_{1A} agonist compounds. 5-HT_{1A} agonists failed to selectively suppress burying behavior only once, at a very high dose of buspirone (64 mg kg^{-1}) that also suppressed activity in general (Craft et al. 1988; Meert and Colpaert 1986). Antidepressants have not been extensively tested in this model, although some tricyclic antidepressants (e.g., desipramine) and a mixed SSRI/5-HT_{1A} agonist produced anxiety-reduction in the model (Table 3).

A variant of the defensive burying model – "marble burying" in mice – was developed by Broekkamp et al. (1986) and Njung'e and Handley (1991a, b). In this test, 20 glass marbles are distributed evenly on the bedding material that covers the floor of a plexiglass chamber. The mice are placed individually in the chamber and are taken out 30 min later. The number of marbles "buried" is used as an index of anxiety, although the actual "burying behavior" of mice is not measured in this version of the test. A large number of classical anxiolytics, such as diazepam, chlordiazepoxide, alprazolam, clonazepam, and flunitrazepam, have been shown to reduce marble burying in mice (Broekkamp et al. 1986; Li et al. 2006; Young et al. 2006; Nicolas et al. 2006). Although 5-HT_{1A} agonists also reduce marble burying (Young et al. 2006; Nicolas et al. 2006; Njung'e and Handley 1991a, b), the effect was observed only at high doses that tend to reduce activity levels in general (Li et al. 2006; Nicolas et al. 2006).

Both tricyclics and SSRIs have been shown to reduce marble burying (Broekkamp et al. 1986; Li et al. 2006; Njung'e and Handley 1991a, b; Ichimaru et al. 1995; Nicolas et al. 2006; Harasawa et al. 2006). However, the suppressive effect of tricyclic antidepressants on marble burying is not always robust (e.g., Ichimaru et al. 1995; Nicolas et al. 2006), and in studies where the effect is clear, the reduction in burying is produced at doses that also suppress general locomotor activity (e.g., Broekkamp et al. 1986). Thus, while the SSRIs have a relatively

Table 3 Summary of findings with the defensive burying test following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Beardslee et al. (1990), Blampied and Kirk (1983), Degroot and Nomikos (2004), Fernandez-Guasti and Martinez-Mota (2003), Fernandez-Guasti et al. (2001), Gomez et al. (2002), Perrine et al. (2006), Picazo et al. (2006), Rohmer et al. (1990), Sikiric et al. (2001), Treit (1985, 1987, 1990), Treit and Fundytus (1988), Treit et al. (1981), Tsuda et al. (1988), Violle et al. (2006), Wilson et al. (2004)	Fernandez-Guasti and Lopez-Rubalcava (1998), Fernandez-Guasti and Picazo (1990, 1997), Fernandez-Guasti et al. (1992), Groenink et al. (1995, 1997), Korte and Bohus (1990), Lopez-Rubalcava (1996), Lopez-Rubalcava et al. (1996, 1999), Picazo et al. (2006), Treit et al. (2001), Treit and Fundytus (1988)	Degroot and Nomikos (2004), Treit et al. (2001)	Bondi et al. (2007), Fernandez-Guasti et al. (1999), Martinez-Mota et al. (2000)	
(−)					
(0)		Craft et al. (1988)		Beardslee et al. (1990)	

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

selective inhibitory effect on marble burying, the effect of tricyclics is less clear. To our knowledge, only one study (Nicolas et al. 2006) reported the effects of MAOIs, and in this case phenelzine reduced marble burying.

Overall, marble burying in mice has been fairly well validated as a correlational model of anxiolytic drug action. However, there are several nonpharmacological issues that detract from its use as an animal model of anxiety. The main problem is that the marbles used in the test may not in fact be anxiogenic. The number of marbles buried is strongly correlated with digging and burrowing behavior, which mice display in the absence of marbles, or any other anxiogenic stimulus; this behavior is nondirected but can coincidentally cover glass marbles. On the basis of these careful observations, Gyertyan (1995) concluded that mice are not "burying" an aversive or threatening stimulus, merely engaging in a species-typical response elicited by a movable substrate. In line with this, Costa et al. (2006) have reported that mice classified into two groups according to their performance in the

marble-burying test (i.e., low-burying, high-burying) did not differ on any measure of anxiety in three other models (i.e., elevated plus-maze, light–dark, hole board).

Njung'e and Handley (1991a, b) suggested that marble burying is a useful screening test for the detection of anxiolytic drugs, even if it did not represent an isomorphic fear response. As a screening test, however, marble burying is less than ideal, since nonanxiolytic and anxiogenic compounds alike can produce "false positives" (Nicolas et al. 2006; Broekkamp et al. 1986). The suggestion that marble burying in mice is more appropriately viewed as a specific model of obsessive-compulsive disorder (Gyertyan 1995; Millan et al. 2002) is interesting, but seems to push the problem of an undefined anxiogenic stimulus beyond the limits of observation, and into the "obsessive thoughts" that presumably drive the mice's "compulsive" digging! This notion is not only fanciful; it cannot be falsified and therefore is not subject to scientific test. Furthermore, more recent research has shown that clomipramine, an "antiobsessional" compound, reduces marble-burying behavior only at doses that also reduce general activity (Nicolas et al. 2006; Li et al. 2006). Thus, the pharmacological evidence for marble burying as a model of obsessive-compulsive disorder is less than compelling.

1.4 Light–Dark Exploration

In the light–dark exploration test, rodents avoid the brightly lit side of a two-compartment chamber, spending most of their time exploring the dimly lit side. Anxiety reduction in this test is indicated by increased transitions between the two compartments and/or increased exploration (i.e., time spent and number of line crossings) in the bright compartment, whereas nonspecific effects are indicated by changes in general locomotor activity (Blumstein and Crawley 1983; for a recent review, see Bourin and Hascoet 2003). The test takes advantage of rodents' natural aversion to brightly illuminated spaces, which itself may represent an adaptive defensive response against daytime predation. Nevertheless, photophobia is not given as an example of a "specific phobia" in the DSM-IV-revised edition, and in humans it is normally related to other medical conditions. As a defensive against predation, however, it may have some homology with other human fears and phobias (e.g., spiders, snakes).

In the hands of most researchers, the light/dark test is sensitive to the effects of benzodiazepines and 5-HT_{1A} agonists, with the exception of Bill et al. (1989), who were unable to show an anxiolytic effect of gepirone (Table 4). Nonanxiolytic agents, such as caffeine, amphetamine, or chlorpromazine did not produce an anxiolytic-like effect (Crawley 1981; Hascoet and Bourin 1998; Shimada et al. 1995). Antidepressants, on the other hand, did not have consistent effects in this model, regardless of the subclass of antidepressant administered (Table 4). It should be noted, however, that the studies reported so far using this test involve acute administration of antidepressant compounds, and until chronic studies are conducted, conclusions about the overall pharmacological validity of the light–dark test may be premature.

Table 4 Summary of findings with light–dark exploration test following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Both et al. (2005), Costall et al. (1987), Costall et al. (1988a, b, 1989), Costanzo et al. (2002), Crawley (1981), Crawley and Goodwin (1980), de Angelis (1992), Kilfoil et al. (1989), Mi et al. (2005), Shimada et al. (1995), Uriguen et al. (2004), Zanoli et al. (2002)	Bill et al. (1989), Costall et al. (1989), Crawley (1981), Hascoet and Bourin (1988), Imaizumi et al. (1994a, b), Lopez-Rubalcava et al. (1992), Onaivi and Martin (1989), Uriguen et al. (2004), Young and Johnson (1991a, b)	Hascoet et al. (2000b)	Bourin et al. (1996), Shimada et al. (1995), Young and Johnson (1991a, b)	Bourin et al. (1996), De Angelis and Furlan (2000)
(−)			Griebel et al. (1994)	Costall et al. (1989)	Crawley (1981), De Angelis and Furlan (2000)
(0)		Bill et al. (1989)	Brourin et al. (1996)	Onaivi and Martin (1989)	

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

1.5 Social Interaction

In the social interaction test, naïve rats are placed in pairs in an open arena, and the time they spend in active social interaction (e.g., sniffing, grooming) is measured. Social interaction is suppressed when animals are tested under bright lights or in an unfamiliar test environment, relative to low light/familiar conditions. This suppression is the index of anxiety (File and Hyde 1978). Line crossings are counted as a measure of nonspecific changes in locomotor activity. Disinhibition of social interaction is the measure of anxiety-reduction in this test (for a review see File 2003). In so far as anxious humans display "social phobia," the model may have some degree of isomorphism. Avoidance of social conflict is in some way related, and may be under positive selection pressure in many social species, from rats to humans.

Table 4 shows that benzodiazepine and 5-HT_{1A} anxiolytics were generally effective in increasing social interaction, whereas antidepressants had varying

effects. The only exception to this general pattern of drug effects is a study by Rex et al. (2004), where diazepam was anxiolytic in Wistar but not Sprague Dawley rats. Since many other studies have administered benzodiazepines to Sprague Dawley rats and produced anxiolytic effects in the social interaction test (e.g., Kantor et al. 2005), Rex et al.'s failure to do so is suspect. SSRIs have been uniformly ineffective or anxiogenic following acute administration in the social interaction test, but anxiolytic when administered chronically for 21 days before the test. Tricyclic antidepressants did not produce anxiolytic effects in this test, even following chronic administration (e.g., Popik and Vetulani 1993). Nonanxiolytic drugs such as amphetamine, caffeine, yohimbine, or naxolone did not produce anxiolytic-like effects in the social interaction test (File and Hyde 1979; Pellow et al. 1985; File 1980).

In addition to lighting levels and familiarity of the environment, the novelty of the testing partner can act as an anxiety-inducing parameter (Gardner and Guy 1984; Guy and Gardner 1985). Gardner and Guy (1984) reported that the suppression of social interaction when faced with a novel rather than a familiar partner was reversed by benzodiazepines and the mixed anxiolytic–antidepressant drug alprazolam, whereas nonanxiolytic agents did not affect this measure. Another more recent variant, based on the general idea that anxiogenic stimuli reduce social interaction, is the "*stress-induced social avoidance*" test (Haller et al. 2002; Haller et al. 2003). In this test, rats subjected to electric shocks or conspecific aggression avoid social contact for up to 10 days. Initial validation studies indicate that the test may be sensitive to the effects of benzodiazepines, 5-HT_{1A} agonists, and SSRIs (Leveleki et al. 2006). These are promising developments and suggest that some variant of the social interaction test may yet be found sensitive to all major classes of anxiolytic drug.

1.6 Elevated Plus-Maze

In the elevated plus-maze, rodents normally avoid the two open arms of the maze, and restrict most of their activity to the two closed arms. Open-arm avoidance appears to be driven by an aversion to open spaces, leading to thigmotaxic behavior (Treit et al. 1993). An antianxiety effect is indicated by an increase in the proportion of activity in the open arms of the maze (i.e., an increase in the percentage of time spent in the open arms and in the percentage of entries into the open arms). Changes in total entries and/or changes in the number of closed arm entries indicate nonspecific drug effects on locomotor activity. (For reviews of procedures and methods see Pellow 1986; Hogg 1996; Treit et al. 2003; Carobrez and Bertoglio 2005.) Other ethologically driven behaviors such as "risk assessment" (i.e., the "stretched attend" posture) have also been measured in the elevated plus-maze to complement the original spatial measures of anxiety (e.g., Rodgers and Dalvi 1997), although their current use is not widespread. Anxious humans can also

display fear of heights and open spaces, and may even display thigmotaxis and risk assessment under these conditions, suggesting some degree of isomorphism or homology. In smaller animals, avoidance of open spaces may have evolved as a defense against larger mammals and/or avian predators (Treit and Fundytus 1988).

Benzodiazepine anxiolytics increase the proportion of activity in the open arms, whereas nonanxiolytic agents (e.g., amphetamine, caffeine) generally do not (Baldwin et al. 1989; Handley and Mithani 1984; Johnston and File 1989; Pellow et al. 1985). Mixed anxiolytic-antidepressant compounds such as alprazolam also have reliable anxiolytic effects in the elevated plus-maze (Griebel et al. 1996; Johnston and File 1988; Jones et al. 1994; Pellow and File 1985). However, the effects of standard antidepressant drugs in the plus-maze have been inconsistent. On the one hand, the tetracyclic antidepressant mianserin produced significant anxiolytic effects after chronic administration (Rocha et al. 1994), and MAOIs such as phenelzine and befloxatone produced anxiolytic effects whether given acutely (Caille et al. 1996; Paslawski et al. 1996) or chronically (Johnston and File 1988). On the other hand, both acute and chronic administration of TCAs (imipramine, amitriptyline) failed to produce anxiolytic profiles in the plus-maze (e.g., Cole and Rodgers 1995; Lister 1987; Luscombe et al. 1990), and SSRIs such as fluoxetine have been reported to be *anxiogenic* (e.g., Handley and McBlane 1992; Silva and Brandao 2000; Silva et al. 1999), *anxiolytic* (e.g., Cadogan et al. 1992; Kurt et al. 2000), or *ineffective* (e.g., Linnoila et al. 1987). The inconsistent effects of SSRIs have been found after both *acute* (e.g., Griebel et al. 1999; Silva and Brandao 2000) and *chronic* (Kurt et al. 2000; Silva et al. 1999) drug administrations.

The effects 5-HT_{1A}-type compounds in the elevated plus-maze are also mixed. There are reports of clear anxiolytic effects (e.g., Dunn et al. 1989; Griebel et al. 1997; Hallar et al. 2000), or no anxiolytic effects (e.g., Pellow and File 1986; Pellow et al. 1987; Silva and Brandao 2000), even after chronic drug administration (e.g., Moser 1989; Moser et al. 1990). Although chronic regimens with buspirone or ipsapirone did not produce anxiolytic effects in the plus-maze (e.g., Moser 1989), there is some evidence that these negative findings may have been related to dose. Soderpalm et al. (1993) found that 5 weeks of buspirone (10 mg kg^{-1} b.i.d.) significantly increased open-arm activity whereas the same regimen at lower doses (2.5 or 5.0 mg kg^{-1}) was without effect. A number of other studies support the hypothesis that high doses of 5-HT_{1A} compounds may be necessary for their anxiolytic effects to emerge after chronic treatment in the elevated plus-maze (Cole and Rodgers 1994; Maisonnette et al. 1993; Motta et al. 1991; Silva and Brandao 2000).

In summary, the elevated plus-maze is clearly sensitive to benzodiazepine-type anxiolytics. However, the effects of antidepressant drugs (both chronic and acute) are mixed, as are the effects of 5-HT_{1A} compounds. There is some evidence that high doses of chronically administered 5-HT_{1A} compounds may be necessary to detect their anxiolytic effects in the elevated plus-maze.

A recent modification of the original-plus maze test is the "stress-potentiated plus-maze" test, in which subjects are prestressed before exposure to the standard elevated plus-maze test (Korte and DeBoer 2003). The addition of prestress (e.g., restraint,

social defeat, electric shock) seems reminiscent of the fear-potentiated startle paradigm, although its motivation seems to have been to increase the sensitivity of the test to the anxiolytic effects of experimental compounds. For example, demonstrating the putative anxiolytic effects of CRF receptor antagonists in models such as the standard plus-maze has proven difficult (Martins et al. 2000). By adding prestress, it was possible to shown that intra-PAG CRF receptor antagonists can block fear behavior in the elevated plus-maze, whereas this was not possible in the standard test (Martins et al. 2000). Nevertheless, the precise role of "pretest stress" in the anxiolytic effects of CRF antagonists remains to be determined. This is especially true given the fact that the sensitivity (or lack thereof) of the "fear-potentiated" plus-maze test to the anxiolytic effects of standard anxiolytic compounds such as diazepam has not yet been demonstrated. Furthermore, "prestress" effects seem to occur only when baseline levels of open-arm activity (the index of fear) are particularly high, indicating low anxiety (for examples, see Korte and DeBoer 2003). If these problems can be rectified or rationalized, however, the stress-potentiated plus-maze paradigm may see special use in unveiling the anxiolytic effects of neuropeptide antagonists and other experimental compounds.

1.7 Separation-induced Ultrasonic "Distress" Vocalization

Rat pups emit high frequency (30–50 kHz) "distress calls" when separated from mother and littermates, which elicits retrieval behavior from the mother (Noirot 1972). In this model, a reduction in the high-frequency calls in the absence of behavioral sedation is taken as the index of anxiety reduction (Insel et al. 1986; Gardner 1985). The eliciting stimulus (separation), under certain conditions (e.g., dependency) seems capable of producing fear or anxiety in humans, and in this sense the model may be analogous to separation anxiety (for a review see Igor et al. 2001).

As seen in Table 5, benzodiazepines, 5-HT_{1A} agonists and SSRIs all reduce ultrasonic vocalizations in rat or mouse pups, a finding that has recently been replicated in juvenile rhesus monkeys (Winslow et al. 2007). The findings with tricyclic antidepressants, however, are not quite as clear. An early paper showed that desipramine actually *increased* USVs (Winslow and Insel 1990), although this finding was not replicated in subsequent studies (e.g., Kehne et al. 2000; Iijima and Chaki 2005). Iijima and Chaki (2005) have suggested that ultrasonic vocalizations are more sensitive to antidepressants that selectively block the reuptake of serotonin (e.g., fluoxetine) and less sensitive to antidepressants that inhibit norepinephrine reuptake (e.g., desipramine). This is an interesting and testable hypothesis, which may partially resolve the equivocal sensitivity of ultrasonic vocalizations to tricyclic antidepressants, which affect both serotonin and norepinephrine to some degree. However, the hypothesis also needs to account for the fact that tianeptine, which has the opposite effect to SSRIs on serotonin reuptake (i.e., facilitation of reuptake; Olivier et al. 1998b) also suppresses rat pup ultrasonic vocalizations.

Table 5 Summary of findings with the social interaction test following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Bhattacharya et al. (2000), Cheeta et al. (2001), Eguchi et al. (2001), File and Hyde (1978, 1979), File et al. (2001), Kantor et al. (2005), Kita and Furukawa (2008), Louis et al. (2008), Millan et al. (2001), Mizowaki et al. (2001), Rex et al. (2004), Salome et al. (2006), Si et al. (2005), Wood et al. (2001)	Cheeta et al. (2001), Costall et al. (1992), Cutler (1991), Dunn et al. (1989), Haller et al. (2000, 2001), Louis et al. (2008), Millan et al. (2001), Picazo et al. (1995), Salome et al. (2006)	Bristow et al. (2000), Dekeyne et al. (2000), Duxon et al. (2000) (chronic), File et al. (1999), Lightowler et al. (1994) (chronic), Starr et al. (2007) (chronic), To and Bagdy (1999), To et al. (1999)	Pellow and File (1987)	
(−)			Cheeta et al. (2001), Bagdy et al. (2001)		Johnston and File (1988)
(0)	Rex et al. (2004)		Lightowler et al. (1994), Duxon et al. (2000), Salome et al. (2006), Louis et al. (2008)	Eguchi et al. (2001), File (1985), Popik and Vetulani (1993) (chronic)	

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

Additional studies that directly compare the effects of chronic and acute antidepressant treatments on ultrasonic vocalizations would also be useful. Because of the relative fragility of rodent pups, however, as well as pharmacokinetic differences between pups and adults, chronic drug treatments would more advisable using an adult ultrasonic vocalization model (see below).

Blumberg et al. (2000) have proposed that ultrasonic vocalizations in rat pups are not anxiety responses per se, but by-products of the "abdominal compression reaction," which increases venous return to the heart when its return is compromised. Blumberg et al. demonstrate that cold temperatures can elicit ultrasonic

Table 6 Summary of findings with the elevated plus maze test following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Kapus et al. (2008), Lister (1987), Pellow et al. (1985), Naderi et al. (2008), Ognibene et al. (2008), Rocha et al. (2007), Seo et al. (2007), Yoon et al. (2007), Albrechet-Souza et al. (2007, 2005), Stemmelin et al. (2008), Felipe et al. (2007), Wesolowska and Nikiforuk (2007), Wei et al. (2007), Bradley et al. (2007), Lolli et al. (2007), Grundmann et al. (2007), Drapier et al. (2007), Chen et al. (2006), Violle et al. (2006), Byrnes and Bridges (2006), Cui et al. (2007), Vignes et al. (2006), Vargas et al. (2006), Wesolowska et al (2006), Gonzalez-Trujano et al. (2006), Gonzalez-Pardo et al. (2006), Kumar and Sharma (2005), Xu et al. (2006), Perrine et al. (2006), Atack et al. (2006), Mora et al. (2006), Carr et al. (2006), Hagenbuch et al. (2006), Papp et al. (2006), Mora et al. (2005), Tokumo et al. (2006), Cha et al. (2005), Chen et al.	Cao and Rodgers (1997), Critchley and Handley (1987a, b), Dunn et al. (1989), Graeff et al. (1990), Griebel et al. (1997), Hallar et al. (2000), Mendoza et al. (1999), Pokk and Zharkovsky (1998), Soderpalm et al. (1989), Grundmann et al. (2007), Jung et al. (2006), Vaidya et al. (2005), Kim et al. (2004), Peng et al. (2004), Majercsik et al. (2003), Escarabajal et al. (2003), Lin et al. (2003)	Cadogan et al. (1992), Griebel et al. (1994), Griebel et al. (1999), Kurt et al. (2000), Kuan et al. (2008), Matsuzawa-Yanagida et al. (2007), Chaki et al. (2005)	Matsuzawa-Yanagida et al. (2007), Aricioglu and Altunbas (2003)	Caille et al. (1996), Paslawski et al. (1996), Johnston and File (1988)

(*continued*)

Table 6 (continued)

Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(2004, 2005), Yasui et al. (2005), Park et al. (2005), Rabbani et al. (2005), Mi et al. (2005), Fernandez-Guasti et al. (2005), Clenet et al. (2004), Savic et al. (2004), Soman et al. (2004), Peng et al. (2004), Rabbani et al. (2004), Wilson et al. (2004), Molina-Hernandez et al. (2004), Cryan et al. (2004), Klodzinska et al. (2004), Kurt et al. (2003), Huen et al. (2003), Rabbani et al. (2003), Cechin et al. (2003), Dal-Co et al. (2003), Silva and Brandao (2000)				
(−)		Handley and McBlane (1992), Koks et al. (2001), Pollier et al. (2000), Silva and Brandao (2000), Silva et al. (1999), Drapier et al. (2007)		
(0)	Collinson and Dawson (1997), Critchley and Handley (1987a, b), Pellow and File (1986), Pellow et al. (1987), Moser (1989), Moser et al. (1990), Rodgers et al. (1997a)	Handley and McBlane (1992), Linnoila et al. (1987), Rodgers et al. (1997b), Adamec et al. (2004), Holmes and Rodgers (2003)	Cole and Rodgers (1995), File and Johnston (1987), Lister (1987), Luscombe et al. (1990), Pellow et al. (1985), Drapier et al. (2007)	Holmes and Rodgers (2003)

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

vocalizations through an increase in venous pressure, as can clonidine, an α2 adrenoceptor agonist. Whether or not this cardiovascular mechanism can explain the suppression of ultrasonic vocalizations by anxiolytic drugs is questionable. It is just as likely that these cardiovascular perturbations themselves are aversive or anxiogenic, or a by-product of anxiety, in which case these data only reinforce the model's general utility.

Ultrasonic vocalizations in the range of 22 kHz can also be induced in adult rats by subjecting them to a variety of stressors, including inescapable foot shocks (DeVry et al. 1993) As seen in Table 6, shock-induced ultrasonic vocalizations are sensitive to the effects of all major classes of anxiety-reducing compounds. However, it should be noted that the effects of diazepam, as well as fluoxetine, were in some cases observed only at doses that were higher than the anxiety-reducing doses ordinarily used in humans (Bartoszyk et al. 1997; Sanchez and Meier 1997; Sanchez 2003).

USVs induced by stressors other than foot shock, such as air puffs, conspecific intruders, acoustic startle stimuli, or drug withdrawal are also reduced by benzodiazepine anxiolytics and 5-HT_{1A} compounds, although there is considerable variability (Naito et al. 2003; Vivian and Miczek 1993; Kaltwasser 1990, 1991; Knapp et al. 1993, 1998; but see Becker et al. 2001). Cold-induced USVs can be reduced by several SSRIs (Fish et al. 2004). However, it is hard to imagine that stimuli as diverse as cold temperatures, attack by an intruder, air puffs to the head, and withdrawal from cocaine would produce similar distress, either quantitatively or qualitatively. Thus, variability in drug effects may be associated with the specific stimulus used to induce the USVs.

2 Summary and Conclusions

In summary, the models reviewed in this chapter show at least some sensitivity to a variety of agents known to produce anxiolysis in humans (i.e., the benzodiazepines, antidepressants, and 5-HT_{1A} compounds). All of the models show good sensitivity to benzodiazepine anxiolytics. Light/dark exploration, social interaction, elevated plus-maze, shock-probe/marble burying, and the conflict tests have shown some sensitivity to antidepressants and 5-HT_{1A} compounds, but to varying degrees. The conflict and ultrasonic vocalization tests seem to be broadly sensitive to the anxiolytic effects of antidepressants. Ultrasonic vocalization appears to be sensitive to all classes of therapeutically effective anxiolytic compounds. Fear-potentiated startle, although sensitive to both benzodiazepine and 5-HT_{1A} anxiolytics, has thus far failed to detect the anxiolytic effects of traditional antidepressants.

While the majority of these models showed at least some sensitivity to antidepressant and 5-HT_{1A} compounds, the anxiolytic effects of these drugs were often more variable than the effects of benzodiazepine anxiolytics. In addition, there were a number of instances in which antidepressant and 5-HT_{1A} agents produced effects

Table 7 Summary of findings with the ultrasonic vocalizations test following peripheral administration of different classes of anxiolytic drugs

	Benzodiazepines	5-HT1A agonists	SSRIs	Tricyclics	MAOIs
(+)	Engel et al. (1987), Fish et al. (2000), Gardner (1985a, b), Hodgson et al. (2008), Iijima and Chaki (2005), Kehne et al. (2000), Miczek et al. (1995), Millan et al. (2001), Olivier et al. (1998), Podhorna and Brown (2000), Rowlett et al. (2001)	Benton and Nastiti (1988), Fish et al. (2000), Hodgson et al. (2008), Iijima and Chaki (2005), Kehne et al. (1991, 2000), Millan et al. (2001), Nastiti et al. (1991), Olivier et al. (1998), Siemiatkowski et al. (2001)	Hodgson et al. (2008), Iijima and Chaki (2005), Kehne et al. (2000), Olivier et al. (1998)	Kehne et al. (2000), Podharma and Brown (2000)	
(−)				Winslow and Insel (1990)	
(0)				Iijima and Chaki (2005)	

(+) anxiolytic; (−) anxiogenic; (0) no effect; *SSRI* selective serotonin reuptake inhibitor; *MAOI* monoamine oxidase inhibitor

opposite to those of standard anxiolytics, suggestive of an "anxiogenic" action. There are several possible explanations for these inconsistencies, which have more general implications for animal models of anxiety and anxiolytic drug action.

A drug may have very reliable effects in an animal model of anxiety, but unless that drug also has reliable antianxiety effects in humans, it cannot be used to validate the animal model. Conversely, a drug that has inconsistent or unreliable anxiolytic effects in humans cannot be used to invalidate an animal model of anxiety. In this regard, there is little clinical evidence that 5-HT_{1A} agents, other than buspirone, produce reliable antianxiety effects in humans and even the effects of buspirone appear to be more variable than the effects of benzodiazepine anxiolytics (e.g., Pecknold et al. 1985; Sheehan et al. 1988, 1990; Wheatley 1982). A number of clinical trials (e.g., Olajide and Lader 1984; Sheehan et al. 1990) suggest that the efficacy of buspirone across different human anxiety disorders (see DSM-IV) is not as robust as the benzodiazepines (Hoffman and Mathew 2008). For a summary of comparative clinical findings see; Argyropoulos et al. 2000, Table 1. These clinical data are certainly not definitive but, if anxious humans respond more variably to buspirone than to benzodiazepine anxiolytics, one might expect the effects of 5-HT_{1A} agents in animal models of anxiolytic drug action to be more variable than the effects of benzodiazepines.

The clinical efficacy of antidepressant drugs in the treatment of anxiety disorders is far more convincing, but there is still some variation in efficacy (see Tyrer and Tyrer 1994; Hoffman and Mathew 2008; Borsini et al. 2002). There is also some disagreement about whether specific antidepressants are required for particular anxiety disorders (e.g., agoraphobia, panic), or are superior to benzodiazepine anxiolytics for these disorders. Furthermore, anxiety in humans often overlaps with depression, so that interpretation of a therapeutic drug effect as being either anxiolytic or antidepressant can sometimes be difficult. Perhaps the most important clinical finding in this literature, however, is that unlike classical benzodiazepines, the anxiolytic effects of traditional antidepressants in humans are normally delayed (2–4 weeks), and the initial (acute) response may sometimes be an exacerbation of anxiety (Argyropoulos et al. 2000). Thus, acute antidepressant treatment in an animal model of anxiety is of questionable relevance to its pharmacological validation.

Chronicity may be equally relevant to the effects 5-HT_{1A} compounds in these models. Whereas chronic administration of 5-HT_{1A} or antidepressant drugs often resulted in reliable, anxiolytic effects in a variety of animal models (Commissaris et al. 1990; Duxon et al. 2000; Fontana and Commissaris 1988; Griebel et al. 1994; Rocha et al. 1994; Soderpalm et al. 1993; Yamashita et al. 1995), acute administration resulted in less reliable anxiolytic effects, or even anxiogenesis (e.g., Griebel et al. 1994; Handley and McBlane 1992; Moser 1989).

Another possibility is that different animal models represent qualitatively different types of "anxiety" or fear, only some of which are reliably inhibited by 5-HT_{1A} agents or antidepressants. Thus, one could speculate that the social interaction test primarily reflects a type of social phobia, which is reliably suppressed by 5-HT_{1A} agents and certain antidepressants, whereas the elevated plus-maze test reflects a type of acrophobia, which is not as reliably suppressed by 5-HT_{1A} agents or antidepressants. This would imply that animal fears can be pharmacologically dissected, which in turn would support the pharmacological dissection of human anxiety. Although these speculations seem to be consistent with some of the animal data reviewed in this chapter, at this time there is no convincing clinical evidence that specific anxiety disorders are differentially affected by benzodiazepine, 5-HT_{1A} or antidepressant anxiolytics (Hoffman and Mathew 2008; Argyropoulos et al. 2000; Tyrer and Tyrer 1994).

Thus, a number of factors, including clinical effectiveness, chronicity, and model-type, may alter the correspondence between the effects of benzodiazepines, 5-HT_{1A} agents, and antidepressants in animal models of anxiety. On the whole, however, the data summarized above suggest that there is enough correspondence between drug-effects across these tests that future paradigmatic studies may ultimately establish their validity as general models of antianxiety drug action. While all models may not attain this ideal, it should be remembered that "class-specific" models could serve as a valuable tools for studying the mechanisms by which benzodiazepine, 5-HT_{1A}, or antidepressant drugs produce their anxiolytic effects.

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Genetic Approaches to Modeling Anxiety in Animals

Laura H. Jacobson and John F. Cryan

Contents

Abstract Anxiety disorders are a growing health problem world-wide. However, the causative factors, etiology, and underlying mechanisms of anxiety disorders, as for most psychiatric disorders, remain relatively poorly understood. The current status of clinical research indicates that anxiety traits and anxiety disorder in man have a genetic component, and therefore genetic modeling in animals is a logical approach to gain a greater insight into their neurobiology. However, it is also clear

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that the nature of these genetic contributions is highly complex. Moreover, the success of this approach is largely contingent upon the utility of available behavioral paradigms for modeling anxiety-related behaviors in mice. Animal genetic models provide a unique and comprehensive methodological tool to aid discovery into the etiology, neurobiology, and ultimately, the therapy of human anxiety disorders. The approach, however, is challenged with a number of complexities. In particular, the heterogeneous nature of anxiety disorders in man coupled with the associated multifaceted and descriptive diagnostic criteria, create challenges in both animal modeling and in clinical research. In this article, we describe some of the powerful modern genetic techniques that are uniquely amenable to the laboratory mouse and thus provide a strategy for approaching some of these challenges. Moreover, we focus on recent advances which have highlighted the relative contribution of genetic modeling in animals to the understanding of underlying neurobiology and genetic basis of anxiety disorders.

Keywords Anxiety disorders · Knockout · Translational · Endophenotype · Psychiatric genetics · Animal models

1 Introduction

Anxiety disorders are common, serious, and a growing health problem world-wide, and currently represent the highest prevalence of any of the DSM-IV disorders in Europe and the USA (Kessler et al. 2005b; Alonso and Lepine 2007; Kessler 2007; Nutt et al. 2007). According to the recently completed National Comorbidity Survey Replication (NCS-R) conducted in the USA (Kessler and Merikangas 2004), anxiety disorders had the highest lifetime prevalence estimates (28.8%) and the earliest age of onset (11 years) relative to other DSM-IV psychiatric disorders (Kessler et al. 2005a, b). Emerging data also demonstrates that patients with anxiety disorders are at high risk not only for comorbid psychiatric disorders such as depression (Merikangas et al. 2003; Kessler et al. 2005b), but also for medical illness such as chronic pain, asthma, cardiovascular disease, hypertension, migraine, and gastrointestinal disorders (Harter et al. 2003; Roy-Byrne et al. 2008). There is no doubt then, that anxiety disorders represent an enormous global societal and economic burden (Miller 2006; Kessler 2007; Nutt et al. 2007). However, the causative factors, etiology, and underlying mechanisms of anxiety disorders, as for most psychiatric disorders, remain relatively poorly understood (Wong and Licinio 2004; Cryan and Holmes 2005), and it is clear that improvements in understanding these factors and the development of better treatments are imperative (Wong and Licinio 2004; Cryan and Holmes 2005).

Animal genetic models provide a unique and comprehensive methodological tool to aid discovery into the etiology, neurobiology, and ultimately, the therapy of human anxiety disorders. The approach, however, is challenged with a number of

specific complexities. In particular, the heterogeneous nature of anxiety disorders in man, and the associated multifaceted and descriptive diagnostic criteria, create challenges in both animal modeling and in clinical research (Gottesman and Gould 2003; Cryan and Holmes 2005; Schulze et al. 2005). Compartmentalization of symptomatology and validated behavioral and translational modeling, in combination with powerful modern genetic techniques that are uniquely amenable to the laboratory mouse, provide a logical and objective strategy for approaching these challenges. Here within, we describe these techniques and postulate on the contribution of genetic modeling in animals to the understanding of underlying neurobiology of anxiety disorders.

2 Basic Concepts in Animal Modeling of Anxiety Disorders

It is widely acknowledged that the full spectrum of a human anxiety disorder, or for that matter other psychiatric disorders, can never be fully recapitulated in any one animal model (Cryan and Holmes 2005; Arguello and Gogos 2006; Crawley 2007). The capacity of humans for processing complex psychological and cognitive concepts, which are evident in the symptomatology of anxiety disorders (e.g., "sense of a foreshortened future" (PTSD), "fear of losing control or going crazy" (Panic Disorder), or fear of acting in a way that will be embarrassing or humiliating (Social Phobia); DSM-IV 1994) are impossible to measure, and therefore to model, in animals. There is, however, substantial genetic, neurochemical, and neuroanatomical evolutionary conservation across mammalian species (Jones 2002; Tecott 2003; Arguello and Gogos 2006). Consequently, as has been well-documented since the scientific endeavors of Charles Darwin (Darwin 1871; Darwin 1872), there are also many fundamental physiological and behavioral responses that are well conserved between the species. By inference, it is therefore theoretically possible to objectively study behavioral responses in animals and the neural circuits and genetic factors subserving them, as a means to understand the basis of human behavior and disease (Geyer and Markou 2002; Cryan and Holmes 2005; Crawley 2007). It is worth reiterating, however, that an animal model of a specific disorder can only be as robust as the clinical measures used to diagnose and monitor the disorders in man. Advances in animal modeling, therefore, require parallel development in clinical measures that facilitate translational comparisons between species. Studies investigating normal humans and animals, in order to improve the understanding of genetic, behavioral, and neurobiological homology across species, are thus a vital component in the validation of animal models of human disorders (Geyer and Markou 2002; Markou et al. 2008).

Specific criteria have been proposed for determining whether or not an experimental paradigm in animals may be considered as a valid model of a human psychiatric disorder (or a component thereof). McKinney and Bunney (1969) were among the first researchers to propose standardized criteria; in this case for the animal modeling of depression although they are equally applicable to anxiety

research. They suggested that a valid animal model needed to be "reasonably analogous" to the human disorder in its manifestations or symptomatology, cause a behavioral change that can be monitored objectively, produce behavioral changes that are reversed by the same treatment modalities that are effective in humans, and be reproducible between different investigators (McKinney and Bunney 1969). More recent theoretical constructs encompass the concepts of different types of validity: face (symptomotology appear similar to the clinical condition), construct (similarities in underlying biology), predictive (appropriate responses to clinically efficacious therapeutics or induction of translational symptomatology), etiological validity (similar inducing agents), convergent (where correlation with other construct-based models is observed), and divergent (where measures are phenomenologically different from those measure in other models) (Geyer and Markou 2000). It has been argued, however, that the most important criteria to satisfy are the reliability and predictive validity of the model system (Geyer and Markou 2002).

In preclinical anxiety research, by far the most commonly used species used in animal modeling are laboratory rats and mice. Rats have historically been the animal of choice for psychopharmacological research, mainly due to the superior performance of rats in many cognitive and operant tasks that have formed the basis of modern behavioral pharmacology and for practical reasons related to the size of the animal and ease of surgical interventions. Many of the behavioral paradigm in use in anxiety research were originally developed and validated on the basis of their ability to predict the effects of clinical anxiolytics in rats, and have since been adapted for application in other species, and particularly mice (Cryan and Holmes 2005).

In the past decade, there has been a dramatic increase in the use of mice in neuropsychiatric research, as in many other branches of biomedical research. Mice as experimental animals hold many practical and economic advantages over rats and other laboratory species for animal modeling. They are easy to breed, have a short generation turnover, low maintenance costs in terms of housing, and their small size is sympathetic to researchers and medicinal chemists alike with regard to amounts of compounds required for drug applications. It is their unique amenability to a broad array of new genetic manipulation techniques, however, that has seen the rising popularity of mice in psychiatric research, including the study of anxiety disorders (Joyner and Sedivy 2000; Tarantino and Bucan 2000; Phillips et al. 2002; Tecott 2003; Cryan and Holmes 2005; Jacobson and Cryan 2006; Crawley 2007).

3 Measurable Outcomes in Anxiety Disorders and Animal Models

In order to model the genetic contributions to anxiety-like behavior in animals, it is fundamentally important to utilize relevant experimental endpoints that are either translatable between species, or at least that measure species-specific behaviors that share a common underlying neurobiology and/or neuropharmacology in the

anxiety-like state. The measurable outcomes used in clinical and preclinical studies are summarized in brief below for the purposes of this chapter. Interested readers should refer to other chapters in this book for a more detailed critique.

3.1 Measuring Anxiety in Man and Diagnostic Criteria in Anxiety Disorders

Fear and anxiety are common, normal emotions in the majority of animal species, including laboratory animals and man, and transient anxiety responses to a real danger are an appropriate, adaptive response in anxiety disorder patients and nonpatients alike. The transition to an anxiety disorder in man, as described in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV 1994) is "marked, persistent, and excessive or unreasonable fear" that is experienced to such a degree that it significantly interferes with everyday life. The diagnosis of anxiety disorders themselves may be considered as an in-depth behavioral analysis, characterized by a broad range of diverse, overlapping symptom clusters which are classified into categories and subcategories based mainly on the subjective descriptions of symptoms (DSM-IV 1994; Geyer and Markou 2002; Sramek et al. 2002; Merikangas et al. 2003; Lam et al. 2006; Bartz and Hollander, 2006).

The most commonly diagnosed anxiety disorders are generalized anxiety disorder (GAD), panic disorder (with or without agoraphobia), specific phobia, social phobia, obsessive-compulsive disorder (OCD), and post-traumatic stress disorder (PTSD). These disorders are distinguished from one another mainly by the specific nature of the anxiety and by the provoking stimulus if one exists. They also have distinguishing features based on the temporality of symptom manifestation and in the underlying and/or coincident neurobiological and neuroendocrine responses and markers. Clinical psychiatrists have several rating scales to assist in the diagnosis and classification of different anxiety disorders, and these scales are also of use in quantifying the severity of the disorder and to monitor treatment progress and outcomes. Examples of scales used in GAD may include the Hamilton Anxiety Scale Rating, Beck Anxiety Inventory, the Clinical Global Impression Scale, whereas OCD may be evaluated using the Yale-Brown Obsessive Compulsive Scale, and for PTSD, the Posttraumatic Stress Diagnostic Scale (Sramek et al. 2002; Lam et al. 2006).

Currently, no biochemical or electrophysiological criteria are used in the diagnostic procedure, although such findings may be used in support of diagnoses. A number of experimental models of human anxiety are utilized with readouts based on alterations in body temperature, HPA axis, and startle in addition to self report (Graeff et al. 2003; Grillon 2008; Nordquist et al. 2008; Vinkers et al. 2008), which is advancing the translational aspects of animal models of anxiety. These coupled with continuous progress in neuroimaging, are sure to provide an informative platform for advancing the understanding of the physiological basis and nosology of anxiety disorders (Bremner 2004; Brown and Hariri 2006; Rauch et al. 2006; Etkin and Wager 2007; Damsa et al. 2009).

3.2 *Measuring Anxiety in Laboratory Animals*

There are a number of different types of tests that have been employed in the animal modeling of anxiety disorders. Small rodents have an innate aversion to exposed, well-lit spaces, presumably because of natural selection pressures against predation. Modeling of pathological anxiety has therefore been considered by some as an extreme state of this continuum, and many animal models have been designed with this in mind (Cryan and Holmes 2005). Furthermore, rats and mice are foraging, exploratory species, and exploration-based anxiety paradigms capitalize on the conflict between natural avoidance and the exploratory drive of rodents, and hence include an ethological foundation in these tasks (Rodgers 1997). Examples of these approach-avoidance conflict tasks include the avoidance of bright, open or elevated areas of the open field test (Prut and Belzung 2003), the light–dark box test (Crawley 2007), the elevated plus maze (Rodgers 1997; Holmes 2001; Crawley 2007), elevated zero maze (Lee and Rodgers 1990; Shepherd et al. 1994), the staircase test (Simiand et al. 1984), and the mirrored arena (Rodgers 1997; Rodgers et al. 1997; Belzung and Griebel 2001; Crawley 2007). "Ethological parameters" are often included as readouts in exploratory tasks, and involve the quantification of species-typical behaviors and postures adapted during exploration. Reductions in the number of stretch-attend postures, rearing and head-dipping over the edges of elevated apparatuses have been interpreted as heightened anxious responses in various apparatuses (Shepherd et al. 1994; Rodgers 1997; Rodgers et al. 1997; Belzung and Griebel 2001). The ethological approach has probably been taken to its greatest extent in the Mouse Defence Test Battery (MDTB), where mice are exposed to a predator and panic, defensive threat/attack and risk assessment behaviors quantified (Blanchard et al. 2003). Ethological parameters are thought to be of particular advantage in animal models due to their basis in risk-assessment behaviors, and thus are thought to model aspects of apprehension and excessive vigilance seen in human anxiety disorders (Rodgers 1997; Blanchard et al. 2003; Cryan and Holmes 2005).

A variation on approach-avoidance tests are punishment-based conflict tests. Examples include the four-plate test, where exploratory drive conflicts with avoidance of foot-shocks delivered though the floor-plates; and the Vogel test, a conflict drinking paradigm where a thirsty animal must overcome the apprehension of a shock delivered through the drinking spout in order to slake its thirst (Vogel et al. 1971). All of the aforementioned tests rely on a passive avoidance strategy to provide an indication of anxiety. The marble-burying and defensive burying (i.e., of a shock probe) tests, in contrast, requires the engagement of active behaviors (burying the offending object with bedding) for the expression of anxious behaviors, and thus are useful inclusions in test batteries (Broekkamp et al. 1986; Sluyter et al. 1996; Sluyter et al. 1999; Spooren et al. 2000; Jacobson et al. 2007). Additional interesting tests include novelty-suppressed feeding, separation-induced ultrasonic vocalizations in pups, and stress-induce hyperthermia (SIH; where body temperature increases in response to acute stressors are quantified). These tests are

relatively free of bias that may be introduced by genetic alterations which also affect locomotor activity, which can be an important factor introducing confounds in the interpretation of exploratory-based tests (Cryan and Holmes 2005; Holmes and Cryan 2006; Jacobson and Cryan 2006). The SIH test in particular is an ideal inclusion in an anxiety test battery as it provides an autonomic readout and is a translational response across a variety of strains and species, including man (Bouwknecht et al. 2007; Vinkers et al. 2008).

All of the aforementioned tasks are responsive to the clinically efficacious benzodiazepine anxiolytics, such as diazepam and chlordiazepoxide, and in this regard they meet the predictive validity criterion for a valid animal model (Cryan and Holmes 2005; Crawley 2007). However, perhaps the main criticism of many of these tests is their predictive validity with regard to other classes of anxiolytic compounds. In particular, this arises mainly from the observation that the selective serotonin reuptake inhibitor (SSRIs) antidepressants, which are clinically efficacious in the treatment of a number of anxiety disorders, show variable effects in many anxiety paradigms, with the possible exception of the MDTB, marble burying and ultrasonic vocalization tests (Rodgers 1997; Borsini et al. 2002; Blanchard et al. 2003; Markou et al. 2008).

Anxiety disorders are characterized by specific cognitive deficits such as misappraisal and overattention to threatening stimuli in panic disorder, GAD and phobias, and persistence of traumatic memories in PTSD (DSM-IV 1994; Lang et al. 2000). Approaches to modeling these aspects has focused on conditioned tests of anxiety, such as Pavlovian fear conditioning (Cryan and Holmes 2005; Ledgerwood et al. 2005; Delgado et al. 2006). Recently, as supported by human imaging evidence of insular cortex dysfunction in anxiety disorders (Paulus and Stein 2006), the insular cortex-dependent task conditioned taste aversion (CTA) has also been growing in popularity (Bermudez-Rattoni 2004; Guitton and Dudai 2004; Mickley et al. 2004; Yasoshima and Yamamoto 2005; Jacobson et al. 2006; Hefner et al. 2008). In conditioned paradigms, associative fear or aversion are induced by exposure to a previously innocuous stimulus (the "conditioned stimulus", e.g., a tone or sucrose drink) that has been paired with an innately aversive stimulus (the "unconditioned stimulus", e.g., foot-shocks, or experimentally induced malaise). Immobility (conditioned freezing), startle (fear potentiated startle, FPS), tachycardia, defensive burying, ultrasonic vocalizations, and sucrose preference (CTA), are the main types of experimental outputs in these tests (Fendt and Fanselow 1999; Cryan and Holmes 2005). However, disturbances in sleep has also been shown to be a sensitive readout of fear conditioning (Sanford et al. 2003a, b), which is relevant to the symptoms of sleep dysfunction that are included as diagnostic criteria for both GAD and PTSD (DSM-IV 1994). Extinction of aversive association tasks, applicable to both fear conditioning and CTA paradigms, has been applied to the modeling of anxiety disorders with persistent aversive memory and associations, such as PTSD and panic disorder (Ressler et al. 2004; Barad 2005; Cryan and Holmes 2005; Ledgerwood et al. 2005; Delgado et al. 2006; Jacobson et al. 2006). This approach has met with some success in predictive validation, as demonstrated by the efficacy of D-cycloserine in facilitation of extinction in both rats in the fear-potentiated

startle paradigm and in acrophobic patients (Ressler et al. 2004; Ledgerwood et al. 2005; Davis et al. 2006).

With all the behavioral paradigms heretofore described, interpretation of "anxiety" in animals requires careful attention to the interpretation of data to avoid confounding effects on other aspects of behavior (Bouwknecht and Paylor 2008). The importance of assessing locomotor activity in mutant animals had been mentioned above, and is of particular significance in interpreting data from anxiety paradigms that rely on a locomotion-based readout, such as exploratory-based paradigms and freezing behavior in fear conditioning tests (Cryan and Holmes 2005; Holmes and Cryan 2006; Jacobson and Cryan 2006). Mutations influencing cognition likewise may influence associative learning and exploratory behavior, and thus affect behavior in both conditioned and unconditioned anxiety paradigms (Jacobson and Cryan 2006; Bouwknecht and Paylor 2008). Potential alterations in other sensory-motor modalities must also be taken into account, for example, pain sensitivity in tests where foot-shock-based tests are employed, or impaired taste or olfactory senses in conditioned-tasted aversion or odor-paired fear learning tasks, respectively. The advice of Jacqueline Crawly in her landmark book "What's Wrong With my Mouse?" (Crawley 2007) to "give your mouse a physical" is practical and fundamentally necessary advice for avoiding erroneous interpretations of behavioral data. Previous test history, interactions with early-life environment, and compensatory changes for constitutive genetic mutations may also influence results in anxiety models (Cryan and Holmes 2005; Holmes and Cryan 2006; Jacobson and Cryan 2006). With these factors in mind, a test-battery approach has been advocated for detecting genuine phenotypes in behavioral modeling with mutant mice (Cryan and Holmes 2005; Arguello and Gogos 2006; Bouwknecht and Paylor 2008).

4 Genetics of Clinical Anxiety Disorders: Setting the Scene for Animal Models

In this section, we discuss the basic characteristics of the genetics of clinical anxiety disorders by way of introducing specific genetic techniques used in animal modeling of anxiety traits. It is widely accepted that clinical anxiety disorders have a significant genetic contribution (Finn et al. 2003; Gordon and Hen 2004; Gross and Hen 2004; Leonardo and Hen 2006). Individual and meta-analyses of family and twin studies show significant familial aggregation for most anxiety disorders, and indicate that the major source of familial risk is genetic in origin (Hettema et al. 2001a; Kendler 2001; Gross and Hen 2004). Conservative estimates for the heritability of panic, GAD, phobias, and OCD are in the 30–40% range (Hettema et al. 2001a). Although relatively modest compared to the heritability of other psychiatric and neurological disorders such as schizophrenia or Huntington's Disease (Leonardo and Hen 2006), this significant genetic component indicates that candidate genes which predispose to anxiety disorders do indeed exist, and many

research efforts have been launched to find them or associated loci, using linkage, association and genome-wide screening techniques (Finn et al. 2003).

Overall, however, this endeavor has met with strikingly little success in the identification of singular genetic candidates in the etiology of anxiety disorders (Lesch 2001; Finn et al. 2003; Gordon and Hen 2004; Flint 2006; Leonardo and Hen 2006; Abbott 2008). Examples of research avenues include those motivated by the success of benzodiazepines and SSRIs in the clinic, with investigations into the genetic relationship between anxiety disorders and the $GABA_A$ receptor (particularly the benzodiazepine site), the serotonin transporter (5-HTT; SERT), or serotonin receptors. For the most part, these studies have either failed to detect any significant relationships, or at best results are inconsistent between different investigators (Finn and Smoller 2001; Finn et al. 2003; Gordon and Hen 2004). The robust capacity of the administration of exogenous cholecystokinin (CCK) peptides in the induction of panic attacks in man (Rehfeld 1992) has similarly prompted studies to examine genetic variations in the CCK peptide or in the CCK_A and CCK_B receptor genes and their relation to panic disorder, and has met with some success (Wang et al. 1998; Kennedy et al. 1999; Hattori et al. 2001; Hosing et al. 2004; Maron et al. 2005), although a negative study showing no association also exists (Hamilton et al. 2001). Similarly, variable or negative results have been reported for the relationship between OCD, panic disorder, GAD, and phobia with either monoamine oxidase-A (MAOA) or catechol-*O*-methyl transferase (COMT) (Finn et al. 2003). An association with a tandem repeat polymorphism in the dopamine transporter (DAT) and PTSD were reported in one study (Segman et al. 2002), although this finding has yet to be reproduced. Despite robust preclinical evidence that decreased expression of BDNF is implicated in enhanced stress responses, reduced hippocampal volume and antidepressant responses (see Duman and Monteggia 2006), variable results have also been reported for a relationship between anxiety traits or disorders and the human loss-of-function val66met single nucleotide polymorphism (SNP; where methionine is substituted for valine in the 5′ pro-BDNF domain; Egan et al. 2003). Met-allele carriers have been reported as showing increased harm avoidance and higher prevalence in anxiety disorder or depressed patients (Jiang et al. 2005), decreased personality trait anxiety and neuroticism (Sen et al. 2003; Lang et al. 2005), and no association with life prevalence of major depressive disorder, GAD or anxious or depressed mood (Surtees et al. 2007), OCD (Wendland et al. 2007) or panic disorder (Lam et al. 2004; Shimizu et al. 2005). Mixed, weak, or negative results have also been reported for other candidate gene studies including glutamic acid decarboxylase 65, estrogen receptor α, and dopamine receptors D2, D3, D4 for the major anxiety disorders (see Finn et al. 2003; Smoller et al. 2008a). Several genome-wide scans have been published on anxiety disorders or anxiety-related personality traits (see Hovatta and Barlow 2008 for review). Surprisingly, little overlap exists between loci identified in different studies which may be due to relying on an underpowered design, and it is becoming clear that the number of patients required for such analysis is larger than anticipated (Hovatta and Barlow 2008).

It is evident from these aforementioned studies that the genetics underlying the various anxiety disorders is significantly more complex than a "single causative gene" etiological model, and a number of hypotheses for the lack of discovery of single candidate genes or variants have been proposed. In a comprehensive study of 90 different SNPs, Maron et al. (2005) concluded that genetic variants of several candidate genes of different neurotransmitter systems could contribute to the susceptibility to panic disorder, although individually with a relatively minor effect. Studies aimed at detecting smaller effects for any given gene's contribution, therefore, may require many thousand participants in order to detect significant associations (Surtees et al. 2007). In addition to polygenic contributions, pleiotropic, epistasic, epigenetic, and gene–environment interactions have been proposed as potential genetic mechanisms at work in anxiety disorders (Kendler 2001; Lesch 2001; Finn et al. 2003; Gordon and Hen 2004; Flint 2006; Canli and Lesch 2007; Murphy and Lesch 2008).

When examining anxious personality traits in man, rather than anxiety disorders *per se*, some more encouraging genetic relationships are apparent. First, there is evidence that anxious personality traits may increase the risk for developing an anxiety disorder (Kendler et al. 1995; Finn and Smoller 2001; Finn et al. 2003; Smoller et al. 2003; Smoller et al. 2005). Such studies indicate that the investigation of anxious traits in nonpatient populations may be highly informative in the search for the genetic architectures of anxiety disorders. This is of particular interest when viewed from an animal modeling perspective, as it forms a foundation for the utility of unconditioned tests of anxiety in model animal species. In animal modeling of anxiety traits, the behavioral analysis of inbred mouse strains, and of selectively-bred rats and mice, are well positioned for modeling aspects of trait anxiety and, by use of tools such as quantitative trait loci analyses (QTL), to the discovery of the genetic architecture of anxiety traits (Flint and Mott 2001). Comparable studies in nonpatient human populations will ultimately build an essential bridge to the validation and development of translational models of anxiety-like behavior and hence to anxiety disorders (Geyer and Markou 2002).

A second consequence of the association between anxious traits and anxiety disorders in man is that some specific candidate genes show reproducible findings in linkage and association studies with anxious personality traits in man, for example, CRF and allelic variations in the 5-HTT.

There has been substantial interest in CRF as a prospective genetic causative or predisposing factor in anxiety disorders, given that CRF is centrally involved in stress responsiveness and control of the hypothalamic-pituitary-adrenal axis (HPA), which is highly reactive to stressful stimuli, and therefore implicated in the mechanism of action of both depression and anxiety (Shekhar et al. 2005; Todorovic et al. 2005). Unexpectedly, there are relatively few studies examining the genetic relationship between CRF or its receptors in relation to anxiety disorders, and these few do not indicate a direct genetic etiology (Finn et al. 2003; Tharmalingam et al. 2006). However, Smoller et al. reported an association with a microsatellite marker linked to the CRF gene and behavioral inhibition in children, which was particularly apparent in the children of parents with panic disorder (Smoller et al. 2003;

Smoller et al. 2005). These studies suggest that the CRF gene affects inhibited temperament, and therefore may be a risk factor for trait anxiety predisposing to panic and phobic anxiety disorders (Smoller et al. 2005).

Many studies have investigated the contribution of polymorphisms of the 5-HT transporter (*SERT*) to the risk for anxiety disorders. The short allele of the 5-HTTLPR variant has been associated with anxiety-related traits and anxiety disorders in many studies (Denys et al. 2006; Grados et al. 2007; Lesch et al. 1996; Melke et al. 2001; You et al. 2005), although many other studies have also reported a lack of association (Billett et al. 1997; Camarena et al. 2001; Hamilton et al. 1999; Lang et al. 2004; Meira-Lima et al. 2004; Samochowiec et al. 2004; Schinka et al. 2004). However, it is important to note that such discrepancies may well be a function of which anxiety traits were measured in a particular study (Schinka et al. 2004). Neuroimaging studies have suggested that S-allele carriers exhibited hyperactivity of the amygdala in response to fearful stimuli (Hariri et al. 2002), thus further supporting a role for the S-allele of the 5-HTTLPR polymorphism in anxiety disorders. The SERTin2 polymorphism has been associated with OCD (Baca-Garcia et al. 2007), anxiety-related personality traits (Vormfelde et al. 2006), but not GAD (You et al. 2005). Moreover, a rare polymorphism in the ninth exon, Ile425Val, has been found in unrelated families with OCD (Ozaki et al. 2003; Wendland et al. 2008). Taken together, genetic association studies suggest an association of *SERT* gene variants with risk for anxiety disorders in humans.

4.1 Gene–Environment Interactions

As mentioned above, heritability estimates for anxiety disorders are relatively modest. In theory, this indicates that a great deal of familial variation is not explained by genetic factors, and indeed, individual environmental factors are considered as a major contribution in nonfamilial variation. This environmental component, however, also includes variability explained by gene–environment interactions that are not accounted for in the estimates of genetic contributions alone (Hettema et al. 2001b; Gordon and Hen 2004). It has been estimated from twin studies that up to 20% of the genetic influences in psychiatric disorders may be mediated in such a way (Kendler 2001), and that interactions between genes and the environment contribute significantly to the etiology of anxiety disorders and other psychiatric illnesses (Kendler 2001; Gordon and Hen 2004).

Recent studies into gene–environment interactions have been particularly instructive in furthering the understanding of genetic contributions to anxiety disorders. Perhaps the best example of gene–environment interactions in psychiatry is the study of Caspi and colleagues, who demonstrated that carries of the short (*s*/*s*) allele of the 5-HTT were more likely to develop depression when challenged with a significant life stress than carriers of the long (*l*/*l*) allele (Caspi et al. 2003), a finding that has also been replicated by other investigators (Kendler et al. 2005; Uher and McGuffin 2008).

It is plausible that the complexity introduced by gene–environment interactions may be responsible, at least in part, for the lack of progress in the discovery of genetic origins of anxiety disorders. Moreover there is an increasing realization that epigenetic influences can alter behavior significantly (Francis et al. 2003; Weaver et al. 2006; Tsankova et al. 2007; Abel and Zukin 2008; Carola et al. 2008; Darnaudery and Maccari 2008; Seckl 2008; Skinner et al. 2008). The preclinical laboratory, whereby there is control over the genetic background of animal subjects and the environment in which they are raised, is well positioned for advancing discovery in the field of gene–environment interactions (Carola et al. 2006).

4.2 Endophenotypes: Modeling Behavior and Genetics Together?

The heterogeneous findings of genetic and linkage studies in man may arise at least in part due to the current diagnostic criteria which is complex, descriptive and subjective in nature, particularly when combined with a self-report based approach. This has been considered by many in the field to provide challenges in clinical diagnosis itself, and certainly in the translation to animal models (Wong and Licinio 2001; Gottesman and Gould 2003; Hasler et al. 2004; Cryan and Holmes 2005; Schulze et al. 2005; Canli and Lesch 2007; Murphy and Lesch 2008). As a consequence, there have been growing calls for the diagnosis and treatment of anxiety and other psychiatric disorders to focus on individual behavioral, physiological, or neurochemical endpoints, rather than entire syndromes *per se* (Geyer and Markou 2002; Gottesman and Gould 2003; Hyman and Fenton 2003; Hasler et al. 2004; Schulze et al. 2005; Markou et al. 2008). This approach aims to identify putative endophenotypes characteristic of diseases and model them independently rather than the whole syndrome (Hasler et al. 2004; Hasler et al. 2006). The term endophenotype, in a psychiatric context, was coined by Gottesman and Shields in the early 1970s (Gottesman and Shields 1973) where they described it as an internal phenotype that emerges from the pathway between genes and the disease state. Fundamental to the concept is the assumption that the genetic basis of variations of the given endophenotypes between patients and control subjects are fewer than those involved in the manifestation of a complex disorder (Gottesman and Gould 2003; Hasler et al. 2004). Thus, employing endophenotypic approaches provides a means for identifying the genetic basis of specific clinical phenotypes, in addition to aiding the analysis of the phenotypic consequences of genes being turned on or off (Gottesman and Gould 2003; Hasler et al. 2004). The endophenotype approach has led to some success in human anxiety disorder studies but is not without some critique (Flint and Munafo 2007).

A consequence of the endophenotype approach is that it is significantly more applicable to animal modeling than holistic approaches (Cryan and Holmes 2005; Holmes and Cryan 2006). When broken down into individual components, many of the symptoms of anxiety disorders are distinctly compatible with current behavioral techniques used in laboratory animals (Cryan and Holmes 2005). Examples of how

Table 1 Symptoms and characteristic features used in the DSM-IV diagnosis of Anxiety Disorders and how they may be modeled in mice (adapted from (Cryan and Holmes 2005

Symptom	How might it be modeled in mice
Avoidance of places from which escape may be difficult *(Agoraphobia)*	Increased avoidance of exposed, well-lit areas
Sudden onset of intense fearfulness, often with respiratory distress and fear of "going crazy" *(Panic Attack)*	Increased flight from a predator
Anxiety provoked by social situations, leading to avoidance behavior *(Social Phobia)*	Low social interaction with unfamiliar conspecific
Anxiety provoked by a specific feared object, leading to avoidance behavior *(Specific Phobia)*	Exaggerated avoidance behavior to specific stimulus
Re-experiencing a traumatic event, leading to increased arousal and avoidance of stimuli associated with the event *(Posttraumatic Stress Disorder)*	Increased freezing response to fear conditioned cue or context
Anxiety-provoking obsessions, anxiety-reducing compulsions (OCD)	Increased marble burying, excessive grooming
Difficulty concentrating or mind going blank (Generalized Anxiety Disorder)	Impaired sustained attention
Sleep disturbance/insomnia	Abnormal sleep architecture (measured via electroencephalogy)
Autonomic hyperarousal (tachycardia, blushing, sweating, frequent urination)	Radiotelemetric measurement of heart rate dynamics during anxiety-provocation, increased stress-induced hyperthermia
Flashbacks of traumatic events	Impairment in extinction of fear memory
Cognitive bias towards ambiguous or weak threat cues	Increased fear conditioning to partial threat cue
Heightened startle response, particularly in threatening contexts	Increased acoustic startle response, and fear-potentiated startle response
Separation anxiety	Increased ultrasonic vocalizations in pups separated from their mother
Feeling of losing control or going crazy during a panic attack	Cannot be modeled

specific symptoms of anxiety disorders may be modeled in laboratory species are shown in Table 1.

5 Genetic Approaches in Animal Modeling of Anxiety and Anxiety Disorders

The use of targeted mutation approaches in vertebrate mammals to discern the behavioral function of genes has now become relatively common (Crawley 2007). Genetic techniques utilized in anxiety research, similar to other branches of research, include both "genotype-to-phenotype" and "phenotype-to-genotype"

approaches. Genotype-to-phenotype, or reverse genetics, refers to investigations that begin with the targeted mutation of a gene followed by evaluation of the resultant phenotype in the mutant animal. Such approaches include conventional, constitutive mutations of single genes, such as gene deletions (knockouts, also called null mutations), point mutations and insertions (knockins, overexpression and transgenics), which are present from conception and throughout the life of the mutant and exist in all cell types where the gene is expressed (Holmes 2001). Conditional and inducible mutations, as well as viral transfection and siRNA approaches are also included in the reverse genetics category. Reverse genetics techniques are of particular value for the exploration of the role of a single gene (and its genetic interactions) in a particular phenotype.

"Phenotype-to-genotype", or forward techniques, begins with the evaluation of a trait of interest, followed by the subsequent pursuit to determine the genetic architecture of that trait. Such approaches include the study of phenotypically segregating inbred mouse strains in combination with QTL analysis; gene expression profiling with DNA microarray chips to investigate the effects of various experimental conditions on induction or inhibition of gene expression in the brain; phenotypic and genetic analysis of randomly generated mutations *via* radiation or chemical agents; and genetic analysis of animals selectively bred for specific traits of interest. A primary advantage of forward genetic techniques is that they are unbiased with regard to the identity of the underlying genes involved, and indeed to the overall genetic architecture of the trait. This positions the technique particularly well for the elucidation of complex and polygenic traits, an important quality given the nature of the genetic architecture of human anxiety traits and disorders.

5.1 Genotype-to-Phenotype: Targeted Manipulation of Candidate Genes

5.1.1 Constitutive Genetic Manipulations

"Knockouts", in this article, refers to animals with a targeted deletion of a gene for the purpose of removing the protein product encoded by the gene, and are also referred to as a loss of function or null mutations. "Transgenic" animals have an added gene, either extra copies of a gene native to the genome in question, or one from a foreign genome, for example, insertion of human genes into mice or of mouse genes into the rat. "Knockin" is the insertion of a gene or part thereof, while a "point mutation" refers to a relatively small genetic mutation within a gene, such as the insertion of stop codon, or to alter an amino acid in a sequence, for example, to mimic a human genetic mutation (see Papaioannou and Behringer 2005; Crawley 2007 for more information on the generation of mutant mice).

More than 80 mutant strains of mice are described in the literature as presenting an anxiolytic or anxiogenic-like phenotype (Cryan and Holmes 2005). Many of

these mutants have been generated from hypotheses based on the mechanisms of action of clinically efficacious anxiolytics, or to reproduce a human genetic mutation thought to be linked to anxiety (e.g., the val66met BDNF variant; Chen et al. 2006), although there are also numerous examples of phenotypes in knockout and transgenic mice that potentially reveal novel mechanisms involved in anxiety (Cryan and Holmes 2005). Examples of mutants generated from hypotheses based on anxiolytic efficacy include the predominantly null and point mutations of the $GABA_A$ receptor subunits and benzodiazepine binding sites, the GABA synthetic enzyme glutamic acid decaroxylase isoform 65 (GAD65); null mutations in the serotonin system including SERT or the 5-HT receptors ($5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{2C}$, and $5\text{-}HT_{5A}$), and null mutations in the monoamine system such as monoamine oxidase A and B (MAO-A and MAO-B), catechol-*O*-methyltransferase (COMT), norepinepherin transporter (NET), vesicular monoamine transporter 2 (VMAT2), and the receptors α_2-adrenergic receptor subtype *a*, dopamine D3 and D4 receptors. The involvement of the HPA axis in the stress response has likewise stimulated the generation of mutants implicated in this pathway, such as null mutants of corticotrophin releasing factor (CRF), urocortin knockout, CRF-binding protein, CRF receptors 1 and 2 (individually and together), the glucocorticoid receptor (GR), and transgenic overexpression of both CRF and GR. The anxiety-related phenotype of these mutants has been reviewed elsewhere (Holmes 2001; Finn et al. 2003) and readers are referred to these articles for further details. On the whole, however, genetic manipulations of these aforementioned serotonin, MAO-A, NET, and HPA targets (with the notable exception of the CRF knockout) have yielded reasonably strong evidence for a role for these systems in murine anxiety-like behavior.

More recently, single mutation targeting has expanded considerably from serotonin, monoamines, $GABA_A$, and the HPA axis. Further work of particular relevance to the GABAergic hypothesis in anxiety includes investigations in our laboratory into the metabotropic GABA receptor, $GABA_B$. $GABA_B$ receptor agonists/positive modulators have an anxiolytic profile in many preclinical and some clinical settings (see Cryan and Kaupmann 2005). Correspondingly, $GABA_{B(1)}{}^{-/-}$ and $GABA_{B(2)}{}^{-/-}$ mice show a highly anxious phenotype in exploratory paradigms (Mombereau et al. 2004a, b; Mombereau et al. 2005), indicating a role in trait anxiety and potential relevance to the modeling of GAD. Additionally, mice with loss of function mutations of the $GABA_{B(1)}$ subunit isoforms ($GABA_{B(1a)}{}^{-/-}$ and $GABA_{B(1b)}{}^{-/-}$ mice), generated by point mutation knockin of stop codons to prevents transcription of the respective isoforms (Vigot et al. 2006), show differential impairments in acquisition and extinction in aversive memory tasks (Jacobson et al. 2006; Shaban et al. 2006), which may be more applicable to modeling aspects of PTSD.

Examples of other mutations that have been reported to alter anxiety-related behaviors include neuropeptides and their receptors, cell adhesion molecules, metabotropic glutamate receptors and subunits of glutamate ligand-gated ion channels, point mutations and transgenes related to Alzheimer's Disease, second-messenger signaling proteins, proteins associated with dopaminergic function,

apoptotic signaling proteins, circadian signaling genes, vitamin receptors, lipid binding proteins, steroid hormone receptors, and a variety of other gene products.

Overall, however, the 5-HTT knockout and heterozgyotic mice are perhaps the most well studied of all single-gene constitutive mutants in anxiety research, due not only to their construct validity given by the action with SSRI mechanisms of action, but also because of their face validity as a model of relevance to the clinical findings with 5-HTTLRP *s*-allele carriers (see Murphy and Lesch 2008 for a comprehensive overview of 5-HTT mutants). Like human 5-HTTLRP *s*-allele carriers, 5-HTT mutant mice show a gene-dose dependent reduction in 5-HTT binding sites and reuptake capacity (Lesch et al. 1996; Bengel et al. 1998), and both the 5-HTT knockout and 5-HTTLRP *s*-allele carriers show enhanced trait anxiety (Lesch et al. 1996; Holmes et al. 2003b, c; Sen et al. 2004; Munafo et al. 2006), hypersensitivity to stressful events (Carroll et al. 2007; Adamec et al. 2008; Caspi et al. 2003; Kendler et al. 2005), and impairment of fear extinction recall (Pezawas et al. 2005; Wellman et al. 2007).

It is also worth bearing in mind that both 5-HTTLRP *s*-allele carriers, like 5-HTT mutant mice, express a constitutive genetic condition, and therefore may share an etiological basis as well. This raises one of the broadly discussed issues in genetic modeling. It is clear that mutant animals that carry their mutation from conception are appropriate for the modeling of naturally occurring constitutive mutations found in the clinic, in terms of the presence of the mutation during development. However, gene deletions represent the greatest extreme of a genetic alteration, and mechanisms such as compensation by other genes are likely to be induced. These compensations can, at worst mask interpretation of the function of the target gene function, while at best they can theoretically unmask important compensatory mechanisms of interest (Holmes 2001; Crawley 2007). Another feature of classical constitutive mutations is that they are ubiquitously expressed, and for the examination of CNS roles of genes, peripheral effects of the mutation may also have consequences on the behavior of the mutant animal that are unrelated to the gene's CNS function. Finally, development-dependent plieotropic actions of genes should also be considered; that is, the role of a gene may differ in specific stages of ontogeny. One example of this from Alzheimer's Disease research includes the actions of beta-secretase, a key enzyme in the production of beta-amyloid that is thought to be one of the main causative mechanisms in Alzheimer's Disease (Venugopal et al. 2008). Beta-secretase, however, is also involved in myelination during development (Willem et al. 2006). This complicates the interpretation of the phenotype of constitutive knockout mice with regard to Alzheimer's like behavioral alterations, as the mice also show phenotypes that are characteristic of schizophrenia models that may be related to developmental effects on ErbB4 and neuregulin1 signaling (Savonenko et al. 2008).

Conditional and inducible mutations offer methods for addressing developmental and ubiquitous expression issues. Conditional mutations are restricted to expression in specific brain regions, for example, the CaMKIIα promotor in combination with a targeted gene mutation restricts the expression of the mutation to forebrain neurons (Mayford et al. 1996). Inducible mutations, such as the Tet-on and Tet-off

systems, are also compatible with tissue-specific promotors, although their greatest advantage is the provision of control over *when* a mutation is transcribed. This allows the study of functions of specific genes at critical developmental periods, and the prospects of reversing a genetically-induced pathological phenotype after its manifestation (Mansuy and Bujard 2000). In the Tet-off system, a gene of interest is linked to the tetracycline-controlled transactivator (tTA), and is transcribed under normal circumstances, but turned off in the presence of the antibiotics tetracycline or doxycycline (Dox) administered, for example, in the drinking water of mice. In contrast, in the Tet-on system, a gene linked to reverse tTA (rtTA) is normally inactive but transcription is turned on in the presence of Dox (see Papaioannou and Behringer 2005, Crawley 2007, and Mansuy and Bujard 2000 for details). Interesting examples of conditional and inducible mutants in anxiety research include a forebrain restricted CRF-R1 knockout mouse (*Crhr1*$^{loxP/loxP\text{-}Camk2a\text{-}cre}$), which spares the pituitary and HPA axis. These mice showed an anxiolytic-like phenotype in exploratory paradigms, and exaggerated cortisol release to restrain stress, demonstrating a central role of the CRF-R1 in anxiety, and the feedback control of the HPA system and it's adaptation to stress that is independent of the HPA axis itself (Muller et al. 2003).

In a second highly pertinent example, a conditional and inducible knockout approach was used to probe the functions of the 5-HT_{1A} receptor in anxiety. Constitutive, global knockout of the 5-HT_{1A} receptor enhances anxiety-like behavior in exploratory paradigms and the novelty-induced suppression of feeding paradigm of adult mice (Ramboz et al. 1998; Gross et al. 2000). Gross et al. (2002) used conditional (CamKIIα promotor), inducible (Tet-off) 5-HT_{1A} knockout mice to demonstrate that expression of functional 5-HT_{1A} receptors in the forebrain (but not in the serotonergic cell bodies of the dorsal raphé nucleus) are vital in preventing the anxious phenotype of the full 5-HT_{1A} receptor knockout mice. Furthermore, loss of the 5-HT_{1A} postsynaptic receptor during development induced an anxiogenic-like phenotype similar to that of full knockouts, whereas loss of the postsynaptic receptor in the adult was without effect on anxiety-like behavior (Gross et al. 2002). These landmark studies clearly demonstrated the importance of functional postsynaptic 5-HT_{1A} receptors during critical development and early postnatal phases for control of anxious behavior in the adult.

5.1.2 Transgenic Rats

Although mice are generally the species of choice for targeted genetic manipulations, the generation of transgenic rats is feasible (Cozzi et al. 2008), and has been utilized as a model species in the study of anxiety. The development of transgenic rats as a research tool has lagged behind their murine counterparts, primarily because of challenges in isolating puripotent rat embryonic stem cells. For the most part, transgenic rats are generated by injection of a transgene construct into the pronucleus of an isolated embryo at the single cell stage, which are then reimplanted into foster dams. Other methods for generating transgenic rats include

sperm-mediated transgenesis and viral transfection. There is a very limited number of lines of knockout rats, with loss-of-function mutations in rats more usually approached by dominant negative strategies (where an introduced gene protein product blocks the expression of the target gene), siRNA (see below), and chemical mutagenesis (discussed later; see Cozzi et al. 2008 for a recent review on genetic modification techniques in the rat). The first successfully generated transgenic rat carried the mouse *Ren*-2 renin gene, for the purposes of modeling primary hypertension (Mullins et al. 1990). Recently, a second transgenic rat, TGR(AsrAOGEN) 680, also targeted the renin–angiotensin system but at the level of the renin substrate, angiotensinogen, with an overexpressed antisense RNA against angiotensinogen mRNA. In addition to reduced blood pressure, renin-induced drinking, and brain, but not plasma angiotensinogen levels, these rats also showed enhanced anxiety in primary and ethological readouts in the elevated plus maze, social interaction, and open field test (Voigt et al. 2005).

An example of a transgenic rat model generated primarily for anxiety research includes a fivefold overexpression of the rat NPY gene (Michalkiewicz and Michalkiewicz 2000). These rats show elevated CA1 and CA2 field prepro-NPY mRNA and NPY protein, with concurrent reductions in Y-1 autoradiographic binding throughout the hipocampus (Thorsell et al. 2000). These rats failed to respond to restraint stress with reduced open arm entries in the elevated plus maze, and showed anxiolytic-like behavior in the punished drinking test, although corticosterone responses to restraint stress did not differ from wild-type rats (Thorsell et al. 2000). Morris Water Maze performance was also impaired in the NPY over-expressing rats, and it therefore may be debated as to whether the primary phenotype of these rats was anxiolytic, cognitive (e.g., in an aversive associative learning domain), or a result of interactive impairments in both. However, the anxiogenic-like phenotype of the NPY knockout mouse (Bannon et al. 2000) in combination with the rat study cements an interpretation for the involvement of NPY in anxiety (Voigt et al. 2005).

5.1.3 siRNA and Viral Transfection

Short interfering RNA silencing (siRNA) and viral transfection techniques offer alternative methods for studying the physiological and behavioral roles of gene products in animal models (Tenenbaum et al. 2004; Green and Nestler 2006; Thakker et al. 2006). Viral transfection techniques involve the insertion of a targeted gene construct into the genome of a nonreplicating virus. Examples of viruses used in transfection applications that are appropriate for CNS application are herpes simplex virus (HSV-1), adeno-associated virus (AAV), and lentivirus (Green and Nestler 2006). A marker protein, such as green-fluorescent protein (GFP), is usually included in the expression plasmid to allow subsequent visualization of its anatomical expression. The plasmid is transfected into cell culture where it is packaged into capsids (viral protein coats) and amplified to generate sufficient material. After purification, it is ready for injection into a brain region of interest.

The viral promotor incorporates the target construct into the genome of neurons (and to a certain degree, glia, depending on the type of viral vector, promotor, and delivery capsid; Janson et al. 2001) near the injection site, and expression of the mutated construct ensues (see Janson et al. 2001; Green and Nestler 2006 for reviews).

RNA silencing refers to a naturally occurring post-transcriptional silencing phenomenon whereby double-stranded RNA or hairpin RNA triggers the formation of short interfering RNA (siRNA) or micro-RNA (miRNA), respectively. These form a complex with the nuclear proteins RISC or RITS, which then bind in a sequence-specific manner to a corresponding mRNA sequence. The complex directly blocks translation of the mRNA and causes the cleavage and destruction of the mRNA. In the laboratory, siRNA constructs may be applied directly into the mouse ventricular system with an osmotic minipump, where they have been shown to selectively knock down targeted CNS proteins, resulting in measurable behavioral alterations (Thakker et al. 2006).

Viral transfection and siRNA techniques offer specific advantages as genetic manipulation methods (Janson et al. 2001; Green and Nestler 2006; Jacobson and Cryan 2006; Thakker et al. 2006). The risk of contamination by unwanted genetic material is low in comparison to classical knockout techniques, where contamination from the embryonic stem cell can be present even in a number of subsequent generations (Cook et al. 2002). Viral transfection and siRNA are applicable to both rats and mice (Green and Nestler 2006; Thakker et al. 2006; Cozzi et al. 2008), which, as they may be sourced directly from reputable suppliers on a needs-be basis, removes the requirement to maintain an in-house colony of a mutated strain. Indeed, constructs may indeed be applied to as many different strain as desired, and as such, the most appropriate strains for any given behavioral test can be used. Additionally, since viral and siRNA constructs are applied directly in the adult animal, resulting phenotypes are not influenced by developmental complications that may affect constitutive mutants. This is of particular value for studying genes where knockout techniques result in nonviable offspring, for example, BDNF.

Both viral transfection and siRNA techniques also have method-specific disadvantages. Viral packaging limits the size of possible insertions, and there are potential toxicity risks when dealing with viral vectors, for example, via insertional mutagenesis or viral vector–host immune interactions, which can be difficult to predict (Janson et al. 2001; St George 2003). For both methods, control over the type or amount of cells affected and the extent of overall expression is limited. For viral transfection there is the risk of host-directed inactivation via mechanisms such as promoter silencing or immunological reaction. Both techniques are in essence knockdown methods – abrogation is unlikely to ever be complete. Finally, as is the case for all genetic manipulations, there is always the chance of redundancy in a given system and that a residually expressed protein can compensate for the silenced gene.

siRNA and viral transfection methods have both been employed in anxiety-related research to good effect. We have used an in vivo siRNA approach to knock down the metabotropic glutamate receptor 7 (mGluR7), a target for which no

pharmacological antagonists existed at the time (Fendt et al. 2008). Seventeen days of infusion into the dorsal third ventricle of either of two different siRNAs that targeted different regions of the mGluR7 gene, significantly reduced mGluR7 mRNA expression by about 15–20% in specific brain regions, including the basal and lateral (but not central) parts of the amygdala and the hippocampus. This attenuation was accompanied by profound deficits in the extinction of a CTA. Supporting the specificity of the mechanism, the mGluR7 agonist AMN082 facilitated extinction of CTA in a different group of mice, and in two further separate experiments, impaired the acquisition and the extinction of FPS in rats (Fendt et al. 2008). These studies thereby confirmed a specific role for mGluR7 in the extinction of conditioned aversive memory, using siRNA mediated inactivation and pharmacological activation techniques in two different species and behavioral tasks.

Heldt et al. (2007) used a lentiviral approach to investigate the role of BDNF in extinction of aversive memory. Knockdown of hippocampal BDNF was achieved by injecting a lentivirus expressing Cre into the hippocampus of adult mice that were floxed at the BDNF locus. In these mice, hippocampal infection rate was on average 53%, and comparable levels of knockdown of hippocampal BDNF mRNA were achieved. These mice demonstrated normal acquisition of FPS and conditioned freezing, but showed significantly reduced extinction in both paradigms (Heldt et al. 2007). In a viral transfection study at a separate laboratory (Berton et al. 2006), adult floxed BDNF mice were injected with an AAV vector expressing Cre recombinase into the ventral tegmental area (VTA) in order to selectively ablate BDNF in this structure. These mice showed an antidepressant-like phenotype in a social defeat model of chronic psychosocial stress. Together these studies may help to explain some aspects of the divergent clinical findings on the relationship between BDNF val66met SNP and anxiety traits and disorders, as they clearly demonstrate opposing permissive roles for BDNF in different brain structures, and therefore in different stress-related cognitive and socio-behavioral domains that are affected in anxiety-related disorders.

5.2 Phenotype-to-Genotype

5.2.1 Inbred Mouse Strains, Gene–Environment Interactions and Quantitative Trait Loci

One of the greatest advantages of the laboratory mouse as a genetic discovery tool is the availability of a number of different inbred mouse strains. An inbred mouse strain is generated by mating related mice, brother × sister, or parent × offspring, for 20 or more generations. This renders approximately 98.6% of the loci homozygous for each mouse within the strain. Since many of the established inbred strains have a far greater number of generations than this, they can be considered as essentially isogenic and homozygous at all loci (Beck et al. 2000). In theory, the isogenicity of the inbred strain suggests that differences between strain trait means

should primarily be a function of genotype (Crabbe et al. 2002). Certainly, many studies have demonstrated phenotypic differences in anxiety-related behaviors and stress responsiveness between inbred strains; for example, the highly anxious and stress-sensitive phenotype of BALB/c mice, has lead some researchers to propose this strain as a suitable model for pathological anxiety *per se* (Anisman and Zacharko 1992; Griebel et al. 2000; Anisman et al. 2001; Belzung 2001). Inbred strains also show differences in their responses to a number of pharmacological agents, suggesting that genetic-based neurobiological differences underlie the observer behavioral variations (reviewed in Crawley et al. 1997; Jacobson and Cryan 2006; Crawley 2007), and for examples, see (Belknap et al. 1989; Crabbe et al. 1994; Crabbe et al. 1998; Griebel et al. 2000; Lucki et al. 2001; Crabbe et al. 2002; Jacobson and Cryan 2005). As such, the inbred mouse has been recognized as an immensely valuable tool for fundamental genetic research and genetic modeling of human disease states. In response to this recognition, and the growing volume of data generated with different inbred strains, an international consortium, the Mouse Phenome Project, was launched to support mouse genomic discovery research and to provide research tools for complex trait analysis including the Mouse Phenome Database, a shared repository for phenotypic and genotypic data voluntarily contributed by researchers in the field (see http://www.jax.org/phenome and Grubb et al. 2008)

Clearly, one of the advantages to inbred mouse strains in combination with targeted mutation approaches is that they allow the assessment of a specific genetic manipulation against a controlled background genetic variability (Festing 2004). Furthermore, when the same mutation is generated in more than one strain, they can be used to determine the effect of genetic background on the phenotype induced by a single mutation, and by inference, the robustness of the phenotype. It is hoped that the study of single mutations generated in different inbred mouse strains may ultimately assist in the discovery of gene–gene interactions in polygenetic traits (Silva et al. 1997; Fisch 2006). However, the situation is somewhat more complex than this, for example, care must be taken in such approaches to ensure that variations in strain background behavior do not mask phenotypes generated by the mutation. Examples of this phenomenon may be seen with 5-HTT knockout and preproenkephalin-deficient mice (Penk1^{-}/$^{-}$) (Holmes et al. 2003a; Bilkei-Gorzo et al. 2004). In 5-HTT knockout mice, the congenic C57Bl/6J background mice showed the expected enhanced anxiety in the light–dark box and elevated plus maze while the 129S6 did not, although both strains failed to display hypothermia in response to a challenge with a 5-HT$_{1A}$ receptor agonist, confirming *via* 5-HT$_{1A}$ receptor loss-of-function that the 5-HTT null mutation was penetrant in both strains (Holmes et al. 2003a). Similarly, Penk1^{-}/$^{-}$ on a C57Bl/6J background showed enhanced anxiety in the light–dark box and startle paradigms, but not in the social interaction test. In contrast, the Penk^{-}/$^{-}$ mice on the DBA/2J background showed enhanced anxiety in the elevated zero maze and social interaction tests (Bilkei-Gorzo et al. 2004). Both Holmes et al. (2003a, b) and Bilkei-Gorzo et al. (2004) also noted significant, test-specific differences in baseline anxiety between the background strains, indicating that anxiety-like changes may have been expressed in

both backgrounds in response to the mutations, but that different types of strain-specific tests may be needed in order to detect them. Similar results have been reported with 5-HT_{1A} receptor knockout mice generated on three different background strains (C57Bl/6, Swiss Webster × 129/Sv and 129/Sv) (reviewed in Groenink et al. 2003). Together these findings have lead to the observation that targeted mutations in general should ideally be introduced into more than one or two strains, and that phenotypic assessment of the phenotype of such mice requires a test battery approach, rather than reliance on a small number of tests (Cryan and Holmes 2005; Arguello and Gogos 2006; Fisch 2006).

Recently, the notion of phenotypic variation of inbred strains as a primary product of genotype has been challenged. This arises primarily from the demonstration that strain-dependent anxiety-related behaviors can be strongly influenced by environmental factors such as strain-clustered maternal rearing styles, as was elegantly illustrated in the cross-fostering studies of Francis and colleagues (Francis et al. 1995; Francis et al. 1999; Francis et al. 2003). With regard to anxiety-related behavior, Francis et al. (2003) cross-fostered C57Bl/6 pups, a strain with reduced innate anxiety relative to the BALB/c strain, under four different maternal strain rearing conditions (prenatal/postnatal dam): (1) C57Bl/6/C57Bl/6, (2) C57Bl/6/BALB/c, (3) BALB/c BALB/c, and (4) BALB/c/ C57Bl/6. When tested as adults, the C57Bl/6 pups reared in utero and postnatally by BALB/c dams showed enhanced anxiety in the open field and elevated plus maze tests and poor performance in the Morris Water Maze indistinguishable from control BALB/c mice, while all other maternal environments produced behaviors more characteristic of C57Bl/6 control mice (Francis et al. 2003). However, C57Bl/6 pups reared in utero by a C57Bl/6 dam but postnatally by a BALB/c dam showed a phenotype like that of control C57Bl/6 mice. In a subsequent study, BALB/c dams showed significantly less arched-back nursing, licking, and grooming of pups and spent more time away from the nest than C57Bl/6 dams (Priebe et al. 2005), and the anxious behavior of BALB/c pups as adults in the elevated plus maze was mitigated when they were reared by a C57Bl/6 dam. Interestingly, however, the poor maternal care of BALB/c dams did not enhance anxiety in C57Bl/6 pups in the elevated plus maze. Together these results indicated that the pre- and postnatal maternal care can have profound influences on anxiety-related behavior in the adult, but in a way that interacts with genotype in a complex manner. Such studies exemplify the role of epigenetic environmentally-dependent alterations of gene expression which is known to be mediated by DNA methylation and histone modifications independent of the coding sequence (Holmes et al. 2005).

The isogenicity and distinct behavioral clusters that differentiate various inbred strains, and the degree of control available over the pre- and postnatal environment of laboratory animals, in itself provides an otherwise unapproachable facility to study gene–environment interactions (Holmes et al. 2005; Priebe et al. 2005; Kaffman and Meaney 2007).

Perhaps one of the greatest advances provided by inbred mouse strains is their essential contribution to QTL techniques for mapping loci that contribute to behavioral or pharmacological phenotypes (Flint 2003; Festing 2004). QTLs are

chromosomal regions that are associated with traits which are measured as continuous variables. QTL studies in mice are performed with a cross between two phenotypically segregating mouse strains to an F2 generation. The progenitor and F2 generation is assessed for the trait of interest, then genotyped at marker locations. Alternatively, recombinant inbred strains, which arise where progeny of two strains are inbred to 10 or more generations to create a series of substrains, or rats or mice selectively bred for extremes of a behavioral trait, may also be used (Mozhui et al. 2007). Associations between the genetic markers and the different trait phenotypes of the progeny are statistically analyzed using LOD scores (logarithm of the likelihood ratio for linkage) to determine the QTLs (Flint and Mott 2001; Flint 2003). In this respect, QTL is a powerful tool for the assessment of the genetic architecture of a trait, for example, by determining whether a trait is influenced by small contributions from multiple different loci, or by larger contributions from fewer loci. Following the identification of QTLs, further mapping of the linkage sites can be made, with the ultimate aim of identifying candidate genes (Korstanje and Paigen 2002). With regard to anxiety-related behavior, Flint et al. (1995), in one of the earlier searches for anxiety related QTLs, mapped three loci on chromosomes 1, 12, and 15 that influence emotionality in mice, based on correlated activity and defecation in the open field test, activity in a Y-maze and elevated plus maze performance. Since then many other QTL studies have been performed to assess the genetic architecture of murine anxiety-related behavior (Turri et al. 2001a; Turri et al. 2001b; Flint 2003; Henderson et al. 2004; Singer et al. 2005; Ponder et al. 2007; Bailey et al. 2008), although the greatest challenge with QTL analyses in mice remains the fine-mapping of loci, and hence the subsequent identification of quantitative trait genes (see (Hovatta and Barlow 2008) for a review).

One of the recent translational success stories from QTL analysis involves the fine-mapping of a previously determined anxiety-related QTL on chromosome 1, using an outbred mouse strain (MF1) as a fine mosaic to identify the "regulator of G protein signaling 2" gene (Rgs2) as a quantitative trait gene (Yalcin et al. 2004). Based on these results, Smoller et al. (2008b) examined the human ortholog of Rgs2 in a family based group and in unrelated adults. Rgs2 markers were associated with measures of childhood behavioral inhibition, adult self-reported introversion (NEO-Personality Inventory–Revised), and fMRI responses in an emotional faces task (Smoller et al. 2008b). These results give very encouraging evidence that QTL analysis to study the genetic architecture of anxiety traits in mice can ultimately provide a translational platform for anxiety-related genetic discovery in man.

5.3 Gene Expression Arrays

Gene expression arrays, sometimes also called DNA microarrays, allow the identification of genes that are expressed at a certain time in a certain tissue type. The microarray itself is a miniaturized glass or silicone chip with, generally, thousands

of short, specific DNA oligonucleotide sequences (e.g., genes, although arrays for SNPs are also available) adhered to the surface, which hybridize with genes or genetic material in the sample. Hybridization is quantified by fluorescence-based detection, the intensity of which indicates the relative abundance of expression in the sample. Microarrays can be used in experiments to compare the gene expression profile between strains or mutant lines of rats and mice, or to evaluate the effects of environmental manipulations (e.g., stress), drug treatments, in whole brains or different microdissected brain regions (Crawley 2007; Hovatta and Barlow 2008). The volume of data generated from microarray experiments can be overwhelming, and appropriate statistical filtering steps and algorithms are important for the management of data and identification of significantly altered gene expression patterns.

A comprehensive example of gene expression profiling relating to anxiety behavior is the study by Letwin et al. (2006). In this study, mirodisssected brain regions from eight mouse strains selected from the A priority group of the Mouse Phenome Project (Bogue and Grubb 2004) were analyzed using a cDNA microarray. These strains exhibit large genetic and behavioral diversity, making them ideal for relating gene expression differences to behavior. Moreover, the advantage of such an approach is exemplified by the access to a large online database of physiological and behavioral measurements available for them (http://www.jax.org/phenome). In the study a total of 35 genes in the periaqueductal grey were associated with total time spent in open quadrants of an elevated zero maze. Moreover, several of these correlated genes could be mapped to surrogate phenotypic QTLs related to anxiety. Examples include the upregulated gene Rims3 and the downregulated gene transcriptional activator Egr-1 in mouse strains exhibiting lower anxiety. Interestingly, increased Egr-1 expression has been reported after fear conditioning in the rat, and the anxiolytic diazepam has been shown to block the fear conditioning increase in Egr-1 as well as to reduce anxiety (Malkani and Rosen 2000).

Gene array studies are also highly complementary with other genetic techniques, as elegantly demonstrated in the recent study by Hovatta et al. (2005). In their study, six inbred mouse strains (129S6/SvEvTac, A/J, C3H/HeJ, C57BL/6J, DBA/2J, and FVB/N) were assessed for anxiety-related behaviors in the exploratory anxiety light–dark box and open-field tests. Seven brain regions implicated in anxiety neurocircuitry were dissected from the different strains and assessed using oligonucleotide arrays. Seventeen genes were identified as correlated with anxiety behavior and which varied robustly between the most and least anxious strains, and were verified by RT-PCR. The antioxidant enzymes glycoxylase-1 (Glo1) and glutathione reductase (Gsr) were selected for further investigation. High levels of Glo1 and Gsr enzyme activity was correlated with elevated anxiety in the open field performance of A/J, C57Bl/6J, their interbred F1 progeny and the noted high anxiety strain BALB/cByJ. Furthermore, manipulation of cingulate cortex expression of Glo1 and Gsr using lentiviral overexpression and siRNA knockdown, increased and decreased anxious behaviors, respectively (Hovatta et al. 2005). Together these experiments thoroughly validated a role for Glo1 and

Gsr in anxious behaviors and highlight an interesting approach to elucidate the molecular basis of anxiety. However, a proteomic and microarray investigation in another laboratory also identified Glo1 as differentially regulated in anxiety, using mice from an outbred strain (CD1), selectively bred for 15 generations for either high (HAB-M) or low (LAB-M) anxiety behaviors in the elevated plus maze (Kromer et al. 2005). Interestingly, in contrast to the studies of Hovatta et al. (2005), in this study Glo1 was more highly expressed in the low anxiety strain than in the high anxiety strain. Differences in the two studies results may originate from the use of different genetic models, or from the use of different test apparatuses. Given that diametrically opposite conclusions were drawn from both studies on the role of Glo1 in anxiety a considerable degree of caution is required when extrapolating data such as these from preclinical genetic studies to the human situation. Overall, these two studies illustrate that identifying genes and gene products that influence anxiety-related behaviour is the first step to begin unraveling the complexity of their actions.

Gene expression analyses are also applicable to other model species. In a chronic mild stress study with rat frontal cortex, RT-PCR-confirmed downregulation of 6 genes involved in synaptic transmission and second messenger signaling (*Itga6*, *Camk2a*, *Plcb1*, *Cart*, *Gad1*, *Homer1*, and *Th*) and the upregulation of two genes (*Egr2* and *Ptgs2*) was determined in anhedonic rats (Orsetti et al. 2008). Sabatini et al. (2007) investigated amygdala gene expression changes in young group-housed macaque monkeys separated as infants from their dams at1 week or 1 month of age, in comparison to normally-reared controls. These two early social stresses produce different types of anxious social behaviors in later life, being self-comforting and social-comforting behaviors, respectively. GUCY1A3 (guanylate cyclase 1α3) which is involved in the signaling cascade of nitric oxide, was the only gene found to be differentially regulated in the two treatment groups. Follow up in situ hybridization studies confirmed this result, and demonstrated that GUCY1A3 was highly expressed in the amygdala, and that its reduced expression in the 1 week deprivation group was positively correlated with social comfort behaviors and negatively correlated with self-comfort behaviors (Sabatini et al. 2007).

5.4 *Random Mutagenesis: Radiation and ENU Screens*

Since the early 1990s, when Takahashi and colleagues (Vitaterna et al. 1994; and see Takahashi et al. 1994, 2008) used a novel chemical mutagenesis approach to identify a mouse gene relevant to behavior, the Clock gene, which is essential for circadian behavior, there has been an upsurge in such genetic strategies. Forward genetic approaches are underway whereby a mutant line of animals is induced randomly by mutagens such as ethylnitrosonurea, and subsequently these animals' behavior is analyzed (Nolan et al. 2000a, b; Tarantino et al. 2000; Moldin et al. 2001; Ohl and Keck 2003; Keays and Nolan 2003). Positional cloning would then enable the identification of gene targets relevant for the behavioral response.

Clearly, mutagenesis screens for behavioral mutations require careful consideration of background strains for use in mutagenesis and subsequent mapping of the affected gene or genes. The use of inbred mouse strains greatly enhances the utility of this approach (Tarantino et al. 2000). A particular advantage of this approach is that no a priori assumptions are made about the nature of the underlying genes in any biological pathway under investigation (Nolan et al. 2000; Wells and Brown 2000). It is clear that these are large-scale projects and can only work within a multidisciplinary collaborative framework and with a strong bioinformatics support. In this context, the German National Genome Research Network was established in collaboration with the pan-European consortium EUMORPHIA, a mouse phenotyping center with open access to the scientific community, called the "German Mouse Clinic" (www.mouseclinic.de, Gailus-Durner et al. 2005). Across the US there are a number of different consortia developed each with a selective focus (Clark et al. 2004). Another advantage of the ENU approach is that it can also be applied to rats (Smits et al. 2006). Recently, a SERT deficient rat was generated using such a strategy (Homberg et al. 2007), whereby a premature stop codon caused a nonsense-mediated decay of the SERT transcript. Loss of SERT induced anxiety-like behavior in all tests conducted (Olivier et al. 2008), and thus may be an additional important tool to uncover the molecular basis of serotonergic function in anxiety.

5.5 Selective Breeding of Rats and Mice

Selective breeding has also proved to be a useful strategy to unravel the genetic basis of psychiatric disorders, including anxiety disorders. A breeding program begins with the evaluation of variation of a trait of interest in a genetically heterogeneous population, for example, a commercially available outbred strain, or in a heterogeneous strain generated from the crossbreeding of inbred strains. Individuals with responses at either extreme of the response curve are then selectively bred together over multiple generations. Ultimately, new strains are generated that express the trait of interest with, for example, an increased frequency, or extent, of expression in comparison to unselected animals. Heritability characteristics of the trait can be evaluated, and later generations of these new inbred strains can then be examined for underlying neurobiology, and polygenetic or pleiotropic correlates of the trait of interest (Phillips et al. 2002). This approach, therefore, greatly improves the probability of discovering anxiety-related neurobiological correlates (Singewald 2007). Examples of selectively bred mouse lines include those selected for high and low baseline immobility in the tail suspension test (TST) (Vaugeois et al. 1996, 1997; El Yacoubi et al. 2003), senescence accelerated mice (Takeda et al. 1997), the DeFries mice which were selected for 30 generations for high or low activity in the open-field test (DeFries et al. 1978), high and low anxiety mice (Kromer et al. 2005), mice bred for short or long attack latency in a social aggression test (Sluyter et al. 2003), and mice bred for opposing sensitivity to the

convulsive effects of the benzodiazepine receptor inverse agonist betacarboline-3-carboxylate (b-CCM) (Do-Rego et al. 2002).

A number of rat-based selective breeding programs have also been initiated based on phenotypes or behavioral characteristics relevant to anxiety (see Singewald 2007 for a review). Examples of rat lines founded using selective breeding for differing aspects of emotionality, including anxiety, are the Maudsley Reactive and Maudsley Nonreactive strains (Blizard and Adams 2002), Roman (Steimer and Driscoll 2003; Steimer and Driscoll 2005), Syracuse rat lines (Brush 2003), Tsukuba (Kitaoka and Fujita 1991), Floripa lines (Ramos et al. 2003), lines with infantile high and low ultrasonic vocalizations (Brunelli 2005), as well as High Anxiety Behavior (HAB) and Low Anxiety Behavior (LAB) rats (Landgraf et al. 2007; Singewald 2007).

Investigators are now employing candidate gene approaches in combination with gene expression profiling and proteomics to identify novel genes, SNPs, and haplotypes that are involved in the manifestation of the anxiety phenotype in these selected lines of rats and mice. It is hoped that the newly identified genes and their products will promote a greater understanding of the mechanisms and circuits that underlie normal and pathological anxiety-related behavior in rodents and ultimately in man (Landgraf et al. 2007).

6 Conclusion

The current status of clinical research indicates that anxiety traits and anxiety disorder in man have a genetic component, and therefore genetic modeling in animals is a logical approach to gain a greater insight into their neurobiology. However, it is also clear that the nature of these genetic contributions are highly complex. Over the past two decades there have been substantial technical advances in the field of molecular genetics. Such tools are well positioned to drive forward efforts to gain a better understanding of the underlying pathophysiology of anxiety and other neuropsychiatric disorders. It is hoped that reverse and forward genetic techniques employed in animal models, coupled with the recent elucidation and the ongoing functionalization of the human genome should provide new insights into the etiology, course, and ultimately treatment strategies for anxiety disorders and other psychiatric illnesses.

One of the most important advances in anxiety research has been the development of genetically manipulated mice, which provide control over the level, site, and timing of expression of specific proteins, be they receptors, transporters, enzymes, or signal transduction molecules. These new tools facilitate the examination of novel targets for disorders where few or no established pharmacological tools exist. These mice additionally enable better testing of the validity of current molecular hypotheses of various psychiatric disorders. Advances in mouse genetics have been accompanied by a remarkable upsurge in the development of models based on the selective breeding of both rats and mice for a specific behavioral trait.

These selected lines and the genetically unbiased nature of the approach are poised to serve as powerful substrates for understanding the neurobiological and genetic basis of behavioral processes and by inference, therefore of neuropsychiatric disorders. Likewise, specific manipulations of rearing environments of both genetically manipulated or selectively-bred rodent lines offers great potential for delineating complex gene × environment interactions, the importance of which has been demonstrated as a fundamental underpin in anxiety-related behavioral traits in both man and laboratory animals.

The success of genetic approaches in basic psychiatric research is critically dependent on the usefulness of behavioral models. Animal behavioral models can only ever be as good as the corresponding measures that are used in clinical research, which requires expansion to include both patients and nonpatients alike. Therefore, increasing research efforts must continue to be directed towards developing and refining specific behavioral tests in both man and experimental animals for such analysis, and for the overall success of the field of anxiety disorder research (Cryan and Holmes 2005; Cryan et al. 2007).

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Behavioral Correlates of Anxiety

Victoria Risbrough

Contents

Abstract The tripartite model of anxiety includes three response domains: cognitive (most often identified by self report), behavioral, and physiological. Each is suggested to bring a separate element of response characteristics and, in some cases, potentially independent underlying mechanisms to the construct of anxiety. In this chapter, commonly used behavioral correlates of anxiety in human research, including startle reflex, attentional bias, and avoidance tasks, as well as future tasks

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using virtual reality technology will be discussed. The focus will be in evaluating their translational utility supported by (1) convergent validity with other measures of anxiety traits or anxiety disorders, (2) their use in identifying neural and genetic mechanisms of anxiety, and (3) ability to predict treatment efficacy.

Keywords Anxiety · Fear · Behavior · Review · Startle · Translational

1 Introduction: Why Do We Need Behavioral Correlates of Anxiety?

Lang's tripartite model of anxiety responding suggests that there are three response domains: cognitive (most often identified by self report), behavioral, and physiological (Lang 1968). Each is suggested to measure a separate element of response characteristics and, in some cases, potentially independent underlying mechanisms to the construct of anxiety (Turner and Michelson 1984). So what exactly do behavioral measures bring to the table in furthering our understanding of anxiety? First, behavioral measures of anxious responding aid in interpretation of self report and physiological or biological assessments. For example, they provide a less biased measure of anxiety compared to self report assessment tools, which can be limited by under or over reporting in certain populations (e.g., military populations where stigma may still be attached to reporting symptoms of post-traumatic stress disorder). It has also been suggested that self report measures of anxiety have the potential to bias research towards overt cognitive mechanisms of anxiety or towards specific high reporting disorder classes (e.g., Mansell and Clark 1999). Behavioral measures are also generally valence dependent. Biological or physiological measures of arousal such as changes in heart rate, blood pressure, or skin conductance are excellent objective measures of autonomic activation; however, they can lack specificity, with similar patterns of activation being observed under non-"anxiety-provoking" circumstances, such as novelty or reward (Dawson et al. 2000). Similar limitations apply to imaging using fMRI, as neural circuits involved in fear activation are also activated under nonaversive conditions (for review see Wager et al. 2003). These limitations are not always circumvented in behavioral tasks (e.g., reaction time tasks based on affect), with some behavioral tasks conferring better specificity in distinguishing negative and positive valence than others. Second, some behavioral measures can also provide insight into preconscious anxiety processes, such as attentional biases or reflexes. Behavioral assessments of unconscious reflexes or responses may be particularly useful in obtaining an "unbiased" measure of anxiety, as well as provide measures that are amenable to model organisms where verbal assessments or complex tasks are impossible. Finally, behavioral tests using presentation of mild stressors to elicit acute anxiety responses are now being used as preliminary screens for efficacy of novel anxiolytic compounds in healthy control subjects. Many of these behavioral tests have analogous models in rodents and primates, supporting translation of model organism results to efficacy testing in

humans. Weaknesses of behavioral measures are that they are not as well validated and standardized across laboratories as physiological measures and rating scales; some require expensive equipment and specific expertise (similar to physiological studies), and in some cases, the window to detect effects of anxiety manipulations (e.g., drugs, stressors) on behavioral measures can be very small. Small behavioral windows are likely more prevalent in studies of normal controls using mild stressors, which may then require more subjects for adequately powered hypothesis testing. Overall, the strength of behavioral assessment tools are that they are relatively objective, are generally stimulus and parametrically controllable, can probe specific constructs of anxiety (e.g., in response to specific stimuli), can be used as screens for pharmacological efficacy, and finally, in some cases, have well validated translational applicability between preclinical and clinical results. Here we will discuss behavioral correlates of anxiety in human research focusing on their use in identifying mechanisms of anxiety and predicting anxiolytic treatment efficacy.

2 Startle Reactivity Paradigms

The startle reflex is an unconscious reflex in response to sudden intense stimuli (visual, acoustic, somatic, and vestibular). The reflex invariably includes an eye blink response, and depending upon the intensity of the stimulus, may also include a contraction of the skeletal musculature with a ducking of the head into the neck towards the shoulders (for a historical review of startle reflex research see Wilkins et al. 1986). This reflex is highly conserved across mammalian species. In humans, startle reactivity is usually measured by the eye blink response as indicated by EMG recordings of the orbicularis oculi muscle, as this element of the response is least variable and most resilient to habituation (for review of EMG technique see Fridlund and Cacioppo 1986). Its function is generally regarded as defensive (discussed later), possibly to facilitate the latency to flee or to shield the head and neck from injury (for review of proposed functions see Yeomans et al. 2002). Although it is an unconscious reflex, the magnitude of the response is modulated by emotional state. Startle magnitude is reliably shown to be inhibited in the presence of rewarding or appetitive stimuli (e.g., erotica or food) while being exaggerated in the presence of aversive stimuli (e.g., violent scenes or threatening stimuli) (Lang et al. 2006). Because startle plasticity can reflect negative and positive emotional valence, it has become a popular tool in anxiety and emotional processing research in both human and animal models (Davis 2006; Grillon 2008).

2.1 Startle Reactivity to Aversive Images

A pioneer in emotional modulation of startle behavior has been Peter Lang's group, focusing on modulation of startle reactivity provoked by emotionally evocative images (Lang et al. 1998). This group has amassed a large database of photographic

image stimuli sets with prior normative affective ratings for affective, dominance, and arousal levels (International Affective Picture System, IAPS, http://csea.phhp.ufl.edu/Media.html). These pictures are used widely in behavioral, physiological, and imaging paradigms. Startle responses to IAPS pictures are reliably reported to be increased while viewing unpleasant pictures (e.g., pictures of insects on food, blood, accidents, violent acts) and these effects are linked to state anxiety (Smith et al. 2005). Startle reactivity is also increased significantly when subjects are shown threatening words (Larsen et al. 2002) or angry faces (Springer et al. 2007). Startle reactivity to aversive images has also been linked to activity of the amygdala and other forebrain regions associated with acute and clinical anxiety responding (Buchanan et al. 2004; Pissiota et al. 2003), supporting the notion that this task is probing anxiety-relevant neural circuits. Surprisingly, the affective modulation of startle does not appear to be linked to trait anxiety (Smith et al. 2005), but instead linked to traits of fearfulness or anger (Cook et al. 1992). A failure to show affective modulation of startle has been linked to psychopathic traits (Lang et al. 1993). Also, affective modulation of startle may be dependent on mood state, as clinically depressed and normal subjects selected for negative mood states show blunted affective modulation of startle (Allen et al. 1999; Grüsser et al. 2007). Care must be taken with the choice of images, as startle potentiation to specific image types (e.g., disgust vs. fear) can be modified by personality traits (Caseras et al. 2006) or individual experiences. One way to circumvent the effects of individual differences in processing and responding to the images is to assess startle reactivity during cued anticipation of images. This task prompts the subject (usually with a simple visual cue, such as a colored circle) that a specific image type (negative or positive) is about to be shown, and it is during this anticipation that startle reactivity is probed. Startle reactivity is increased during anticipation of negative imagery, although similar to image probe tasks, the startle reactivity evoked by anticipation does not appear to be related to trait anxiety (Nitschke et al. 2002).

2.2 Startle Reactivity in Darkness

Humans exhibit increased startle reactivity when tested in complete darkness compared to well-lit conditions (Grillon et al. 1997b). This is a similar phenomenon to light-potentiated startle in rodents,[1] and both are reduced by anxiolytic treatment (Baas et al. 2002; de Jongh et al. 2002). Combat veterans and combat PTSD subjects exhibit increased startle reactivity to darkness compared to civilian controls (Grillon et al. 1998b), indicating these conditions may probe learned arousal or anxiety-related to combat experience. Panic disorder subjects do not exhibit increased startle reactivity in the dark (Melzig et al. 2007). The drawback of this paradigm thus far

[1]As opposed to humans, rodents are nocturnal, and lit conditions tend to be more threatening as they increase the chances of being observed by predators.

has been that the potentiation can be relatively weak and habituate rapidly in normal subjects, limiting its use for examining drug effects (Baas et al. 2002).

2.3 *Fear Potentiated Startle to Conditioned Cues*

Pavlovian conditioning has been an important tool in behavioral assessments of anxiety (LeDoux 2000), and stronger fear conditioning may be a component of anxiety disorders (Lissek et al. 2005). In animals and humans, a conditioned fear association is made when a conditioned stimulus (CS; e.g., a tone or light) and unconditioned stimulus (US; e.g., a painful shock), are presented in close temporal proximity. Thus, the CS "predicts" the occurrence of the US. After sufficient training, the presentation of the CS alone will evoke a conditioned fear response (CR; e.g., freezing, exaggerated startle response, autonomic activation). In the fear potentiated startle (FPS) model of conditioned fear, a visual or acoustic CS is presented with a noxious stimulus such as a shock or airpuff. After a sufficient number of pairing trials, the presence of the CS will elicit exaggerated startle responses. The degree to which startle is exaggerated is used as an operational measure of the conditioned fear response (Brown 1951; Davis et al. 1993). FPS is a cross-species paradigm (with its use pioneered by the laboratory of Michael Davis), observed reliably in humans, (pioneered predominantly by the laboratory of Christian Grillon; for review see Grillon 2008), rodents (Davis 2008), and primates (Winslow et al. 2007).

In humans, fear potentiated startle magnitude has consistently been associated with state anxiety (for review see Grillon and Baas 2003), as well as correlated to neuroendocrine responses to stress (Grillon et al. 2006c), indicating convergent validity with psychological and biological markers of acute anxiety or fear. As with the effects of affective images on startle (discussed earlier), magnitude of FPS is not predicted by trait anxiety (Grillon et al. 1993), unless the aversive stimulus used is relevant to a specific phobia of the subject (Cornwell et al. 2006). Studies in humans and animals indicate that amygdala activation contributes to acquisition of FPS (Antoniadis et al. 2007; Funayama et al. 2001; Weike et al. 2005; Winslow et al. 2008); hence, this behavior has been used as a correlate of amygdala function across research disciplines.

As opposed to cued fear, conditioned fear to contextual cues is elicited by associations of the aversive event with a diverse range of cues occurring at the same time (i.e., a specific environment), with each discrete cue that makes up the context having weak to little effects when presented alone. In animals, contextual fear requires both the amygdala and hippocampus, while cued fear is hippocampal independent (LeDoux 2000). Recently, more research has focused on contextual fear learning in humans, based on the hypothesis that contextual fear may be a more accurate measure of "anxiety" (sustained defensive responding in response to weak or ambiguous threat cues) than "fear" (short lived defensive responses in the presence of discrete, highly predictive, threat cues) (Baas et al. 2008; Grillon and

Baas 2003; Grillon et al. 2006b; Winslow et al. 2008). Context potentiated startle appears to have convergent validity with increased avoidance behaviors of contexts predicting shock (Grillon et al. 2006a), and may be more sensitive to anxiolytic treatments than FPS (Grillon et al. 2006b). More research is needed to indicate whether context potentiated startle is predictive of trait anxiety (Baas et al. 2008), or abnormal in anxiety disorder patients (Grillon et al. 2008).

There are some critical differences to keep in mind when comparing results of FPS across humans and animals. First, most human studies train and test the subject in one sitting, whereas animal studies usually have at least 24 h between training and testing; thus, molecular mechanisms underlying FPS may be different across these paradigms. Another variation is that human FPS commonly uses only verbal instructions of the CS–US contingency before testing, with few to no presentations of the aversive stimulus (e.g., shock to the wrist or airpuff to the neck). Few studies in humans rely on experience-dependent learning of the CS–US contingency, presenting multiple CS–US pairing trials before testing, which is the only way to assess FPS in animals. Thus, it is important to bear in mind that across animals and humans, as well as across different human FPS protocols, the FPS measure may probe at least some differential neural circuitry, and require different levels of awareness of the contingency from the subject.

2.4 Conditioned Inhibition and Extinction

One theory of the etiology of anxiety is a reduced ability to inhibit fear responding, either by inability to distinguish safety signals from threat signals, or inability to extinguish previously learned fear responses (Nutt and Malizia 2004; Rothbaum and Davis 2003). In the phenomenon of cued inhibition, subjects are trained to associate a cue (CS+) with an aversive US, and also trained that when a different stimulus is presented (CS−), in addition to the CS+, the US will not occur (Grillon and Ameli 2001), hence the CS− inhibits the fear response to the CS+. Conditioned inhibition was found to be reduced in subjects with high harm avoidance, suggesting harm avoidance traits may be associated with inability to inhibit behavioral fear responses (Grillon and Ameli 2001). A recent study has reported that during the compound CS+/CS− presentation, a conditioned response to the CS+ (e.g., increased startle) continues to be present early during the cue presentation, while the effects of the CS− in inhibiting the response appear to occur later in the cue presentation (e.g., reduced startle) (Wendt et al. 2008). This initial potentiation followed by inhibition supports the hypothesis that separate neural systems may mediate responses to the CS− (safety signal) and the CS+ (danger signal). This protocol may have utility in testing whether anxiety disorders are linked to reduced responsivity to safety cues (the CS−), or rather enhanced reactivity to the threatening cues (CS+), as the two constructs may be distinguished temporally. The human version of the conditioned inhibition paradigm does have limitations;

however, as unlike rodents and monkeys, humans typically learn only that the *compound stimulus* CS+/CS− predicts that the US will not occur. The CS− alone does not inhibit fear responses to other CS+ cues that have not been specifically paired with the CS− during acquisition (i.e., humans do not generalize the individual CS− safety features to other threatening cues) (Winslow et al. 2008). As a consequence these paradigms had not been used extensively although a recent adaptation of this paradigm may be promising (e.g. Jovanovic et al. 2009).

Fear extinction is a complex construct made up of a number of distinct processes that inhibit learned fear responses (Myers and Davis 2002). Fear extinction occurs when a CS previously associated with an aversive US is then presented without the US. The subject learns that the CS no longer predicts the presence of the US and fear responses to the CS are extinguished (e.g., FPS is reduced). In both humans and animals, the magnitude of fear extinction is associated with increased prefrontal cortex activity and reduced amygdala activity (Milad and Quirk 2002; Phelps et al. 2004). Clinically, extinction of fear responses elicited by trauma-related or phobia-specific stimuli is facilitated by exposure-based or cognitive behavioral therapies. Neurochemical substrates of extinction learning in animals has shown predictive validity for human extinction, as the glutamate partial agonist D-cycloserine enhances extinction learning in both rats and in phobia subjects (Norberg et al. 2008; Ressler et al. 2004; Walker and Davis 2002). These findings indicate that glutamatergic transmission in both rodents and humans modulates the extinction of learned fear responses. Extinction paradigms using startle as an operational measure of learned fear responding have been developed (Norrholm et al. 2006; Orr et al. 1997; Vansteenwegen et al. 1998), although drug studies using this paradigm have not yet been published. One study has shown reduced FPS extinction in anxious children compared to nonanxious children (as assessed by clinical interview) (Liberman et al. 2006). PTSD subjects have also been shown to exhibit normal extinction as assessed by startle but retarded extinction as assessed by skin conductance, suggesting that in PTSD physiological measures of arousal may be more resistant to extinction than startle behavior (Orr et al. 2000).

3 Startle Reactivity as a Tool for Predicting Anxious Responses

3.1 Do Startle Reflex Measures Predict Clinical Anxiety?

For an extensive review of startle reactivity changes in anxiety disorders see Grillon and Baas (2003). Here we will briefly touch on the more classical findings and discuss new findings since 2003. Individuals with panic disorder, social phobia, and PTSD have been reported to exhibit increased startle reactivity. Panic disorder subjects have been shown to exhibit increased baseline reactivity and reduced startle habituation and inhibition (Grillon et al. 1994; Ludewig et al. 2005; Ludewig et al. 2002).

The increased baseline startle and inhibition deficits may be partially normalized by medication (note, however, that this was a cross-sectional study, and thus medication effects should be judged cautiously) (Ludewig et al. 2005). Panic disorder subjects do not exhibit significantly greater startle potentiation to aversive words (Larsen et al. 2002) or cued shock compared to controls (Grillon et al. 1994; Grillon et al. 2008), suggesting that responses to specific threats are not altered in this population. A recent study suggests that co-morbid depression may, however, mask observations of increased potentiation in panic disorder subjects (Melzig et al. 2007), as subjects without depression have shown increased fear potentiation. Interestingly, a recent study reported that panic disorder subjects without major depression exhibited greater tactile startle under conditions of unpredictable threat compared to conditions of predictable (cued) threat, suggesting that panic disorder subjects exhibit strongest anxiety behaviors during periods of uncertainty (Grillon et al. 2008).

Increased startle reactivity is also considered a hallmark feature of the arousal domain of PTSD symptoms. Although not observed in all studies (e.g., Orr et al. 1997), a recent meta-analysis indicates that the increased startle reactivity reported by PTSD patients, in particular combat PTSD patients, is generally reproducible in the laboratory (Pole 2006). There is a great deal of inconsistency, however, in whether these increases are in baseline "nonstressed" startle reactivity, or only in response to stressful or anxiety-provoking experimental conditions (for extensive review see Grillon and Baas 2003). Ability to inhibit startle reactivity in the form of prepulse inhibition may also be disrupted in PTSD subjects (Grillon et al. 1998c; Grillon et al. 1996), although these data need to be replicated in larger samples. Three studies have tested whether exaggerated startle reactivity is a vulnerability trait for developing PTSD or manifests only after trauma exposure. In a prospective study of subjects recently exposed to trauma, startle responding was similar in all subjects immediately after trauma; however, those that did not develop PTSD became less reactive over time, while those that went on to develop PTSD continued to be reactive over these repeated sessions (Shalev et al. 2000). Because startle reactivity was similar across subjects immediately after trauma, it was interpreted that startle reactivity increases manifested only after trauma in those that go on to develop PTSD. This explanation does not account for the possibility that subjects developing PTSD had high startle before trauma (high responses due to trait), while those that did not develop PTSD had high startle only in response to trauma (high responses due to state). An additional caveat is that the recording resolution in this study (50 Hz, 1 recording every 20 ms), was not within the usual resolution required (1,000 Hz, 1 recording every 1 ms) for accurate recording of peak EMG responses (peak responses are high frequency and occur usually within a range of 15–20 ms); thus, the conclusions that can be drawn from this study are weakened. A small prospective study of firefighters exposed to trauma found a modest positive correlation between pretrauma startle magnitude and post-trauma PTSD-like symptoms; however, a clear PTSD diagnosis was not found in any of the subjects tested (Guthrie and Bryant 2005). A very recent study examined if startle reactivity to threat predicted development of PTSD in policemen. Pole et al. (2008)

found startle reactivity and skin conductance under mild threat conditions (electrodes were placed on subjects but subjects were not given specific cues that shock was imminent) in cadets predicted PTSD symptoms as assessed by the PTSD check list after 1 year of police service (Pole et al., 2008). These data argue that increased fear responsiveness may be a vulnerability trait for PTSD instead of consequence of trauma. Although more studies are required to test if startle is a vulnerability factor for development of PTSD in different trauma populations, these data support further research in understanding genetic and environmental influences that modulate startle and physiological measures as these markers may be related to vulnerability for developing PTSD.

Not surprisingly, in phobic patients, startle reactivity is consistently increased in the presence of phobic-specific stimuli (e.g., social phobia, Cornwell et al. 2006; e.g., spider phobia, Wendt et al. 2008). There is preliminary evidence that this increased startle reactivity to phobic stimuli is reduced after extinction based therapy (De Jong et al. 1993). Hence, startle reactivity to phobic stimuli may be used as an objective marker of treatment efficacy in phobic subjects.

4 Can Startle Reactivity Be Used as an Endophenotype for Anxiety?

There is some preliminary evidence that heightened startle reactivity is linked with "risk" for anxiety disorder development. Children of patients with anxiety and depressive disorders exhibit exaggerated startle (either baseline or only during threat) compared to children with no family history for these disorders, suggesting that startle responsiveness could be a marker of vulnerability for development of clinical anxiety (Grillon et al. 1997a, 1998a; Merikangas et al. 1999) and depression (Grillon et al. 2005b). This latter finding is somewhat surprising because baseline startle appears to be normal, or even reduced, in adults with depression (Kaviani et al. 2004; Perry et al. 2004; Quednow et al. 2006). One untested speculation is that startle reactivity may change with the onset and progression of depression. Personality traits linked to anxiety and startle have include a study of children classified as "fearless" exhibiting significantly reduced startle responses (Goozen et al. 2004). Generally, links between startle and anxiety-related personality traits like behavioral inhibition have been mixed however (Caseras et al. 2006; Fullana et al. 2006; Hawk and Kowmas 2003; Nitschke et al. 2002), although the sample populations (e.g., infants vs. older children and adults), methodologies (e.g., affective modulation of startle using images or painful stimuli), and measures used (e.g., assessment scales) are very diverse, making comparisons across studies difficult. Startle reactivity has shown convergent validity with state but not trait anxiety, which may account for the inconsistency of baseline startle reactivity findings in anxiety disorder subjects, as it is difficult to control for experimental stress across studies.

The most compelling findings in clinical anxiety populations to date are startle reactivity abnormalities in panic disorder and PTSD subjects (discussed earlier). Interestingly, these disorders have been suggested to have some similar etiology (Kellner and Yehuda 1999; Risbrough and Stein 2006).

It has been suggested that a simple relationship between a gene and complex trait mechanisms such as anxiety are unlikely to be found given the complexity and overlapping nature of the symptoms of neuropsychiatric disorders. Instead, optimally reduced measures of neuropsychiatric function or "endophenotypes" may be more useful than behavioral "macros" or diagnosis to understand genetic contributions to mental health (Gottesman and Gould 2003). Additionally, many disorder-relevant endophenotypes can be modeled in animals (e.g., startle reactivity) as opposed to complex symptom clusters. The identification of specific genetic contributions to variance in startle reactivity is in its infancy. Based on the data discussed above, as well as the fact that startle reactivity shows strong heritability and low within subject variance (Baker et al. 2008), its use as an anxiety endophenotype to identify potential genetic contributions to anxiety is certainly worth pursuing (Carlson et al. 1997; Radant et al. 2001) in its stress- or anxiety-potentiated form. For example, carriers of the short allele of the serotonin transporter, which has been consistently associated with anxiety and depressive disorders, have recently been reported to exhibit increased startle reactivity compared to carriers with the long allele (Brocke et al. 2006). Similarly, a recent study examined the link between startle reactivity and the well studied Val/Met mutation of the catechol-*O*-methyltransferase catecholamine degradation enzyme (Tunbridge et al. 2006) that may be associated with trait anxiety (Stein et al. 2005). Subjects homozygous for the Val polymorphism, which imparts less enzyme efficiency and thus less catecholamine signaling, exhibited greater startle potentiation when viewing negative images compared to Met carriers (Montag et al. 2008). There is uncertainty whether this mutation alone confers risk for clinical anxiety, as many large sample studies have failed to find an association (Baekken et al. 2008; Rothe et al. 2006; Wray et al. 2008). Overall, however, it appears that affective modulation of startle may be a useful tool for delineating genetic mechanisms associated with emotional processing and anxiety.

5 Can the Signal Predict Treatment Efficacy?

One of the more important questions is whether or not startle reactivity is a reliable measure to predict anxiolytic efficacy in humans. Startle reactivity reliably probes functions of the neural circuitry found to be abnormal in anxiety disorders (e.g., amygdala hyperactivation) (Grillon 2008); thus, treatments that act on this neural circuitry would be expected to modulate startle functions (Paulus 2008).

5.1 Benzodiazepines

Benzodiazepine treatment effects on fear potentiated startle (cued shock) has been somewhat inconsistent, with some reports showing benzodiazepine treatment efficacy (Bitsios et al. 1999; Graham et al. 2005) while others have only shown reductions in startle overall (Baas et al. 2002; Riba et al. 2001; Scaife et al. 2005). Baas et al. (2002) did find consistent reductions in self reports of state anxiety with diazepam treatment, indicating the doses used were psychoactive. One potential reason for the discrepancy between negative and positive findings is that the experiments where benzodiazepines where effective removed the shock electrodes during the "safe" condition (Bitsios et al. 1999; Graham et al. 2005), while the electrodes remained on even during presentation of cues that were instructed as "safe" (Baas et al. 2002; Riba et al. 2001; Scaife et al. 2005). Thus, it is possible that even during the "safe" condition, when electrodes are on, there is greater anxiety, and hence stronger effects of benzodiazepine treatment on startle across the entire session, masking a specific effect on cued-startle potentiation. A second difference is that in procedures that observed benzodiazepine effects, threat conditions were longer (90 s) and signaled by the placement of electrodes, while experiments with lack of effects used shorter (30 s) visual cues to alert the subject of oncoming shocks. Hence, another possible explanation is that the more cues predicting the possibility of shock are presented (e.g., electrodes plus visual cue), the less effective the treatment is in inhibiting cued startle. Benzodiazepines have been shown to be effective in blocking startle reactivity to contextual cues or to startle reactivity potentiated by darkness, which are more ambiguous threats than discrete fear cues (Baas et al. 2002; Grillon et al. 2006b). The finding that responses to classically conditioned fear cues are not affected by benzodiazepine treatment conflicts with animal studies that show consistent reductions in fear potentiated startle (Baas et al. 2002; Davis 1979; Risbrough et al. 2003; Winslow et al. 2007). Possibly, the ED50 for sedation is higher in animals allowing higher benzodiazepine dosing, or the effects in animals are due to disruption of cognitive processes and not anxiety (i.e., interferes with fear memory recall as opposed to the fear response). Indeed, benzodiazepine treatment inhibits acquisition of cued fear responses (Scaife et al. 2005). However, the more parsimonious explanation may be that there are fundamental differences in how animal and human tests are designed and experienced by the subjects. For example, the procedure for most rodent studies involves removing the shock grid before FPS testing to reduce contextual fear effects on baseline startle, which eliminates one of the primary conditioned cues for the aversive stimulus. Removal of the electrodes does not occur for any of the human studies during the threat condition, so this difference in contextual environment may also account for diazepam efficacy differences across animals and man.

Diazepam has also been shown previously to reduce startle potentiation induced by aversive images (Patrick et al. 1996); however, these effects were not replicated using lower doses (Murphy et al. 2008). Overall, it appears that startle potentiation

induced by ambiguous cues (contextual, darkness) or cues associated with relatively weak aversive stimuli (images) is sensitive to benzodiazepine treatment, but specific and discrete cues associated with stronger aversive stimuli (e.g., shock) are not[2]. Because baseline startle reactivity is so sensitive to state anxiety however, the ability to separate "sedative" vs. "anxiolytic" effects of benzodiazepines on this behavior will likely always be difficult, especially in experimental designs in which the subject knows from the outset that they are going to experience an aversive stimulus.

5.2 Serotonin Reuptake Inhibitors

Acute treatment with citalopram increases baseline startle reactivity (Browning et al. 2007) without affecting startle potentiation induced by negative images, while subchronic citalopram treatment (7 days) reduces startle potentiation induced by negative images (Harmer et al. 2004). Thus the aversive image task appears to be able to dissociate acute vs. chronic effects of serotonin reuptake inhibitor (SSRI) treatment. These data fit with reports of increased arousal induced by acute SSRI treatment and the evolution of anxiolytic effects of treatment after chronic dosing. As indicated above, medicated panic disorder subjects exhibit significantly lower startle reactivity compared to unmedicated patients (Ludewig et al. 2005). Whether this normalization is associated with improvements in global functioning is still unclear, requiring longitudinal studies pre- and post-treatment.

5.3 Putative Anxiolytics and Behavioral Treatments

Preliminary data also indicate that the putative anxiolytic LY354740, a structural analog of glutamate that shows specificity at the mGluR2/3 receptor, reduces FPS in both rats and humans (Grillon et al. 2003; Helton et al. 1998). This compound has been shown to have efficacy in reducing anxiety symptoms in generalized anxiety

[2]How does this fit with the efficacy of benzodiazepines in the clinic? One might expect then that phobias to specific cues, which on its face would be the most similar form of anxiety to the fear potentiated model, are less treatable by benzodiazepines, and indeed, behavioral therapy has been the most effective first line treatment for specific phobias (Argyropoulos and Nutt 1999; Davidson 1997; Verster and Volkerts 2004). There is evidence, however, that benzodiazepines reduce fear and behavioral avoidance in claustrophobia (Tschirch et al. 2007), agoraphobia (Carter et al. 1995), and public speaking (Graeff et al. 2003; Lee 2004). Thus, although benzodiazepines are not first line treatments for most phobias due to interference with extinction processes(Lee 2004), sedation, toxicity and abuse potential, they are effective in short term reduction of phobic symptoms. Benzodiazepines have been shown to reduce self report of fear but not behavioral measures of fear (reduced approach) in animal phobia (Sartory et al. 1990); however, higher doses of benzodiazepines reduce both anxiety and approach behavior (Whitehead et al. 1978). These data suggest that behavioral manifestations of phobias may be more resilient to benzodiazepine treatment than the conscious fear emotions, although this speculation requires testing.

disorder (GAD) in a small clinical trial (Dunayevich et al. 2007), although possible toxic effects have precluded its development further. As indicated above, small studies have also indicated startle reactivity to phobic stimuli is reduced after extinction based therapy (De Jong et al. 1993). In anxiety disorders, there has been little comprehensive research examining the sensitivity of startle abnormalities to pharmacotherapy or behavioral treatments; thus, it is difficult to make an assessment of the predictive validity of this paradigm.

Due to lack of specificity and sedation confounds, baseline startle has been an insufficient measure of anxiety-like responding. There is also to date a lack of consistent convergent validity with other measures of trait anxiety or anxiety vulnerability for either baseline or stress induced increases in startle except in the case of specific phobias and phobia related stressors. On the other hand, startle potentiation has shown sensitivity to specific anxiety disorders, probe related neural substrates (e.g., amygdala activation), and show sensitivity to anxiolytic compounds (with the exception of sedation confounds with benzodiazepines, discussed above). A significant test for the use of startle reactivity as a translational tool in the future will come with its potential to identify new treatments. Although interpretation can be confounded by sedation side effects, ultimately, the search for novel anxiolytic compounds includes reducing sedative profiles. Hence, it is not clear that the confounding sedative effects of benzodiazepines in the FPS paradigm preclude the paradigm's use in identifying novel anxiolytic drugs. Novel approaches in pinpointing stimulus conditions most amenable to treatment (e.g., contextual cues) may also increase the sensitivity of the task. The use of more complex stimuli, such as virtual reality, may support the use of more ethological paradigms and cues (Baas et al. 2004). Clearly, care must be taken in choosing the right paradigm, as conditions with high potentiation may be resistant to attenuation by some drugs (Baas et al. 2002). Future research will also prove whether startle potentiation is a useful endophenotype for understanding genetic contributions to anxiety, as it is a promising translational tool, in particular for bridging the gap between preclinical and clinical research, in identifying anxiety-related substrates (Merikangas and Pine 2004).

6 Emotional Bias Tasks

Another tool for assessing behavioral responses related to anxiety is the assessment of response bias towards threat cues. Cognitive theories of anxiety posit that anxious subjects have increased likelihood or bias towards detecting, processing, and orienting to threat cues in the environment, even if these cues are ambiguous and pose little actual threat (for review see Bishop 2007; Mogg and Bradley 1998). To operationally measure such bias, a number of tasks have been developed, including the emotional Stroop task and the dot probe task (for review see Mathews and MacLeod 2005). The emotional Stroop task was utilized by Mathews and MacLeod (1985), and involves presenting the subject with positive or negative words on a video screen, and asking the subject to indicate the color of the letters

making up the word. Increased reaction time to name the word color is interpreted as increased processing of the task-irrelevant emotional content of the word. High anxiety subjects showed increased response times to color-name negative words, and thus may devote more processing resources to negative words or constructs. This task had some confounds in the interpretation of attentional bias, however, as viewing negative words may interfere with performance or distract high anxiety individuals, as opposed to increase in processing time per se (Mogg and Bradley 1999). An adaptation of this task, the dot probe task, shows two words or images on the screen, one in the upper portion and one in the lower portion. The subject is instructed to press a button when the screen stimuli disappear and are replaced by a dot. The dot can occur in either location, with the assumption being that when a subject is oriented to a particular word in a given location, the response time will be faster when the dot appears in that word's location.

7 Do Attentional Bias Task Measures Predict Clinical Anxiety?

The association with measures of increased attentional bias towards negative stimuli and anxiety are thoroughly reviewed elsewhere (Mathews and MacLeod 2005). In brief, high trait anxious subjects have been shown to exhibit greater bias towards negative words (Mathews and MacLeod 1994), as well as threatening faces (Mogg and Bradley 1999). GAD subjects have also been shown to orient more quickly towards threatening faces (Bradley et al. 1999; Mogg et al. 2000). Interestingly, an adaptation of these tasks using masked stimuli appears to best differentiate between anxiety and depression patients, with only anxiety patients continuing to show increased measures of attentional bias to masked negative stimuli (Mogg et al. 1995). The attentional bias towards masked threatening faces is also correlated with amygdala activation in GAD but not normal controls (Monk et al. 2008). These data indicate that preconscious attentional bias may be a unique feature of clinical anxiety, at least in GAD subjects.

8 Do Attentional Bias Tasks Predict Treatment Efficacy?

Recently, Mogg et al. (2004) reported that attentional bias assessed by the dot probe task was significantly reduced in GAD patients treated with paroxetine or citalopram. Most importantly, there was a direct correlation between treatment effects on the cognitive bias task and clinical global assessment of anxiety scale scores. Treatment with a low dose of diazepam specifically increased orienting to happy faces without affecting attentional responses to threatening faces, suggesting that diazepam may switch cognitive bias to more positive stimuli (Murphy et al. 2008). Although preliminary, this is an interesting finding, as it addresses whether an anxiolytic is effective because it inhibits responses to aversive stimuli

(e.g., avoidance) or because it facilitates competing responses to appetitive stimuli (e.g., approach). There is also evidence that attentional bias tasks are predictive of cognitive therapy treatment outcome in GAD (Mathews et al. 1995), but not PTSD (Devineni et al. 2004).

Taken together, attentional bias measures appear to have sensitivity towards anxiety specific traits (especially relevant to GAD) and there is compelling preliminary evidence for sensitivity to anxiolytic treatments. Although interpretation of the exact attentional construct being measured in these tasks is under debate (e.g., initial orienting, reduced ability to disengage attention, or increased processing) (Mogg et al. 2008), these tasks have empirical support as behavioral probes for clinical anxiety mechanism and treatments. Future studies of the heritability and genetic contributions to attentional bias responding will be of great value.

9 Behavioral Avoidance

In diagnosing anxiety disorders, phobias in particular, behavioral assessment tests are sometimes used by the clinician. Outside of self report scales of avoidance, commonly employed tests are the behavioral approach test (e.g., presenting the individual with a behavioral choice to approach or avoid a feared object or situation), as well as assessing behavioral responses to specific phobic events (e.g., behaviors such as trembling, truncated speech patterns in social phobics during speech) (Harb et al. 2003). These tests are somewhat limited to disorders with specific "triggers," and thus are not as useful for generalized anxiety disorder or panic disorder (Kaloupek and Levis 1983; Mavissakalian and Hamann 1986), although avoidance behaviors have been suggested to be possible risk factors for panic disorder and agoraphobia (Zvolensky et al. 2006). Although helpful in behavioral therapy techniques (Chorpita and Taylor 2001), these behavioral assessments have not been consistently used for predicting anxiety traits or anxiety treatments.

An interesting paradigm has recently been reported which exploits the use of carbon dioxide presentation as an aversive stimulus (Fannes et al. 2008). CO_2 inhalation induces anxiety-like arousal and symptoms, and can induce panic in some human subjects (Bailey and Nutt 2008). If a cue is associated with the CO_2 presentation subjects exhibit a marked reduction in respiration when the CS+ is presented, and can also extinguish this avoidant behavior after the CS+ is no longer contingent with CO_2 presentation. Other panicogenics may also be useful as stress challenges for behavioral studies and fear conditioning, such as lactate and cholecystokinin tetrapeptide treatments. Scales of anxiety and panic symptoms are consistently sensitive to these challenges (Keck and Strohle 2005); however, there is little data on their use in humans with behavioral measures of anxiety. Although still preliminary, such paradigms may be useful, as the stimuli and some of the response measures (physiological changes, avoidance responses) may be used across animal and human tests (e.g., neurobiological mechanisms of lactate sensitivity; Johnson et al. 2007).

10 Promising Behavioral Tasks in Development

Tasks developed to assess anxiety and fear behaviors in animals are now being adapted in the clinic to assess the neurobiology of anxiety (e.g., Davis 2006). The advent of sophisticated gaming and virtual reality technology has supported more ethological experimental designs to assess anxiety responses. For example, tasks that assess cognitive ability in animals such as the Morris Water Maze have been used to assess neural substrates activated by similar virtual reality tasks in humans (Astur et al. 2006; Astur et al. 2002). This approach has recently been adapted to assess anxiety responding. When presented with scenarios of proximal or distal threat, human beings report similar patterns of defensive behavior as observed in animals (Blanchard et al. 2001). A recent study in nonanxious controls used a virtual reality version of a predator exposure paradigm previously established for rodents (Mobbs et al. 2007) to assess neural circuitry underlying defensive responses in humans. The study found that when the virtual predator (which could elicit shocks when in contact with the subject's virtual self) was distal from the subject, frontal cortex regions were most activated, but when the "predator" became more and more proximal, greater activation switched to the periaqueductal gray region (PAG) of the brain stem. This neural circuitry parallels animal literature in that the PAG is critical for defensive fight or flight behaviors (Fanselow et al. 1995; McNaughton and Corr 2004). The subjects showed graded levels of avoidance of the predator depending on the number of shocks elicited when in contact with the predator. Hence, this task may be useful in quantifying differing levels of avoidance in high anxiety subjects, and how treatments might affect these behaviors.

A similar adaptation of assessing animal locomotor behavior has been developed in humans, called the Human Behavioral Pattern Monitor (Young et al. 2007). This paradigm uses visual tracking software to assess the spatial location of a human subject in a small "waiting" room filled with furniture and objects, but importantly no chair. The subject's movements during a 15-min session are mapped and a number of measures of locomotion and exploration patterns are quantified. Although this paradigm was developed to delineate locomotor and object exploration abnormalities in manic and schizophrenic subjects, it may also be modified for assessments of avoidance, locomotor, or novelty seeking behavior in other patient groups, including anxiety disorders.

11 Final Summary

Behavioral correlates of anxiety in humans have begun to increase their scope from simply describing behavioral phenomena associated with anxiety, towards identification of neurological and genetic factors related to anxiety responding and predicting anxiolytic treatment. Startle reactivity and attention bias tasks appear to be the most advanced in these areas. Other, less developed tasks, still require more

research to determine if they are sensitive to anxiety disorders and anxiolytic treatments. Overall, behavioral tasks have been complementary tools with imaging, self report, and physiological measures, providing valence dependent, unbiased assessment of behavioral responses to anxiety-provoking cues, as well as to control for attention and motivational confounds. Significant limitations to keep in mind are that behavioral assessments often require sophisticated and expensive equipment (although so does physiological measurement), often require more experimental time from the subject, and are rarely adaptable to assess subjects outside of the laboratory. Most importantly, in comparison to self report and to a lesser degree physiological measures, behavioral tasks are not well standardized, with significant divergence in stimuli, task instructions, and analysis across laboratories. Efforts towards standardization must be addressed for these tasks to become exploited to their full potential, both in pharmacotherapy and genetic studies. Finally, the development of new tasks using virtual reality and other novel techniques is truly exciting, and shows great promise for supporting sophisticated and translational behavioral assessments of anxiety in the future.

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Challenging Anxiety: A Focus on the Specificity of Respiratory Symptoms

M.A. Van Duinen, V. Niccolai, and E.J.L. Griez

Contents

Abstract Physiological symptoms are characteristic features of anxiety states. Presumably, specific psychophysiological profiles differentiate between anxiety disorders, which would offer potential for diagnostic purposes. Abundant evidence points to a causal relationship between panic disorder and instability of respiratory regulation. However, the specificity of most measures that indicate aberrant functioning of the respiratory system in PD can be questioned. Possibly, the traditional measures of respiratory functioning are too restricted. The underlying respiratory vulnerability in PD seems to constitute a subtle, unstable trait, which calls for more

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sensitive and sophisticated measures of respiratory variability and chaos. To increase the probability of finding parameters with diagnostic specificity, the application of disorder specific challenge paradigms is recommended.

Keywords Anxiety · Respiration · Panic disorder · Psychophysiology

1 Introduction

The presumably, primal response during the active state of anxiety disorders, shows many resemblances with a normal defensive response. Autonomic changes described for the classic stress response include a wide range of physiological systems including the respiratory and cardiovascular systems. It is conceivable that specific anxiety disorders show characteristic psychophysiological profiles. In case the physiological profile of different anxiety disorders could serve as an objective discriminator, this would not only be of scientific interest, but could even offer potential for diagnostic purposes.

The provocation of anxiety symptoms under standardized conditions has proven to contribute to a better understanding of anxiety disorders, both from a behavioral and biological point of view. Especially in social phobia and panic disorder (PD), well-defined paradigms are available that reliably provoke specific core phenomena in affected persons. However, for other anxiety disorders, specific, standardized challenges inducing the relevant affective state are described to a lesser extent.

Generally, we can state that there are two types of anxiety provocation. Exposure to exteroceptive stimuli, that is exposing patients to feared situations or stimuli, or interoceptive exposure in which bodily sensations or disorder-related thoughts are evoked. One way to evoke bodily sensation is by means of chemical stimulation. Over the years, quite some compounds have been claimed to provoke instances of clinical anxiety. However, most of these models have been criticized for their lack of specificity. In general, biochemical manipulations induce merely a state of general anxiety rather than symptoms resembling an affective state relevant to the anxiety disorder under study.

Compared to other anxiety disorders, the induction of specific symptoms by means of chemical stimulation is best described in PD. It might not come as a surprise that PD is particularly suitable for chemical symptom induction. The unexpected attacks with clear bodily sensations are not induced by an external stimulus but rather appear from an internal distortion.

A noteworthy similarity among most of the pharmacological agents that have been proposed as specific panicogenics, is the ability to exert a strong influence on respiration (Calverley et al. 1983; Liebowitz et al. 1984; Charney et al. 1985; Griez et al. 1987; Bradwejn et al. 1990). Moreover, respiratory symptoms represent a category that is present during the core phenomenon of PD – the panic attack.

Consequently, studies focusing on possible dysregulated respiratory patterns in this particular disorder have strongly increased in the past few decades. Evidence from these studies show that PD subjects are particularly sensitive to respiratory challenges. Especially the respiratory subgroup, characterized by prominent respiratory symptoms during panic attacks, seems to be associated with an increased sensitivity to respiratory challenges (Maddock and Carter 1991; Valenca et al. 2002). In addition, this subgroup might present a distinct endophenotype characterized by an earlier onset of the disorder, more frequent spontaneous panic attacks, a higher familial prevalence of PD, more previous depressive episodes, and a different profile with regard to pharmacological responsiveness (Briggs et al. 1993; Nardi et al. 2006).

The abundant attention for respiration within the field of PD cannot be neglected. However, for other anxiety disorders, respiratory symptoms have not been the primary focus. Although sympathetic activity in general has been studied, respiratory symptoms were often neglected. Is it because of the evident presence of respiratory symptoms during panic attacks that respiratory symptoms are principally reported in studies on PD, or could affected respiration also be an endophenotypic trait of other anxiety disorders? In this chapter, we focus on the specificity of respiratory symptoms for PD and we discuss the peculiarity of the relationship between respiratory symptoms and different anxiety disorders.

2 Panic-Respiration or Anxiety-Respiration Link?

Respiratory symptoms, such as hyperventilation, dyspnea, and a feeling of choking or breathlessness are commonly described as prominent symptoms of a panic attack. With regard to other anxiety disorders, only for generalized anxiety disorder (GAD) a respiratory symptom has been explicitly mentioned in the DSM (APA 1994).

Hyperventilation, in particular, is consistently observed during laboratory-induced panic attacks (Gorman et al. 1986; Papp et al. 1989). Basically, hyperventilation implies breathing in excess of metabolic need, thus lowering arterial pCO_2 and resulting in increased pH. Although a fundamental role for hyperventilation in triggering panic was proposed previously (Ley 1985), it has been demonstrated that hyperventilation is not a causal mechanism in the initiation of panic. It rather constitutes one of the many symptoms that can emerge during an attack (Hornsveld and Garssen 1997).

A second main respiratory symptom in the spectrum of PD is dyspnea, defined as "breathing discomfort consisting of qualitatively distinct sensations that vary in intensity". In an early report of Rapee et al. (1992b), symptom profiles based on DSM-III-R panic symptoms were compared between PD patients and subjects suffering from other anxiety disorders (social phobia, specific phobia, and obsessive-compulsive disorder). Subjects were retrospectively questioned on the occurrence of these specific symptoms during a panic attack or a cued phobic or OCD response.

All panic symptoms were reported to occur during attacks in both groups, with a higher prevalence in the PD group for all items. Seven out of fourteen panic symptoms were reported significantly more often by PD patients among which dyspnea.

Although dyspnea seems to indicate pulmonary obstruction, the occurrence of dyspnea does not seem to be related to structural changes in the cardiopulmonary system. On the contrary, severe structural change is not required for the experience of dyspnea (Dudley et al. 1968). Patients with so-called medically unexplained dyspnea reported more intense dyspnea than patients with organic lung diseases. As a symptom, dyspnea depends for its identification on the subjective judgment of the patient. No objective tests (e.g., blood gas or ventilatory abnormalities) are available to date. However, Han et al. (2004) showed that subjects with medically unexplained dyspnea could be distinguished from subjects with organic dyspnea by using a small set of psychological and physiological measures, including PaO_2 and forced expiratory volume in 1 s. Furthermore, one-third of these patients met the criteria for PD.

In addition to the characteristic occurrence of respiratory symptoms during panic attacks, an increased prevalence of respiratory diseases has been reported in PD patients as compared to both healthy control subjects and patients with other anxiety disorders (Zandbergen et al. 1991). Vice versa, the lifetime prevalence of PD in patients with asthma is higher than expected in comparison with epidemiologic estimates of prevalence in the normal population (Perna et al. 1997; Nascimento et al. 2002; Potoczek 2005). Both severe asthma in the past 4 weeks and severe lifetime asthma were associated with a significantly increased likelihood of PD (Goodwin et al. 2003). It has been reported that up to 40% of PD patients have a childhood history of respiratory diseases, particularly asthma and bronchitis (Verburg et al. 1995b). Furthermore, an increased incidence of PD in patients suffering from chronic obstructive pulmonary disease (COPD) has been reported (Karajgi et al. 1990). Interestingly, respiratory disorders and PD have been found to run in the same families. A common genetic susceptibility for PD and respiratory disorders has been suggested (Coryell et al. 2001; van Beek et al. 2005).

Unlike other anxiety disorders, the link between respiration and PD is explicitly mentioned in the most fundamental theories on the mechanisms of PD. Ley (1985) stated that unexpected somatic events (dyspnea and palpitations, in particular) are the consequence of a rise in blood alkalosis produced by hyperventilation. According to this theory, the occurrence of hyperventilation is a necessary factor for the occurrence of panic. In the past, a so-called hyperventilation provocation test was used to diagnose chronic hyperventilation. This method lacked both sensitivity and specificity and the diagnosis of hyperventilation syndrome appeared untenable (Hornsveld and Garssen 1996; Hornsveld et al. 1996). A second, quite influential, theory that attempts to explain panic suggests that panic attacks result from the catastrophic misinterpretation of certain internal bodily sensations (Clark 1986). The misinterpreted sensations are mainly those involved in normal anxiety responses (e.g., breathlessness). Clark suggested that the catastrophic misinterpretation of sensations accompanying hyperventilation

plays an important role in panic attacks. A third more biological theory proposed that CO_2 hypersensitivity in PD patients results from the malfunctioning of a "suffocation monitor". Normal shifts in physiological states, more specifically increased pCO_2 or decreased pH, are misinterpreted initiating a false alarm, i.e., panic attack (Klein 1993). In case this last theory would proof to describe the actual underlying mechanism of panic, malfunctioning of a critical system directly related to respiration is confirmed. This would explain most of the respiratory variability in PD and, at the same time, it would imply the specificity for this particular disorder.

3 Respiratory Variables in PD

The abundant literature concerning the link between respiration and PD clearly shows that this topic has stimulated researchers and inspired theories. The hypothesis of impaired respiratory functioning has led to studies investigating a plethora of respiratory parameters, such as respiratory rate (RR), partial pressure of CO_2 (pCO_2), tidal volume (TV), minute volume (MV), and more sophisticated techniques including approximate entropy. We recently reviewed the literature on respiratory parameters in PD (Niccolai et al. 2009). The following is based on the outcomes of this review.

3.1 Respiratory Rate

The easiest indicator of respiratory functioning is probably RR. Studies that investigated RR during baseline and resting conditions have consistently shown similar values in PD patients and controls. The same outcome was reported by one ambulatory monitoring study (Hoehn-Saric et al. 2004) and investigations during sleep onset and sleep (Koenigsberg et al. 1994; Stein et al. 1995). Different RR in PD patients compared to control subjects was found in some studies (Maddock and Carter 1991; Munjack et al. 1993; Wilhelm et al. 2001b), but the methodological design of these studies may well have influenced the outcomes.

With regard to panicogenic challenges, a similar result was found. Most studies measuring RR during the challenge phase did not find a different pattern in PD patients compared to controls. These studies involved either lactate infusion or 5% CO_2 inhalation (Schwartz et al. 1996; Rapee et al. 1992a; Bystritsky et al. 2000) as a respiratory challenge. One study reported increased RR in PD subjects 3–6 min after the start of lactate infusion during sleep (Koenigsberg et al. 1994). However, the difference between groups did not reach significance and, according to the authors, the increase was not impressive or consistent. Papp et al. (1993b) found higher RR in PD subjects compared to controls using the 35% CO_2 inhalation for 30 s.

However, this procedure is hardly comparable with others involving CO_2 inhalation. To our knowledge, this has never been replicated.

It has been proposed that PD patients are insufficiently capable of maintaining homeostasis (Perna et al. 2004). In this regard, it is very interesting to consider recovery from challenges when homeostasis needs to be restored. A few studies investigated RR during recovery from respiratory challenges. Two studies found higher doxapram induced RR in PD subjects than in controls (Lee et al. 1993; Abelson et al. 1996). Rapee et al. (1992a) found similar RR between medicated PD patients and controls following 5.5% CO_2 inhalation.

3.2 Partial Pressure of CO_2

Numerous studies have focused on baseline pCO_2. Outcomes show two major directions: towards diminished or towards similar pCO_2 levels in PD subjects compared to controls. The evidence for lower pCO_2 in PD patients than in healthy controls is most convincing as evidence comes from studies involving large sample size and focusing on resting conditions (Gorman et al. 1986; Papp et al. 1997; Liebowitz et al. 1985; Wilhelm et al. 2001b; Munjack et al. 1993; Ponto et al. 2002; Gorman et al. 1988; Rapee et al. 1992a; Holt and Andrews 1989). They provide true basal measurements, unbiased by the expectations of a challenge. Among those who did not report a difference in pCO_2 values between groups are studies with notable limitations concerning small sample size (Maddock and Carter 1991; Ponto et al. 2002; Kellner et al. 1998; Koenigsberg et al. 1994; Wilhelm et al. 2001a) or subjects' medication status (Pain et al. 1988; Holt and Andrews 1989; Rapee et al. 1992a). Antipanic medication can modulate respiratory physiology (Papp et al. 1993a; Gorman et al. 1997). Specifically, successful treatment of PD has been shown to normalize pCO_2 by increasing its values (Gorman et al. 1985).

Studies investigating the challenge phase have used either lactate infusion or inhalation of different concentrations of CO_2. During lactate infusion, PD subjects consistently showed decreased pCO_2 levels (Gorman et al. 1986; Liebowitz et al. 1985; Kellner et al. 1998) or a trend towards lower values (Koenigsberg et al. 1994). Moreover, in these studies nonpanicking patients and controls were compared and lower pCO_2 was reported for the first group.

Studies applying CO_2 inhalation found comparable results. Lower (Papp et al. 1997) or a trend towards lower (Ponto et al. 2002) pCO_2 levels in PD subjects compared to controls have been reported during the continuous inhalation of CO_2 concentrations around 6%. A few studies did not find a group difference, but they showed limitations concerning medication status, sex distribution (Rapee et al. 1992a), and type of challenge (Gorman et al. 1990). A different case is the 35% CO_2 inhalation challenge, in which a single vital capacity breath is taken and hypercapnia is immediate and lasts for about 7–16 s after exhalation (van den Hout and Griez 1985). Studies investigating pCO_2 level during the 35% CO_2 inhalation are limited, which is not surprising since the challenge procedure formally

exists of one inhalation and a breath holding period. Ponto et al. (2002) applied this challenge, followed by 10 s of rebreathing, and found lower pCO_2 levels in PD subjects than in controls.

Few studies have investigated the pCO_2 response during recovery from a challenge. Findings consistently report lower pCO_2 in PD patients compared to controls after both doxapram injection and CO_2 challenge. Lee et al. (1993) found lower pCO_2 in PD subjects compared to controls for the last 7 min of a 9-min recovery period from doxapram injection. Abelson et al. (1996) investigated 20 min of recovery from doxapram and reported reduced pCO_2 in PD subjects compared to controls persisting throughout the recovery phase and reaching significance during the last 5 min. After CO_2 inhalation, results consistently showed that lower pCO_2 in PD patients compared to controls was maintained for several minutes (Gorman et al. 1988, 1990; Papp et al. 1997). To our knowledge, only one study has reported similar pCO_2 values in PD subjects and controls during 7 min of recovery from 5.5% CO_2 inhalation (Rapee et al. 1992a). However, one major limitation of this study was that medication was not stopped before the test day, which makes the results less reliable. Moreover, as shown by other studies focusing on recovery phase, it appears that group differences in pCO_2 levels emerge after a period of time. It is possible that measuring challenge effects during recovery for only 7 min prevent finding a group difference in pCO_2.

3.3 Tidal Volume and Minute Volume

Findings from studies investigating TV and MV during baseline, sleep, or resting condition have mainly shown increased values in PD subjects compared to controls (Pain et al. 1988; Gorman et al. 1990; Stein et al. 1995; Abelson et al. 1996; Wilhelm et al. 2001b) or a tendency toward higher values in the PD group (Martinez et al. 1996; Papp et al. 1997). No evidence of lower TV and MV in PD patients compared to controls has been found. Some studies have reported no differences in TV and/or MV in PD subjects relative to healthy subjects (Gorman et al. 1988; Papp et al. 1993b; Yeragani et al. 2002).

The very few studies focusing on TV or MV during a respiratory challenge and/or the recovery period have shown inconsistent results. Normal TV in PD patients during respiratory challenges has been described (Abelson et al. 1996). Different TV reactivity between groups during recovery from 5% CO_2 inhalation has been reported as well (Papp et al. 1997). When subjects switched to room air, TV restored more slowly in patients than in controls. This was interpreted as a physiological response to CO_2 intolerance. Higher MV in PD subjects than in healthy subjects was found during a 30-s lasting 35% CO_2 inhalation (Papp et al. 1993b) and during recovery from doxapram injection (Lee et al. 1993; Abelson et al. 1996).

Overall, it appears that PD patients show a different reaction than controls to respiratory challenges with regard to their TV and MV.

3.4 Respiratory Variability, Sighing, and Approximate Entropy

Several studies found increased respiratory variability in PD subjects compared to controls during both baseline and resting conditions. Respiratory variability provides insight into fluctuations in respiration, which may play a role in susceptibility to panic (Wilhelm et al. 2001a). Abnormal respiratory regulation appears to render PD subjects more susceptible to heightened concentration of CO_2 in inhaled air (Martinez et al. 2001). In fact, PD subjects who panicked after CO_2 inhalation demonstrated the largest baseline respiratory variability in TV and MV. Other studies have confirmed increased baseline TV or MV variability in PD patients compared to controls (Gorman et al. 1988, 2001). Interestingly, Abelson et al. (2001) found that the increased TV irregularity in PD subjects was observed throughout the three phases of the experiment (adaptation, placebo, and postdoxapram phase). This stable respiratory feature may be suggestive of dysregulated neural circuits in the brainstem or midbrain levels in PD patients. Consistent with this, studies investigating sleep reported higher TV or MV variability in PD subjects compared to controls (Stein et al. 1995; Martinez et al. 1996). Presuming that sleep is a state during which anxiety is limited, these results could suggest that increased erratic breathing found in PD patients during baseline represents a physiological endophenotype. Increased respiratory variability in PD patients has also been found during recovery from the 35% CO_2 challenge. In a recent study from our laboratory, nonmedicated patients showed increased variability in both RR and pCO_2 compared to controls and medicated PD subjects (Niccolai et al. 2008).

More frequent and prominent sighs have been suggested to account for larger TV variability in PD patients compared to controls (Stein et al. 1995; Abelson et al. 2001). A higher frequency of sighs in PD subjects has been reported during resting state (Wilhelm et al. 2001a; Yeragani et al. 2002; Caldirola et al. 2004), baseline, and during/after a challenge (Schwartz et al. 1996; Abelson et al. 2001). It has also been found that patients had greater sighs than controls (Wilhelm et al. 2001a). Only one study did not find differences in the number of sighs between groups during sleep (Stein et al. 1995). However, the authors acknowledged that the enormous variability in sigh frequency shown by normal adults during sleep and the small sample size might explain the negative finding. Breath-by-breath variation in TV manifested by sighing is suggestive of a robust and fairly stable marker in PD patients (Abelson et al. 2001). Although the presence of sighs may not fully explain the irregularity in breathing patterns (Caldirola et al. 2004), it could contribute to the identification of respiratory etiopathological pathways in PD.

Apneic episodes also importantly contribute to respiratory variability. These episodes can be described as the absence of respiratory movement for a period of time that may range from l to 10 s. The higher number and the longer duration of apnea episodes appeared to distinguish PD patients from normal controls during baseline (Bystritsky and Shapiro 1992). Also, more frequent apnea episodes during

baseline seemed to predict panic attacks during CO_2 inhalation, both in patients and controls. Increased length and number of breathing pauses and increased variability in length of breathing cycle were found during baseline in panicking patients compared to the nonpanickers and normal controls by the same authors in a successive study (Bystritsky et al. 2000). The breathing pauses were found to persist or worsen during CO_2 inhalation. More frequent pauses in PD subjects' breathing relative to controls have also been found during sleep (Stein et al. 1995).

Results from studies applying approximate entropy, a measure of regularity of time series, are in line with the traditional measures of respiratory parameters (standard deviation and coefficient of variation). These findings suggest that PD subjects not only have a greater overall variability, but also a more chaotic pattern of breathing. Specifically, greater entropy in the respiratory function is suggestive of an intrinsic instability in the respiratory homeostasis, which would explain why hypercapnic challenges act as "disrupting factors" in PD patients, leading to panic attacks (Caldirola et al. 2004).

4 Respiration and Phobias

Phobias are particularly suitable to be subjected to psychological paradigms. These instances of acute fear are characterized by symptoms that transpire in specific situations. The physiological symptoms of an acute phobic reaction largely resemble the symptoms of a panic attack. In fact, phobic responses are often referred to as panic attacks, even though diagnostically, panic attacks are unexpected, spontaneous attacks, not triggered by a specific stimulus.

In essence, symptoms can be triggered by exposing patients to a phobic stimulus. The drawback of this kind of manipulation is that there is a myriad of possible approaches, resulting in a lack of standardization.

Basically, there are two different approaches – in vivo, and imaginal exposure – each with its own positive and negative aspects. The obvious advantages of imaginal exposure are the relative ease with which it can be conducted and its low costs. There are no special requirements other than a quiet room. However, it is hard to control the actual state of mind of the patient. Concerning in vivo exposure, basically, there are two options. Exposing the subject to the actual feared object or situation, such as a spider in spider phobia, or showing the subject images or movies of the relevant subject. Although the first might evoke the stronger reaction, the procedure has some drawbacks. It is often time consuming, guarantees little control over the environment, and can be costly. A great advantage of movies or pictures, is that it allows to study the responses to visual stimuli in, for example, fMRI paradigms. An alternative that is currently gaining more attention and which can be considered as a third option, is confrontation in a virtual environment.

In the following sections, some striking findings on respiratory symptoms in the different phobias are presented.

4.1 Social Phobia

Some interesting links exist between social phobia and PD (Horwath et al. 1995; Caldirola et al. 1997). From a diagnostic point of view, it can be difficult to distinguish a true unexpected panic attack from a situationally triggered attack in a socially phobic person. Symptomatically, there are many resemblances. The main discriminator are the cognitions of the patient during the attack. Whereas the social phobic will be afraid of the scrutiny and judgment of others, the PD patient will be mainly afraid of dying (suffocation), losing control, or going crazy. Remarkably, there have been some reports that showed increased sensitivity in social phobics for carbon dioxide (Gorman et al. 1990; Papp et al. 1993b; Caldirola et al. 1997). Unfortunately, the specific effect on respiratory symptoms was not addressed. In contrast, Pine et al (2000) subjected children who were diagnosed with social phobia to a 15-min period of 5% carbon dioxide inhalation and found no symptomatically defined hypersensitivity for carbon dioxide. However, this group consisted of only 10 children.

Public speaking tasks have been validated, in particular the Trier Social Stress Test (Kirschbaum et al. 1993), and activation of the sympathetic nervous system in social phobics during social confrontation has been demonstrated (Gerlach et al. 2003). However, despite the possible link between PD and social phobia and the distinct sympathetic activation during exposure, possible respiratory correlates of this disorder have not been addressed.

4.2 Blood Injury Phobia

Generally, acute anxiety states or, in other words, fear states, are known to be accompanied by sympathetic activation. Blood injury phobia (BIF) presents a bit of a difference in this regard. The vasovagal syncope, or presyncope, that often results from confrontation with the feared stimulus in BIF underlines not only a strong sympathetic response, but it could be related to an abnormal hemodynamic response, possibly not specifically related to the phobia itself (Accurso et al. 2001).

Respiratory symptoms have been addressed in some studies involving persons suffering from BIF. Respiration rate was measured under different circumstances among which were fear related and control movies. Sarlo et al. (2002) did not find a difference in RR between those different conditions. This finding was confirmed by Ritz et al. (2009) who also included a healthy control group. Interestingly, the latter authors included other respiratory parameters in addition to RR in which they did find differences. Specifically, during surgery films, MV and TV were significantly increased in BIF patients. In addition, irregularity of TV showed strong increases and there was a trend towards an increased sigh frequency in the movie condition. Concordantly, reduced pCO_2 levels have been reported by the same group (Ritz et al. 2005, 2009). In conclusion, there seems to be some indication that respiration is affected in BIF, especially during the acute state.

4.3 Other Phobias

The number of possible phobias is more or less endless. Even though the acute phobic response can be accompanied by a strong increase in respiration rate, detailed information on respiratory variables is limited. In a very early study of Prigatano and Johnson (1974), respiration rate and respiration amplitude in 11 spider phobic females was described in response to confrontation with pictures of spiders. No increase in RR was found in spider phobics during picture viewing both compared to a control group and to control slides. There was a trend towards significance with regard to respiration amplitude. Spider phobics showed larger respiratory amplitude in response to spider slides as compared to seaside or surgery slides. Yet, there was no difference in amplitude when phobic subject and controls were compared during the spider condition.

Two other phobias that have been described in literature with regard to respiration are driving and flying phobias. In both studies, respiratory variables were measured during real-life exposure. Wilhelm and Roth (1998) determined several respiratory variables in 14 flight phobics during a 12-min flight. Inspiratory pause showed the best discriminant validity for distinguishing phobics from controls, with phobics demonstrating extended pauses after inspiration of more than 20 s. A group effect was found for duty cycle, an index of inspiratory timing defined as inspiratory time/total breath time. This indicates a more tonic difference between the groups, possibly affected by anticipatory anxiety and differences in recovery. The remaining respiratory variables that were measured, knowing RR, MV, inspiratory flow rate, TV irregularity, did not discriminate between phobics and controls. A second study assessed multiple respiratory variables in 21 females suffering from driving phobia (Alpers et al. 2005). Differences between the control and the phobic group with regard to exposure were found for four different respiratory variables. Phobics showed lower ET pCO_2 during driving, and the effect size for distinguishing groups based on pCO_2 was large. Sigh rate showed a group effect with higher rates in phobics and duty cycle increased from quiet sitting to exposure, which was not the case in the control group. Additionally, respiratory variables related to pCO2 and sighing were determined. Larger variability in TV was present in phobics. Differences in mean RR and TV were not statistically significant.

5 Obsessive-Compulsive Disorder

Studies describing respiratory variables in obsessive-compulsive disorder (OCD) are rather scarce. Nevertheless, all of the relevant challenge paradigms have been described with the inclusion of respiratory parameters. Patients were studied under three conditions: relaxation, imaginal flooding, and in vivo exposure (Zohar et al. 1989) and during general stress tasks (Hoehn-Saric et al. 1995).

An early study by Zohar et al. (1989) included both RR and pCO_2. In vivo exposure resulted in anxiety ratings and obsessive-compulsive ratings that were

statistically higher than during imaginal exposure, which might lead to the conclusion that imaginal exposure is not as effective in evoking symptoms as real-life exposure. Blood pressure and heart rate were found to be significantly increased in the exposure trials as compared to the relaxation phase, but for both respiratory parameters no such effect was found. Unfortunately, this study included only 10 OCD patients and the variance in respiratory symptoms was rather large. Nevertheless, in contrast to the other physiological symptoms, pCO_2 values were lower during imaginal exposure than during relaxation. Strangely enough, during in vivo exposure, values equaled pCO_2 values obtained from the relaxation phase. Hoehn-Saric et al. (1995) subjected 23 OCD patients to two nonspecific stress tasks: the Divided Attention Task and the Risk Taking task. Physiological measurements included respiratory frequency but no group × task interaction effect was described for this variable. In this study, a decrease rather than an exaggeration of the physiologic response during psychological stress was described.

6 Generalized Anxiety Disorder

Somewhat more work has been performed on respiratory parameters in GAD. It might not be a coincidence that respiratory variables have received most attention in PD and GAD. In the DSM-II-R, "shortness of breath or smothering sensations" is described as a GAD symptom, whereas in other anxiety disorders other than GAD and PD, distinct descriptions of respiratory symptoms are not explicitly mentioned.

In an early study, Hoehn-Saric et al. (1989) performed a comparable study as described at the OCD section. Twenty female patients suffering from GAD were compared to a matched control group during baseline and during stress tasks. No differences in autonomic activity were reported during baseline. The stress tasks resulted in an increased respiratory frequency in both groups. Although GAD subjects reported increased difficulty in breathing during the tasks, respiratory frequency did not show a significant difference between groups in any of the conditions.

A more extended study (Wilhelm et al. 2001b) measured several respiratory variables during rest, including RR, TV, MV, duty cycle, inspiratory flow rate, ET CO_2, sighs, and apneas. In addition, breath-to-breath variability by Complex Demodulation was assessed as a measure of instability. Fifteen GAD patients were directly compared to PD patients ($n = 15$) and healthy control subjects ($n = 19$). Most respiratory variables were increased in PD patients as compared to the control group. GAD patients showed intermediate values, not significantly different from control values on any of the parameters. Duty cycle was significantly increased in GAD patients as compared to PD subjects. Group × time interactions were found for MV and inspiratory flow rate. Remarkably, GAD subjects showed a different response over time. GAD subjects showed a decline, whereas the other two groups slightly increased. The inclusion of variability indices proved valuable. None of the electrodermal or cardiovascular measures showed group effects,

whereas all of the respiratory variables did. This might indicate that respiratory variables are more sensitive indicators of anxiety or that they show better discriminative ability. Both patient groups showed increased variability as compared to controls on RR, TV, MV, and duty cycle. Variability in TV and MV was even larger in PD patients as compared to GAD patients, although the difference in TV disappeared when data was corrected for sighs. Six out of sixteen PD patients panicked during testing, and one GAD patient reported a symptomatic attack. Analyses were reproduced with exclusion of these subjects. As a result, some variables, among which duty cycle, lost significance. Removing these subjects resulted in an increased discriminative ability for pCO_2, for which this parameter became significantly smaller in PD patients as compared to GAD patients as well. In itself, this might seem counterintuitive. One would expect that during a panic attack, hyperventilation is most pronounced, resulting in decreased pCO_2 values. Yet, removing this specific group leaving only 10 PD, results in a significant difference between PD and GAD subjects. This seems to imply that the panicking subjects were responsible for increased variability.

Another direct comparison between PD and GAD patients was performed by Hoehn-Saric et al. (2004). Forty GAD subjects were compared to twenty-six PD patients and twenty-four control subjects during everyday activities. The only direct indication of respiratory functioning was RR. Subjectively, patients reported more difficulty breathing during the 6-h recording than control subjects. In PD subjects, this showed a sixfold increase during times when panic was reported. RR did not differentiate between groups. In addition, no statistically significant effects were found in the analysis of the relationship between RR and difficulty of breathing.

7 Posttraumatic Stress Disorder

Only limited information exists on respiratory variables in Posttraumatic Stress Disorder (PTSD). In PTSD, vivid memories or flashbacks can be accompanied by different autonomic symptoms including respiratory symptoms. In addition, increased heart rate and RR following a traumatic event have claimed to be predictors of subsequent PTSD (Bryant et al. 2008). One recent study (Blechert et al. 2007) describes different variables during a 5-min baseline period and during threat of shock in 23 PTSD patients, 26 PD patients, and 32 healthy control subjects. The following respiratory parameters were assessed: RR, TV, MV, duty cycle, sigh frequency, inspiratory flow rate, ET CO_2, and ribcage contribution to TV. Variability of respiratory cycle duration and TV was assessed using complex demodulation (frequency band: 0.004–0.14 Hz). Differences in respiratory variables were only found during baseline. Both patient groups had lower pCO_2 values as compared to control subjects, but this difference reached statistical significance only in the PD group. pCO_2 values in PTSD and PD patients were not statistically different. Additional differences in respiration were reported for sighing and ribcage contribution to TV. PTSD patients, but not PD patients, sighed more than control subjects

during baseline. Furthermore, PTSD patients showed less thoracic and more abdominal breathing than the other groups, indicated by lower ribcage contribution. None of the respiratory variables could discriminate between the diagnostic groups.

Unfortunately, to our knowledge there are no reports on respiratory variables in PTSD in which subjects were subjected to a disorder specific provocation paradigm, such as personalized trauma-related imagery, script-driven imagery method, or just simple exposure to trauma-related cues.

8 Discussion

The obvious abundance of literature on respiratory variables in PD is not remarkable considering the evident links between this physiological system and this specific psychiatric disorder. The frequent occurrence of respiratory symptoms seems relatively specific for PD, although respiratory symptoms have been described in other anxiety disorders as well. This is not surprising in itself, since increased RR is a normal physiological response to fear and anxiety – affective states that are characteristic for any anxiety disorder.

An important role of hyperventilation in PD has been suggested in different theories. Even though it has been shown that hyperventilation is not causally related to the occurrence of panic attacks, hyperventilation might be a phenomenon related to PD. As extensively reviewed by Niccolai et al. (2009), PD patients demonstrate decreased pCO_2 values under normal physiological circumstances and during respiratory challenges. With regard to basal values, different explanations have been suggested. Chronic hyperventilation would obviously explain low pCO_2 levels in PD subjects (Gorman et al. 1986). It has been proposed that chronic hyperventilation serves to maintain low levels of pCO_2 in order to avoid triggering of the CO_2 sensors. According to Klein's suffocation false alarm theory, triggering of these sensors would set off false alarms in these hypersensitive subjects. On the other hand, the great majority of studies do not show increased RR in PD subjects, which would be indicative of hyperventilation. However, chronic hyperventilation can also depend on TV. Increased TV has repeatedly reported in PD patients (Pain et al. 1988; Gorman et al. 1990; Stein et al. 1995; Abelson et al. 1996; Martinez et al. 1996; Papp et al. 1997; Wilhelm et al. 2001b). In other words, this indicates PD patients do not breathe faster, but breathe deeper than unaffected persons. Moreover, using disorder-specific challenges, MV and TV were increased in panicking patients as compared to nonpanicking PD subjects (Gorman et al. 1988). However, this is true for some specific phobias as well (Prigatano and Johnson 1974; Ritz et al. 2009), questioning the specificity of this measure. In contrast to resting conditions, increased RR has been found during panicogenic challenges in PD (Gorman et al. 1988, 2001). To our knowledge, this has not been reported for other anxiety disorders, with the limitation that data on respiratory parameters during disorder-specific challenges is scarce. Before drawing definite conclusions from this, one has to keep in mind that the validity of these indices as diagnostic

tools of chronic hyperventilation is questionable. The ultimate measures of hyperventilation are pCO_2, pH, and bicarbonates (Munjack et al. 1993). These very parameters have been investigated in PD patients by Gorman et al. (1986) using venous blood samples in rest. Mixed chronic and acute respiratory alkalosis was found, both characterized by low pCO_2 values and normal and elevated pH, respectively. In a subsequent study by the same group (Papp et al. 1989), arterial instead of venous pCO_2 was measured. Results were consistent with acute, but not chronic hyperventilation. In concordance, absence of chronic hyperventilation in PD patients was also reported in a more recent study investigating arterial blood gases (Zandbergen et al. 1993).

Clear insight into the issue of chronic hyperventilation may be provided by ambulatory monitoring studies. While experimental respiratory challenges directly influence respiration by provoking alkalosis or acidosis, ambulatory studies measure transcutaneous pCO_2 in a naturalistic setting. Therefore, data from ambulatory studies have greater external validity than those obtained in the laboratory, which, as a stressor-condition, may also influence psychophysiological recordings intrinsically. One study focusing on PD subjects reported no difference in transcutaneous pCO_2 between panic attacks and nonpanic control periods (Garssen et al. 1996). Despite the great value of ambulatory studies, to our knowledge no studies on pCO_2 in PD that included a control group, are available to date, possibly with the exception of one sleep study. During sleep onset, which is likely to be a good reflection of physiological activity at rest, no difference in pCO_2 was found between PD patients and control subjects (Koenigsberg et al. 1994). Even though more research needs to be performed, from the available data we can conclude that there are no clear indications of chronic hyperventilation in PD.

In addition to PD, reduced pCO_2 has been reported in BIF (Ritz et al. 2005) and driving phobia (Alpers et al. 2005) during disorder specific stress. Driving phobia has been associated to PD (Curtis et al. 1989), which could explain overlapping symptom profiles. However, this has been disputed by others (Antony et al. 1997). Lower baseline pCO_2 was found in both PD and in a mixed non-PD anxiety group consisting of mainly OCD patients and social phobics (van den Hout et al. 1992). In addition, Blechert et al. (2007) found decreased baseline pCO_2 values in PTSD patients that were not statistically from values of a group of PD patients. This indicates that decreased pCO_2, indicative of hyperventilation, is not specific for PD. Holt and Andrews (1989) suggested that hyperventilation may be intimately tied to anxious affect. The more anxious patients are, the more likely they are to show reduced pCO_2 levels. Furthermore, an explanation for decreased pCO_2 levels in anxiety disorders in general could be provided by increased levels of anticipatory anxiety. This is especially relevant when baseline values preceding an experimental stress paradigm are considered. Bystritsky et al. (2000) suggested that larger fluctuations in baseline breathing patterns in PD patients compared to controls are likely to reflect anticipatory anxiety. This could also apply to other anxiety disorders. The above seems to suggest that decreased pCO_2 does not constitute a specific trait marker of PD, but might be an indication of the current anxiety level.

In addition to decreased pCO_2 levels, both an increased frequency of sighs and greater sighs has been reported in PD (Schwartz et al. 1996; Abelson et al. 2001; Wilhelm et al. 2001a; Yeragani et al. 2002; Caldirola et al. 2004). It has been suggested that sighing adaptively keeps pCO_2 values below a depressed suffocation alarm threshold (Klein 1993). However, it should be noted that, in one of the studies reporting a higher rate of sighs in PD patients, the sighing pattern did not appear to be stimulated by increased pCO_2 or reduced TV (Wilhelm et al. 2001a). Another possible explanation for the adaptive effect of sighs is that they are efforts to overcome breathlessness (Klein 1993). Abelson et al. (2001) suggested that frequent sighing is a compensatory response in an attempt to reduce the sensation of dyspnea. According to others, it is more specific since sighing would distinguish between patients with chronic anxiety having frequent episodes of dyspnea from patients with various lung diseases (Tobin et al. 1983). However, with regard to the specificity of sighing for PD as compared to other anxiety disorders, some reservations have to be mentioned. A higher frequency of sighs in PD subjects has been reported (Schwartz et al. 1996; Abelson et al. 2001; Wilhelm et al. 2001a; Yeragani et al. 2002; Caldirola et al. 2004). However, increased levels of sighing as compared to control subjects has been reported in BIF (Ritz et al. 2009), driving phobia (Alpers et al. 2005), and PTSD (Blechert et al. 2007) as well, which seemed to be trait dependent and most pronounced for the latter two. In fact, the study in PTSD even showed increased sighing in PTSD as compared to PD subjects during a baseline period. Even though there is only limited data available on sighing in anxiety disorders, this does raise doubt on the specificity of this symptom for PD.

The increased comorbidity with respiratory disorders such as asthma and COPD that is often reported in PD, has also been described in other anxiety disorder but far less pronounced (Brenes 2003; Goodwin et al. 2003; Patten and Williams 2007). The fact that PD and respiratory disorders run in families, points to a shared genetic susceptibility, a trait that has not been reported for other anxiety disorders. This could indicate a specific vulnerability in PD patients related to the respiratory system. As a result, exaggerated fluctuations in biological substrates important for the regulation of respiration may emerge, that do not reach the threshold of conscious awareness. In line with this, increased variability and more chaotic breathing patterns have been described in PD (Wilhelm et al. 2001b; Yeragani et al. 2002; Caldirola et al. 2004). Although increased variability seems to be a promising candidate for a specific marker of panic, it has been reported in other anxiety disorders as well. In GAD, increased variability compared to values in control subjects was reported for RR, TV, MV, and duty cycle during quiet sitting (Wilhelm et al. 2001b). This study included both GAD and PD subjects. Only TV irregularity showed significantly increased values in PD subjects caused by sighing. Another study comparing healthy controls, PTSD and PD subjects during rest and during anticipation of electrical shock describes measures of variability in TV and respiratory cycle duration. No differences between either patient group or controls was described and it was reported that none of the respiratory variables discriminated between diagnostic groups. The only study describing respiratory variability during the active state was performed in driving phobics. A larger

variability in TV was reported in phobics as compared to control subjects during in vivo exposure.

Respiratory output is a physiological signal with complex dynamics that are not easily captured by linear statistics such as mean and standard deviation. A number of studies have, therefore, focused on nonlinear measures of respiration, which are likely to unravel the breath-by-breath complexity of respiratory dynamics. These more sophisticated measures include approximate entropy and other measures of chaos. Some studies have reported greater instability and higher levels of respiratory irregularity and complexity in PD subjects compared to healthy subjects in pCO_2, RR, TV, MV, and/or duty cycle (Wilhelm et al. 2001b; Yeragani et al. 2002; Caldirola et al. 2004).

The suggested inability of PD patients to preserve respiratory homeostasis explains the increased vulnerability of PD patients to specific panicogenic challenges. As stated before, these experimental procedures, in particular CO_2 inhalation, lactate infusion, and cholecystokinin infusion are known to exert a strong influence on respiration (Calverley et al. 1983; Liebowitz et al. 1984; Charney et al. 1985; Griez et al. 1987; Bradwejn et al. 1990). PD patients specifically show a more pronounced response to these challenges than both healthy control subjects and, in many instances, people suffering from other anxiety disorders (Den Boer et al. 1989; Cowley and Arana 1990; Bradwejn et al. 1991; van Megen et al. 1996; Perna et al. 1995; Verburg et al. 1995a). Comparable to other medical conditions, these provocation tests challenge the affected system and magnify subtle aberrations that otherwise may remain undetected. In addition, the fact that PD patients of the respiratory subtype show the most prominent response might point to a relationship between the degree of malfunctioning of the respiratory system and the response to provocation of this system. In fact, even in healthy volunteers the affective response to different dosages of carbon dioxide shows a dose response relationship, which is best predicted by respiratory symptoms (Colasanti et al. 2008).

In conclusion, even though disturbed respiration seems related to PD in many ways, simple, linear, measures of respiration do not discriminate between PD and other anxiety disorders. Since sympathetic activation, including respiratory adaptation, is such a general response to many situations, it should not come as a surprise that other conditions characterized by high anxiety levels show signs of affected respiration as well. Responses to general stress can differ substantially from responses to specific challenges. In PD, respiratory responses to panicogenic challenges have received major attention. Unfortunately, this is not the case for other anxiety disorders. Therefore, it seems too early to draw final conclusions on the specificity of respiratory symptoms with regard to the acute state. A related topic that deserves more attention is recovery. Assuming that PD is characterized by instability of the respiratory system, recovery after strong provocation of this system should be affected. Indeed, investigation of the recovery phase after either doxapram injection or CO_2 inhalation revealed lower pCO_2 and higher RR in PD patients compared to controls (Gorman et al. 1988, 1990; Lee et al. 1993; Abelson et al. 1996; Papp et al. 1997). To our knowledge, there are no reports on respiratory variables during recovery from acute state symptoms in other anxiety disorders.

Moreover, the underlying respiratory vulnerability in PD might not be a stable trait, which calls for more sensitive and sophisticated measures of respiratory variability, such as approximate entropy. Unfortunately, again this has been insufficiently investigated in other anxiety disorders to draw any conclusions.

At this point, there does not seem to be concrete evidence of a specific respiratory marker in PD. In fact, most parameters proved not to be specific. However, there are a few promising candidates left, coming from more sensitive and sophisticated methods that needs to be assessed in other anxiety disorders, preferably during or following disorder specific challenge paradigms.

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Functional Neuroanatomy of Anxiety: A Neural Circuit Perspective

Amit Etkin

Contents

Abstract Anxiety is a commonly experienced subjective state that can have both adaptive and maladaptive properties. Clinical disorders of anxiety are likewise also common, and range widely in their symptomatology and consequences for the individual. Cognitive neuroscience has provided an increasingly sophisticated understanding of the processes underlying normal human emotion, and its disruption or dysregulation in clinical anxiety disorders. In this chapter, I review functional neuroimaging studies of emotion in healthy and anxiety-disordered populations.

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A limbic-medial prefrontal circuit is emphasized and an information processing model is proposed for the processing of negative emotion. Data on negative emotion processing in a variety of anxiety disorders are presented and integrated within an understanding of the functions of elements within the limbic-medial prefrontal circuit. These data suggest that anxiety disorders may be usefully conceptualized as differentially affecting emotional reactivity and regulatory processes – functions that involve different neurobiological mechanisms. While the neural bases of several anxiety disorders are increasingly better understood, advances have lagged significantly behind in others. Nonetheless, the conceptual framework provided by convergent findings in studies of emotional processing in normative and anxiety-disordered populations promises to yield continued insights and nuances, and will likely provide useful information in the search for etiology and novel treatments.

Keywords Amygdala · Anterior cingulate · Anxiety · Circuit · Dorsomedial prefrontal · Emotion · Emotion regulation · Fear · GAD · Generalized anxiety disorder · Hippocampus · Hypothalamus · Insula · Limbic system · Medial prefrontal · Meta-analysis · Obsessive-compulsive disorder · OCD · Panic disorder · Periaqueductal gray · Posttraumatic stress disorder · PTSD · Social anxiety disorder · Social phobia · Specific phobia · Ventromedial prefrontal

1 Introduction

"Anxiety" describes a wide range of subjective, often unpleasant, sensations that likewise reflect responses to a wide range of inciting events or stimuli. Anxiety can be part of a contextually appropriate and adaptive response, as it alerts an organism to salient events in its environment or internal milieu. Anxiety, however, can also be triggered to an inappropriate degree or at inappropriate times, and when prolonged, severe enough, and disruptive to the individual, is then considered a clinical disorder of anxiety. In this chapter I will review the neural basis of anxiety in healthy and clinical populations, with primary focus on functional neuroimaging studies in humans. The goal of this chapter is not to provide a comprehensive review of all such studies, but rather to provide a coherent understanding of the functional neuroanatomy of anxiety in its various manifestations from a neural circuit perspective, through a selective review of the literature. While there are many ways in which to describe, categorize and compare mental states or psychiatric disorders, I have chosen to focus on a cognitive neuroscience approach, as this literature is sufficiently broad, both in healthy subjects and in those with anxiety disorders, to fuel a substantive discussion. Ultimately, however, the fullest view of anxiety will require simultaneous integration across multiple levels of investigation.

Fear and avoidance of trigger cues are common to many anxiety disorders (APA 1994) and resemble the arousal and avoidance responses shown by normal subjects to conditioned fear cues (Grillon 2002). Thus, a common element of anxiety

disorders may be an abnormally elevated fear response. Furthermore, some anxiety disorders appear to involve a more generalized dysregulation of negative affect, suggesting that a rich explanatory model of anxiety also has to take these factors into consideration. In order to understand the neurobiology of anxiety, we must therefore first elaborate on the neural circuitry underlying the normal response to negative emotional stimuli and the regulation of their effects. This broad field of study has seen a dramatic expansion over the last decade, attributable largely to a proliferation of functional neuroimaging methods, and very usefully informs interpretation of alterations in this circuitry in patients with anxiety disorders.

Moreover, though anxiety can be seen as a shared overriding category for diverse clinical situations, the attractiveness of that concept belies the complexity of clinical presentations and neural bases of anxiety disorders. I will explore both shared and disorder-specific features of anxiety disorders, and through that suggest a different way of understanding the neural basis of anxiety. Finally, I will review emerging knowledge on neurobiological changes associated with, or prognostic of, treatment-related improvement of symptoms in patients with anxiety disorders.

1.1 Neural Circuitry of Anxiety-Relevant Negative Emotion: Reactivity and Regulation

Human and animal studies of the processing of negative emotions, such as fear, have implicated a large number of cortical and subcortical areas, and these have been quantitatively summarized by a number of meta-analytic surveys of the literature (Kober et al. 2008; Phan et al. 2002; Wager et al. 2003). A simplified information-flow conceptual model of some of the regions and their functional interactions is shown in Fig. 1, and the details are given in the sections below. Central to a neural circuit of negative emotion are a set of core limbic structures, which include the amygdala and insula, as well as interconnected structures such as the periaqueductal gray (PAG) and hypothalamus. These regions interact with a number of cortical areas, amongst which are several medial prefrontal subregions. Finally, the processing of emotion modulates other cognitive systems involved in perception, motor planning, and memory.

1.2 Core Limbic System

The amygdala is a complex structure and is composed of multiple subnuclei, each with its own distinct pattern of afferent and efferent projections. Our understanding of the organization of the amygdala is derived largely from animal work investigating the functions of subnuclei within the amygdala, and the distinct brain networks that they take part in. Of particular interest for anxiety are the basolateral complex

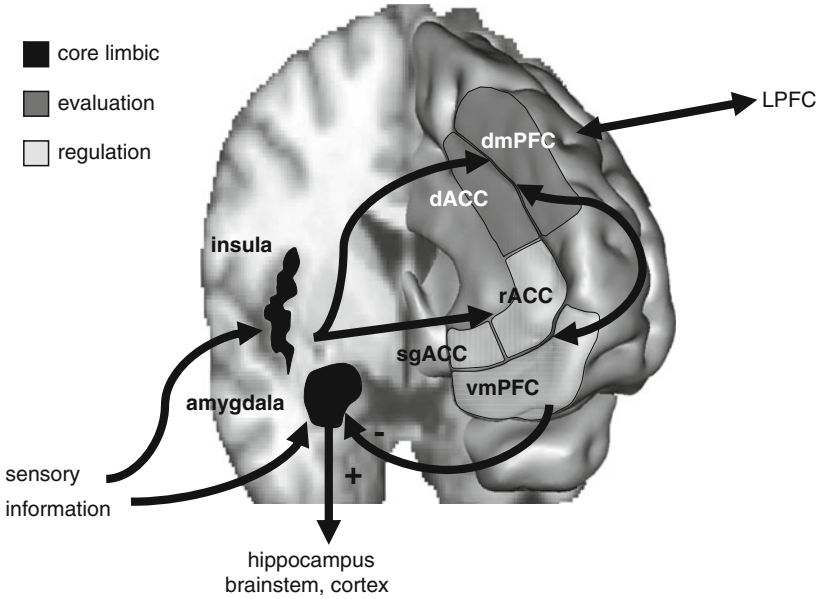

Fig. 1 A limbic-medial prefrontal circuit view of negative emotional processing. Relevant structures are separated into three functional groups. A core limbic group includes the amygdala, insula, as well as subcortical areas like the hypothalamus and periaqueductal gray (not depicted on this figure). These regions register emotional stimuli and initiate coordinated species-specific physiological and behavioral responses to them. A second group includes dorsal medial prefrontal structures including the dorsal anterior cingulate (dACC) and dorsomedial prefrontal cortex (dmPFC), and is involved in more extensive evaluation of emotion and may gate its access into conscious awareness. Finally, a third region, which includes the rostral and subgenual anterior cingulate (rACC, sgACC), and ventromedial prefrontal cortex (vmPFC), is involved in contextually appropriate regulation of emotion and core limbic processing. The processing of an emotional stimulus may lead to modulation of regions important in other cognitive functions (e.g., perception, motor planning, and memory) by core limbic regions. Additionally, lateral prefrontal (LPFC) executive regions may engage medial prefrontal emotion processing circuitry to aid in deliberate regulation of emotion

(BLA, a composite of the functionally related basal, lateral, and accessory basal nuclei) and the central nucleus, which is part of the centromedial amygdala (CMA) (Heimer et al. 1999). The BLA is the primary input site in the amygdala, receiving sensory information from the thalamic nuclei and sensory association cortices (Amaral et al. 1992), and also provides the majority of the thalamic and cortical projections from the amygdala. By contrast, the central nucleus is an output region that projects to the brain stem, hypothalamic and basal forebrain targets (Paxinos 2003), and is located dorsal to the BLA (Mai et al. 1997). In rodents, the basolateral amygdala encodes the threat value of a stimulus, while the central nucleus is essential for the basic species-specific defensive responses associated with fear (Davis and Whalen 2001).

Central to the circuit outlined above are anatomical connectivity findings in rodents and nonhuman primates, which differentiate the largely cortical connectivity pattern of the BLA from the largely subcortical connectivity pattern of the central nucleus or CMA (Amaral et al. 1992; Pitkanen 2000; Price et al. 1987). The anatomical connectivity of human amygdalar nuclei, however, is currently unknown. We recently examined the differential connectivity patterns of these amygdalar subregions in healthy subjects and patients with GAD to investigate the functional brain networks in which the amygdala is involved, and which underlie the distinct functions of these amygdalar subregions (Etkin et al. 2009). Indeed, we found that the two key subregions of the amygdala can be dissociated using conventional human fMRI data, and that their patterns of connectivity closely matches those found in anatomical tract-tracing studies in nonhuman primates.

The human amygdala is activated by negatively valenced emotional stimuli (Phan et al. 2002; Wager et al. 2003). Lesions of the amygdala are associated with an inability to accurately label fearful facial expressions (Adolphs et al. 1994), and an inability to encode fear-based memories (Bechara et al. 1995). Invasive stimulation of the human amygdala with microelectrodes produces subjective reports of fear and anxiety (Lanteaume et al. 2007). Beyond its activation by negative emotional stimuli, the amygdala also responds to anxiety-provoking environmental cues that are themselves neutral in valence. Herry et al. (2007) compared the effects of predictable and unpredictable sequences of neutral tones in mice and humans and found that amygdala activation was increased in both species in response to the unpredictable sequence. Unpredictable tone sequences were also associated with increased anxiety-related behavior in mice and an attentional bias in response to emotional facial expressions in humans.

Activation of the amygdala can even occur in response to emotional stimuli processed outside of awareness, or under very limited attentional resources. We have found, for example, that activation in a region consistent with the basolateral amygdala to unconsciously presented fearful faces can be detected in healthy volunteers in a manner that varies with their baseline anxiety, such that activation is greatest for the most anxious subjects (Etkin et al. 2004). Along with amygdala activation, we found that individual differences in baseline anxiety predicted subjects' performance in a concomitant cognitive task. Others have found a similar relationship between amygdala activity and anxiety when evaluating the emotional content of fearful faces in the context of limited attentional resources (Bishop et al. 2004). The amygdala, therefore, plays an important role in both the subjective and attentional-vigilance aspects of threat processing, and thus abnormalities in this system may be associated with hyperarousal and hypervigilance to threat in anxiety disorders. Moreover, these data also suggest that there may be a conceptual overlap between amygdala activation observed in response to negative emotional stimuli presented outside of awareness, which cannot therefore be extensively consciously evaluated, and amygdala activation by anxiety-producing unpredictable or ambiguous stimuli. This is particularly important, since many anxiety disorders are associated with intolerance of uncertainty or ambiguity (Boelen and Reijntjes 2008;

Grillon et al. 2008; Holaway et al. 2006) and negative interpretations of ambiguous material (Bishop 2007; Eysenck et al. 1991).

The insula, another of the core limbic regions, is heavily interconnected with the amygdala, hypothalamus, and PAG matter (Paxinos 2003). The insula regulates the autonomic nervous system (Oppenheimer et al. 1992), and like the amygdala is activated during the processing of a variety of negative emotions (Phan et al. 2002). Though the insula has received less intense study than the amygdala in the context of negative emotional processing, its important role is suggested by its more frequent association with activation in the amygdala than with activation in other cortical regions, thereby suggesting a high degree of functional similarity to the amygdala (Kober et al. 2008). Studies have found, for example, that the insula plays a particularly important role in the monitoring and interpretation of internal physical sensations. Its activation during a task in which subjects have to attend to their heartbeats, for example, was greater in individuals with greater interoceptive sensitivity and in those with higher levels of anxiety (Critchley et al. 2004). Similar to what has been noted for the amygdala, insular activation is greater in negative facial expression (e.g., disgust) than neutral expressions even in the context of limited attentional resources (Anderson et al. 2003).

The importance of both the amygdala and insula in anxiety-relevant negative emotion processes was investigated in a recent meta-analysis of human fear conditioning experiments (Etkin and Wager 2007). Current influential models of anxiety draw heavily on human and animal work on experimentally induced conditioned fear states, and enough of these studies have now been undertaken to allow for a quantitative meta-analysis of the results. Etkin and Wager examined ten well-controlled fear conditioning studies and found that consistent activation was seen, as predicted, in both the amygdala and insula, confirming a role for both of these regions in anxiety-relevant emotional processing. Finally, the PAG and hypothalamus are involved in the regulation of the autonomic nervous system, as well as characteristic species-specific motor response patterns, suggesting an important role for these regions in anxiety as well. Though the PAG and hypothalamus have not been the focus of studies on clinical anxiety, they likely play an important role by virtue of their normal functions in responses to negative emotional stimuli.

1.3 Medial PFC

Consistent cortical activations to emotional stimulation are found throughout the medial wall of the PFC, including the anterior cingulate cortex (ACC), as well as in parts of the lateral PFC. In the largest and most recent meta-analysis of neuroimaging studies of emotion, Kober et al. (2008) used multivariate clustering methods to discriminate between groups of coactivated regions. This data-driven approach identified two distinct medial prefrontal groups of activation clusters. The first group consisted of the presupplemental motor area in the posterior medial PFC, and was associated with coactivation in several lateral PFC structures. This pattern

of coactivation suggests that this group, labeled by the authors as "cognitive/motor" in function, is likely to reflect attentional or executive processes involved in emotion, but not specific to it. The second group was more anterior and consisted of the dorsal ACC, overlying regions of the dmPFC, and extended into the rostral (pregenual) ACC and portions of the subgenual ACC (together labeled the "medial prefrontal" group). Regions within this group consistently coactivated with the amygdala and PAG, suggesting an important role in emotion. Interestingly, the ventromedial PFC (including the medial orbitofrontal cortex), another region commonly implicated in the processing of emotion, did not appear in this meta-analysis. There may have been various reasons for this, including the frequent loss of signal in this region due to magnetic susceptibility artifacts in functional magnetic resonance imaging (fMRI) studies, or the limited contexts in which it is activated during emotion processing (e.g., only when certain types of emotion regulation are engaged, see below).

It has, however, become increasingly apparent that the large area of activation in the "medial prefrontal" group, along with the ventromedial PFC, can be functionally divided into meaningful subregions, though the precise roles of these subregions are not fully clear. One influential view, derived from studies of the ACC, holds that the dorsal regions are "cognitive" in function, in part through their connectivity with "cognitive" regions in the lateral PFC involved in cognition and executive functioning. Ventral regions, meanwhile, are argued to be "affective" in nature (Bush et al. 2000), in part through their connectivity with core limbic structures such as the amygdala. The meta-analysis results above, however, argue against this view, as both dorsal and ventral medial prefrontal regions appear to be similarly engaged in emotion processing. Indeed, while cognitive (i.e., nonemotional) processing appears to engage the dorsal more than the ventral regions of the medial PFC and ACC (Ridderinkhof et al. 2004), affective processing appears to be more widespread. Consistent activation is seen in the dorsal ACC, for example, during fear conditioning (Etkin and Wager 2007). An informative alternative view comes from studies of evaluation and regulation of emotion.

Converging work suggests that the dorsal ACC and overlying dorsomedial PFC are involved in appraising and monitoring emotion, and may be important for the conscious subjective experience of emotion. Kalisch et al. (2006a) induced anxiety in healthy volunteers by signaling that they may receive a shock and found that increased anxiety was associated with activation of the dorsomedial PFC. If subjects, however, also simultaneously carried out a challenging working memory test, activation in the dorsomedial PFC diminished, likely reflecting the role of this structure in higher level emotional appraisal, which can be interrupted when attentional load was high enough. Consistent with this, another recent meta-analysis found that dorsomedial PFC activation was more commonly noted in studies in which subjects were induced to experience emotion, rather than just perceiving it (e.g., through judgments about attributes of an image) (Wager et al. 2008).

Etkin et al. (2006) recently developed an emotional analog to the color-word Stroop task to test how individuals respond to and regulate the impact of emotional conflict. They showed subjects images of fearful or happy facial expressions and

asked them to identify the affect. Written across the faces were the words "fear" or "happy," which were either of the same affect (congruent) or of a different affect (incongruent) as the facial expression. As in the color-word Stroop task, subjects were to ignore the text but were unable to avoid involuntarily reading the word and extracting its meaning. The emotional meaning of the words thus led to direct conflict with interpretation of the facial affect. As a result, incongruent stimuli interfered with affect identification, and were associated with dorsomedial PFC activation.

During conflict tasks, however, the degree of behavioral interference by an incongruent trial varies in a predictable manner based on previous trial history. It has been found that there is less reaction time interference (i.e., less conflict) for incongruent trials if they are preceded by an incongruent trial than if they are preceded by a congruent trial – a phenomenon termed conflict adaptation (Botvinick et al. 1999; Egner and Hirsch 2005a, b; Gratton et al. 1992; Kerns et al. 2004). These findings suggest that the conflict generated by an immediately prior incongruent trial activates a regulatory mechanism, which leads to improved handling of conflict on the next trial (Botvinick et al. 2001). Incongruent trials can thus be separated depending on whether they are associated with high conflict regulation and consequently less conflict (an incongruent trial preceded by an incongruent trial) or low conflict regulation and thus more conflict (an incongruent trial preceded by a congruent trial). Neural activity in regions responsible for either generating or monitoring conflict should reflect the amount of behavioral conflict, resulting in higher activity for low conflict regulation than for high conflict regulation trials in these regions (i.e., low>high conflict regulation trials). By contrast, for brain regions implicated in conflict resolution, reduced conflict should be associated with increased neural activity (i.e., high>low conflict regulation trials).

Activation in the dorsomedial PFC decreased during high conflict regulation trials, confirming a role for this region in the monitoring and evaluation of negative emotion. Interestingly, a follow-up study, which compared the emotional conflict paradigm above to a matched nonemotional conflict task, found that activation in the nearby dorsal ACC was common to the monitoring or evaluation of both emotional and nonemotional conflict (Egner et al. 2008), suggesting that aspects of dorsomedial PFC function in emotion may be part of a greater role for this region in monitoring ongoing processing demands for the purpose of recruiting context-appropriate control processes. Along these lines, activation of the dorsomedial PFC or dorsal ACC is seen during interpretation of affective ambiguity (Simmons et al. 2006), and is similarly also engaged during interpretation of nonemotional ambiguity.

More ventral portions of the ACC however, show a different pattern of activation from the dorsomedial PFC and dorsal ACC, suggesting that while they may be part of the "medial prefrontal" group in the Kober et al. meta-analysis (Kober et al. 2008), they can be functionally dissociated from the more dorsal structures. Activation of the rostral ACC was associated with regulation of emotional conflict in the task of Etkin et al. 2006, described above. Activation of the rostral ACC was also accompanied by a simultaneous and correlated reduction in amygdala activity.

These results are consistent with a recent study of the extinction of conditioned fear responses, in which subjects evaluate and override expectations for aversive stimuli. Fear extinction involved increased activity in the rostral and subgenual ACC, and the ventromedial PFC, along with decreased activity in the amygdala (Phelps et al. 2004). Likewise, rostral ACC activation has also been observed during placebo anxiety reduction, a process in which control over an emotional stimulus (an aversive picture) is recruited to diminish the effect of the emotional stimulus (Petrovic et al. 2005). These data suggest that the ventromedial PFC and rostral/subgenual portions of the ACC may have an important role in the regulation of emotion, which is different from the evaluation and monitoring role of the dorsomedial PFC and dorsal ACC.

Beyond functionally differentiating between core limbic and medial prefrontal subregions, evidence indicates that these regions communicate with each other in a coherent and meaningful way. Emotional evaluation by the dorsomedial PFC and dorsal ACC is mediated through direct projections from core limbic regions (Ghashghaei et al. 2007) and indirectly through limbic-ventromedial prefrontal projections (Stein et al. 2007). Emotion generation or evaluation signals are in turn able to recruit emotional control processes. Emotional conflict-related activation during incongruent trials in the amygdala and dorsomedial PFC, for example, predicted conflict regulation-related activation in the rostral ACC on subsequent trials (Etkin et al. 2006). Activation by ventral ACC and ventromedial PFC regions in turn modulated activity in areas such as the amygdala, which are involved in generating aspects of the emotional response of the individual. This model of emotional reactivity and regulation is consistent as well with studies in the likely rodent homologs of these areas, which suggest that amygdala activity can be dampened by stimulation of the medial PFC due to activation of top-down inhibitory projections from the medial PFC to the amygdala (Quirk et al. 2003). Moreover, lesions of the medial PFC in rodents impair extinction of conditioned fear, leading to persistent fear responses in the absence of an aversive stimulus (Morgan et al. 1993). Drawing on the data presented above, Fig. 1 summarizes the flow of information processed within the limbic-medial prefrontal circuit.

1.4 Explicit and Implicit Emotion Regulation

Where do these data fit into the current understanding of emotion regulation circuitry? Gross (Gross 2002) has proposed a framework for classifying different emotion regulation strategies. One important distinction is between "antecedent-focused" strategies, which aim to alter emotional responses before they begin, and "response-focused" strategies, which suppress the expression of emotion. Antecedent-focused strategies include willful detachment, distraction, and cognitive reappraisal; response-focused strategies include voluntary suppression of positive or negative emotional reactions. Orthogonal with this framework is the idea that a given emotion regulation strategy may be "deliberate," requiring conscious top-down

intentionality, or may be "implicit," engaging top-down regulation of emotional processes without requiring conscious intentionality.

Several recent neuroimaging studies of the neural circuitry associated with deliberate efforts at emotion regulation (Beauregard et al. 2001; Kalisch et al. 2005; Kalisch et al. 2006b; Levesque et al. 2003; Ochsner et al. 2004) found that deliberate emotion regulation involves consistent activation of the dorsolateral PFC, an area associated with top-down cognitive control, regardless of whether an antecedent-focused or a response-focused strategy was being employed. These findings suggest that some of the same regulatory circuitry is involved in the cognitive control of emotion as in nonemotional forms of cognitive control. Consistent with this view, adaptation to nonemotional conflict, even if in the context of task-irrelevant emotional stimuli, involves activation of lateral prefrontal control mechanisms (Egner et al. 2008). This pattern of activation was dissociable from the one described above in which rostral/ventral ACC activation was associated with the regulation of emotional conflict, and dampening of amygdalar reactivity (Egner et al. 2008). Thus, it appears that the role in emotion regulation of the limbic-medial prefrontal circuit described above and depicted in Fig. 1 is different from the role of the dorsolateral PFC in deliberate, instructed forms of emotion regulation.

Insight about the nature of emotion regulation by the limbic-medial prefrontal circuit is suggested by the details of the tasks which trigger it, none of which involved a deliberate instruction to regulate emotion. It is highly unlikely, for example, that subjects in the emotional conflict task of Etkin et al. 2006 were aware of the effect of previous trial emotional incongruence on their response to an emotionally incongruent stimulus on the current trial. Thus, emotion regulation by the limbic-medial prefrontal circuit may represent an implicit and reflexive form of emotion regulation, dissociable both psychologically and neurobiologically from explicit and instructed strategies for emotion regulation. Thus, implicit emotion regulation may be based on an individual's expectation or anticipation of emotional stimuli, which may operate outside of their awareness, and be independent of an explicit goal of emotion regulation. This view would therefore predict that abnormalities in the circuitry mediating implicit emotional regulation may be demonstrated in psychiatric disorders with prominent emotional dysregulation, but in which no concurrent dorsolateral PFC deficits are observed.

Though the circuit in Fig. 1 describes the limbic-medial PFC circuit as recurrent, or feedback, in nature, it is likely that it is not entirely inhibitory with respect to effects on emotion generating limbic structures. In fact, there may be instances when increasing negative emotion, through positive ventral frontal-limbic interactions, is more contextually appropriate than decreasing it. One such example is a recent study in which subjects played a video game in which they were chased, and if captured would receive a shock to their hand (Mobbs et al. 2007). When the threat was relatively distal, it would seem appropriate to activate and enhance fear or threat responses in order to better shape behavior to avoid the threat. Consistent with this idea, this phase of the chase was associated with coactivation of the ventromedial PFC and basolateral region of the amygdala. Increased confidence of escape was associated with increased ventromedial PFC activation. By contrast,

when threat was near and defensive responses might be hypothesized to take precedence over fear-motivated avoidance responses, activation shifted to the dorsal region of the amygdala, where the central nucleus is located, and the PAG, an important brainstem target of the central nucleus. Likewise, engaging in contextually appropriate negative emotion-relevant behavior (shooting an aggressive assailant or healing a wounded bystander) resulted in greater activity in the ventromedial PFC, amygdala, and insula than matched contextually inappropriate behavior (shooting the bystander or healing the assailant)(King et al. 2006).

1.5 Hippocampus

Though the hippocampus is not commonly considered to be a central region in human emotional processing, apart from the relevance of its mnemonic role in emotional modulation of memory, a growing body of animal work implicates it in anxiety, and is thus worth a brief discussion. Studies in rodents differentiate between the function of the dorsal hippocampus, which is primarily involved with memory and other cognitive functions, and the function of the ventral hippocampus. Unlike the dorsal hippocampus, the ventral hippocampus is heavily interconnected with the amygdala and the hypothalamus (Bannerman et al. 2004). Lesions or inactivation of the ventral hippocampus results in a reduction of endogenous anxiety-like behavior in rodents, an effect that is not seen if similar manipulations are made of the dorsal hippocampus (Bannerman et al. 2004; Kjelstrup et al. 2002). In humans, a nonmnemonic role for the hippocampus in emotional processing has not clearly emerged, nor has its relevance for anxiety disorders been delineated. Thus, unlike limbic and medial prefrontal circuitry, the lack of a significant role for the hippocampus in human anxiety currently stands in contrast to the relative conservation of fear- and anxiety-related circuitries across phylogeny.

1.6 Summary

In the preceding sections I have outlined the neural circuitry central to the processing of negative emotional material in humans, focusing on the specific functions of each region and how information flows within the circuit. The focus on negative emotion and its regulation reflects an assumption that this material is of special relevance to anxiety, and also benefits from the broad wealth of neuroimaging and related studies in this area. As shown in Fig. 1, registration and reactivity to a negative emotional stimulus is carried out in the amygdala and insula, two core limbic structures. These regions can direct and modulate activity in a range of target regions, including the PAG, hypothalamus, hippocampus, and sensory cortex. Further monitoring and evaluation of the negative stimuli is carried out by the dorsal ACC and dorsomedial PFC, which are informed directly by core limbic

regions, and indirectly through projections from ventral frontal regions which receive innervation from core limbic regions. Engagement of these dorsal structures leads to a detailed appraisal of the emotional stimulus not possible by the amygdala and insula, and can involve conscious awareness of that emotional appraisal. Information on the stimulus is relayed to regulatory regions in the ventral ACC (rostral and subgenual) and ventromedial PFC, either through direct projections from core limbic regions, or through projections from dorsal ACC and dorsomedial PFC, which likely reflect different aspects of the emotional reaction to a stimulus. These regulatory regions in turn provide feedback onto core limbic areas, resulting in context-appropriate regulation, which may take the form of either inhibition or enhancement of limbic processing.

I have also emphasized that this limbic-medial prefrontal loop appears to result in the regulation of emotion in the absence of a specific goal for emotion regulation and likely outside of subjects' awareness. This model stands in contrast to the prevailing view of deliberate emotion regulation, wherein lateral prefrontal circuitry important in cognitive control in nonemotional contexts is thought to mediate the cognitive control of emotion. Since lateral prefrontal structures have little direct projections to core limbic regions, indications are that cognitive control of emotion by the lateral PFC is achieved by its engagement of medial prefrontal areas within the limbic-medial prefrontal loop described above (Johnstone et al. 2007; Urry et al. 2006). Thus, understanding the differences between implicit and explicit emotion regulation processes may be an important avenue for interpreting neural abnormalities in anxiety disorders and the mechanisms of their treatment.

While informative and helpful in understanding anxiety, the model above remains simplistic, and narrow in its focus. I have neglected other functions of some of these regions, including in reward (amygdala and ventromedial PFC), and in self-related processing (throughout the medial PFC). Basic research in these areas is generally not as well detailed as for negative emotional processing, and few investigations exist in these areas in anxiety disorders. Others have recently emphasized a unifying role for the medial PFC in functions such as reward or self-referential processing, with particular relevance to understanding the neural bases of anxiety disorders (Liberzon and Sripada 2008). Thus, understanding these aspects of anxiety will be a topic for future research.

1.7 Negative Emotional Processing in Anxiety Disorders: A Meta-Analytic Framework

The number of functional neuroimaging studies of negative emotion in clinical anxiety disorders have accumulated at a rapid pace, now reaching a point at which a quantitative meta-analytic review is feasible. Much of the clinical neuroimaging literature, particularly in its earlier periods, was carried out on small groups of subjects, with significant sample heterogeneity between studies and methodologies. This has led to inconsistencies of findings across studies, even for the brain regions,

which are most heavily hypothesized to be important for anxiety. One advantage of a meta-analysis is that it allows for a quantitative summary of the findings by accounting for across-study variability. In addition, robust meta-analytic findings can help define the regions of greatest interest and support specific hypotheses for future studies, such that these studies can approach their questions in the most direct and nuanced manner possible. Etkin and Wager (2007) have recently reported a meta-analysis of negative emotional processing in posttraumatic stress disorder (PTSD), social anxiety disorder (SAD), and specific phobia, and compared these findings to experimentally induced anxiety to discrete cues in healthy individuals through fear conditioning. These disorders were chosen as they were the only anxiety disorders for which a sufficient number of relatively homogeneous publications were available to allow for a reliable meta-analysis. I will review these findings and then highlight other relevant findings in each anxiety disorder, including those that were not part of the meta-analysis.

The studies included for each disorder were a combination of symptom-provocation studies, in which scripts, images, or sounds were used to specifically evoke disorder-specific anxiety symptoms, and studies using generally negative, but not disorder-specific, emotional stimuli. The latter were most often pictures of aversive scenes or emotional facial expressions. Common to all three anxiety disorders was consistent hyperactivation of the amygdala and insula in patients, compared to matched controls (see Fig. 2). A similar pattern of activation was noted during fear conditioning, suggesting that amygdala and insula hyperactivation in patients reflects excessive engagement of fear- or negative emotion-related circuitry. This finding is important because it identifies a core phenotype for at least these three

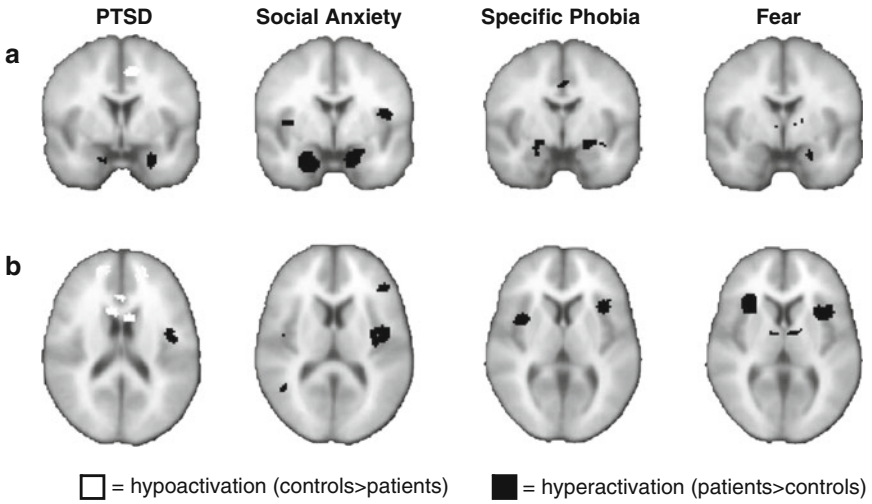

Fig. 2 Clusters in which significant hyperactivation or hypoactivation was found in patients with PTSD, Social Anxiety Disorder (SAD), and Specific Phobia relative to comparison subjects and in healthy subjects undergoing fear conditioning. Notable is common hyperactivation in the amygdala and insula. Adapted from Etkin et al. (2007)

anxiety disorders and supports an understanding of anxiety derived from animal fear conditioning studies. Moreover, it settles debate in the literature about whether, as hypothesized, amygdalar hyperactivity is a hallmark of at least some common forms of clinical anxiety. Finally, it brings the insula, which had not been a central part of previous neural circuitry conceptualizations of anxiety, into prominence alongside the amygdala.

In addition to the shared findings across disorders, there are also important differences between disorders. Most strikingly, for SAD and specific phobia, only clusters showing greater activity in patients compared to controls were noted. By contrast, for PTSD, there were large regions of both hyper- and hypoactivation in patients. Extensive hypoactivation was found in the dorsal ACC and dorsomedial PFC as well as in ventral portions of the ACC and the ventromedial PFC (see Fig. 3). These hypoactivations were significantly more common in PTSD than in the other two anxiety disorders. There was also a small cluster of hypoactivation in the dorsal part of the amygdala, spatially distinct from the more ventral cluster showing hyperactivity in patients. Interestingly, no differences between anxiety disorder patients and matched controls were found in lateral prefrontal regions involved in cognitive control. The results above also held true when only symptom provocation studies were used in the analysis.

Of the three disorders, PTSD is considered to be more severe, and has more diverse symptomatology. In addition to symptoms of hyperarousal and hypervigilance to trauma-related cues, and avoidance of trauma reminders, all of which may be consistent with a model of anxiety based on inappropriately exuberant fear conditioning, PTSD also presents with a range of symptoms reflecting generalized emotional dysregulation. The latter include emotional numbing, generalization of anxiety reactions to stimuli not closely related with the trauma, intrusive thoughts

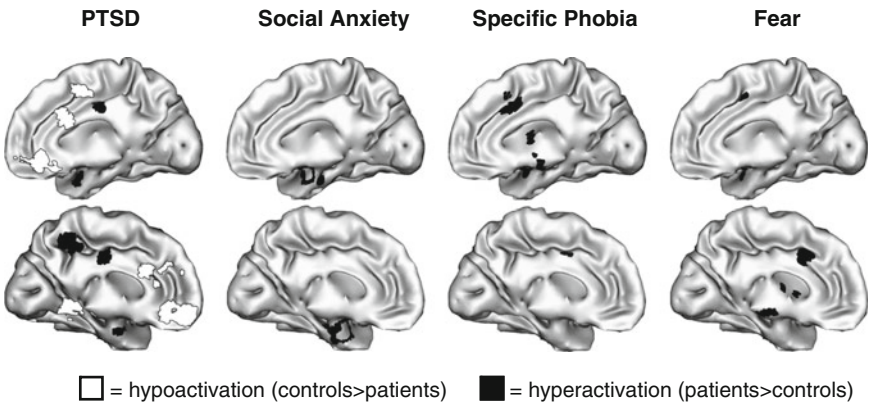

Fig. 3 Clusters in which significant hyperactivation or hypoactivation was found in patients with PTSD, SAD, and Specific Phobia relative to comparison subjects and in healthy subjects undergoing fear conditioning. Notable are PTSD-specific hypoactivations in the dorsal and ventral portions of the medial prefrontal cortex. Adapted from Etkin et al. (2007)

and memories, rumination, affective instability (e.g., anger outbursts), anhedonia, and a sense of negative foreboding (APA 1994).

In the light of the previous discussion on emotional processing and implicit regulation by the limbic-medial prefrontal circuit, Etkin and Wager (2007) proposed that the robust hypoactivation in the medial PFC in PTSD reflects a deficit in implicit emotion regulation occurring in the absence of deliberate attempts at emotional control. This neural abnormality would therefore be reflected clinically in symptoms of emotion dysregulation and anxiety generalization. In the framework provided by the information processing model for the limbic-medial prefrontal emotion circuit shown in Fig. 1, patients with PTSD appear to have dysfunction in both the dorsal monitoring/evaluation and the ventral regulation components.

Others have recently proposed that medial PFC deficits in PTSD reflect a core abnormality in extinction of learned fear (Milad et al. 2006; Rauch et al. 2006). It would seem, however, that dysfunction of a more general implicit evaluation and regulation system for negative emotional stimuli by the medial PFC would more readily explain the range of emotion dysregulation symptoms in PTSD, and may subsume within it a deficit in fear extinction. Much work, however, needs to be done in this area before any firm conclusions can be drawn. In particular, theoretical and experimental attention must also be paid to the distinction between dorsal medial frontal regions involved in the monitoring, evaluation, or experiencing of emotion, and ventral regions involved in emotion regulation, as this distinction is growing clearer in the basic science literature.

An important role for the medial PFC in PTSD is supported as well by results from structural imaging studies. A number of these studies have reported decreased gray matter volumes in patients with PTSD in both the dorsal and ventral portions of the ACC or medial PFC (Karl et al. 2006; Kasai et al. 2008; Yamasue et al. 2003). One study examined variation in ACC volume as a function of trauma exposure or a putative genetic vulnerability for PTSD, assessed by comparing identical twin pairs discordant for combat exposure or PTSD, and found that decreased ACC volumes reflected the presence of PTSD symptoms rather than simply exposure to trauma without resulting symptoms or a genetic vulnerability to PTSD (Kasai et al. 2008). Interestingly, studies in rodents suggest that exposure to an uncontrollable stressor, which may reflect some aspects of the effects of traumas on patients with PTSD, resulted in dendritic retraction in a rodent analog of human medial PFC, potentiated fear conditioning, and interfered with fear extinction (Amat et al. 2005; Izquierdo et al. 2006). Thus, medial prefrontal dysfunction may be an important factor maintaining the symptoms of PTSD, and may to some degree have resulted from long-lasting effects of the trauma on basic aspects of medial prefrontal neuronal architecture.

1.8 Generalization of Anxiety Beyond Disorder-Related Material: PTSD and Specific Phobia

As discussed above, functional neuroimaging studies of anxiety have employed both disorder-specific and generally negative stimuli. These experiments afford an

opportunity to determine the extent to which specific types of anxiety manifest through abnormal responses to any negatively valenced stimulus, or whether a response is only elicited to disorder-specific stimuli. For example, presentation of faces with different expressions can trigger limbic system activation in healthy subjects, and can thus be used as a probe for emotional processing in disorders where abnormal social signaling is not a central feature (i.e., not SAD, in which face stimuli are disorder-specific symptom triggers). Patients with PTSD displayed the characteristic pattern of medial prefrontal hypoactivity when viewing fearful compared to neutral or happy faces (Shin et al. 2005; Williams et al. 2006), counting emotionally negative compared to neutral words (Shin et al. 2001), recalling anxious or sad autobiographical events using script-guided imagery (Lanius et al. 2003) or viewing pictures of aversive compared to neutral visual scenes (Phan et al. 2006). Viewing fearful expression faces likewise also resulted in amygdalar hyperactivity in PTSD (Shin et al. 2005). Patients with specific phobia, meanwhile, showed similar amygdalar responses to emotional faces as controls (Wright et al. 2003). These data suggest that dysregulation within the limbic-medial prefrontal circuit during the processing of disorder nonspecific negative stimuli may be characteristic of states of generalized emotional dysregulation, such as those seen in PTSD, and does not merely reflect the presence of anxiety *per se*. Comparable experiments, however, have not yet been reported in other anxiety disorders, and will be important for further testing of this hypothesis.

It is now also clear, based on a number of imaging studies in healthy subjects, that understanding disorder-related alterations in amygdalar functioning requires separate analysis of emotional processing within and outside of awareness. Several recent studies have shown that elevated generalized anxiety (e.g., trait anxiety) in nonpsychiatric populations is associated with exaggerated amygdalar activation, most sensitively detected when emotional stimuli are processed outside of awareness or in the presence of limited attentional resources (Bishop et al. 2004; Etkin et al. 2004). In PTSD, fearful faces can activate the amygdala even when processed outside of awareness (Bryant et al. 2008b; Rauch et al. 2000). While similar manipulations of attention or awareness have not been reported in other anxiety disorders, this type of approach will be useful to probe the level at which vigilance or hypersensitivity to threat is already evident in each anxiety disorder. By extension, this information will also be useful in understanding the interplay between emotional reactivity and regulation processes in shaping symptomatology.

2 Generalized Anxiety Disorder

Despite its prevalence, generalized anxiety disorder (GAD) has not been a focus of neuroimaging studies on anxiety, and thus very little is known about the neural abnormalities associated with it. The data discussed above can establish several hypotheses about the neurobiology of GAD, and a handful of studies have recently emerged which can begin to address these hypotheses. Several recent GAD studies

have used fearful expression faces to probe amygdalar activity, and found that consciously presented fearful faces do not result in amygdalar hyperactivation in GAD patients (Blair et al. 2008; Whalen et al. 2007). In fact, in one study, angry faces were even associated with amygdalar hypoactivation in patients (Blair et al. 2008). Of note, in one of these studies, a cohort of subjects with SAD were scanned using the same task and, as expected, showed exaggerated amygdalar responses to fearful faces, providing a positive control (Blair et al. 2008).

While no study has as yet been reported using unconsciously presented threat in adults with GAD, one such study has recently been published in children and adolescents (Monk et al. 2008). In this study, subjects were presented with fearful face stimuli presented only outside of awareness, and thus a direct comparison of conscious to unconscious threat processing was not possible. As predicted, however, patients with pediatric GAD showed exaggerated amygdalar responses to unconsciously processed disorder nonspecific threat (Monk et al. 2008). It will be important in future work to extend this paradigm to adults and to directly compare neural responses to conscious and unconscious threat.

We recently found evidence of the involvement of the amygdala in several ways in adult GAD (Etkin et al. 2009), by analyzing the differential connectivity of the BLA and CMA, as described earlier. This analysis revealed an intra-amygdalar abnormality at the subregional level in GAD, which was accompanied by abnormal amygdalar coupling with several brain networks.

Finally, Etkin et al. 2008 have recently reported preliminary data on GAD using the emotional conflict task described above. They found that patients with GAD failed to regulate emotional conflict, and as a consequence displayed exaggerated responses to conflict. At a neural level, patients failed to activate the ventral ACC and, failed to modulate the dorsomedial PFC. If these results hold up for a larger group, they would suggest that the similar dorsal monitoring/evaluation and ventral regulation PFC deficits seen in GAD and PTSD reflect a generalized dysfunction in implicit emotion regulation. Indeed, an essential part of the DSM-IV criteria for GAD is difficulty controlling worry (APA 1994), suggesting that dysfunction in general emotion regulation systems may be central to this disorder. There is also considerable controversy regarding the nature of GAD: whether it reflects an extreme temperament or a distinct psychiatric condition, and what its relationship with major depression is, given their high degree of comorbidity. Neuroimaging studies of GAD will be in an important position to address these issues.

3 Panic Disorder

Very little is currently known about the neurobiology of panic disorder, in part because neuroanatomical models of panic disorder have been in flux, and in part due to a lack of sufficient neuroimaging data. Earlier models have emphasized abnormal brainstem responsiveness to carbon dioxide, termed the “false suffocation

alarm" (Klein 1993). Subsequent models have employed the fear conditioning paradigm just as other anxiety disorders like PTSD have (Gorman et al. 2000). Spontaneous panic attacks have been hypothesized to arise from overly sensitive fear circuitry that either inappropriately reacts to minor stimuli, or cannot restrain minor anxiety responses, which then develop into severe panic attacks (Gorman et al. 2000). Evidence of this and related hypotheses is largely lacking, though a recent imaging study of a single subject who had a spontaneous panic attack during fMRI scanning reported increased amygdala activity during the attack (Pfleiderer et al. 2007). Previous studies have induced panic attacks through pharmacological means during PET scanning, producing mixed results. These studies are also difficult to interpret in the context of global cerebral vascular perfusion changes during a panic attack, which can confound neural activity-related regional blood flow changes.

More conceptually clear is the phobic avoidance of reminders and possible triggers or exacerbating stimuli for panic attacks, which may be more readily understood within the context of a traditional fear conditioning model, though this too remains largely theoretical. The generalization of fear and anxiety beyond specific stimuli in panic disorder suggests some similarity to PTSD and GAD. A medial prefrontal deficit may therefore account for the persistence of free-floating anxiety and emotional dysregulation in these disorders, which is not seen in SAD or specific phobia – disorders in which medial prefrontal function appears to be largely intact.

4 Obsessive-Compulsive Disorder

Unlike the anxiety disorders discussed above, obsessive-compulsive disorder (OCD) appears to have a distinct neural basis. In fact, many features of OCD differ from those of the other anxiety disorders, suggesting that it may no longer be part of the anxiety disorder category in revisions for DSM-V (Hollander et al. 2008). While amygdala activation has been reported in OCD (van den Heuvel et al. 2005), this appears to be the exception, rather than the rule. More prominently, abnormalities have been identified in cortico-striato-thalamic-cortical circuits that mediate motor planning and learning, as well as habits, but also play a role in a variety of cognitive and affective functions. The striatum receives input from a wide range of cortical regions, and projects to the thalamus, which gates neurotransmission back to the cortex. A particular focus for OCD has been on the lateral orbitofrontal cortex, anterior cingulate, and caudate nucleus. Abnormalities in the orbitofrontal cortex and ACC have been proposed to relate to obsessions in OCD, while striatal abnormalities have been suggested to lead to stereotyped or ritualistic behavior (Graybiel and Rauch 2000; Menzies et al. 2008; Saxena and Rauch 2000). Early resting metabolism studies demonstrated increased activity in the orbitofrontal cortex and caudate, an effect that has also been observed in symptom provocation studies (Menzies et al. 2008). A recent meta-analysis of performance of patients with OCD on a wide range of fMRI tasks found support for elevated orbitofrontal

and caudate activity in OCD, but also found involvement of several other regions in the ACC, medial and lateral PFC, as well as regions of striatal hypoactivation (Menzies et al. 2008). Neurosurgical interventions for severe OCD also reflect some of the regions consistently identified as abnormally engaged in imaging studies of OCD, with promising results reported for dorsal ACC lesions and deep brain stimulation in the striatum (Aouizerate et al. 2004; Dougherty et al. 2002). Abnormalities in OCD, therefore, do not readily fit into the limbic-medial PFC emotional processing circuit described above, further supporting its differentiation from the other anxiety disorders.

5 Treatment Studies

Compared to depression, relatively few neuroimaging-coupled intervention studies have been reported for each anxiety disorder. Of these, most are difficult to interpret because of an absence of important controls. Nonetheless, there are several suggestive studies that open the way to increasingly better designed and more sophisticated approaches. In one such study, Furmark et al. (2002) examined patients with SAD treated with either citalopram or cognitive-behavioral therapy (CBT), measuring brain activity in response to having to give a prepared speech in the scanner while in the presence of others – a potent symptom provocation paradigm (Tillfors et al. 2001). Improvement in symptoms with treatment was accompanied by decreased activity in the amygdala and the medial temporal lobe. No such changes were seen in waiting-list control subjects. Comparing treatment groups with a control group of waiting-list patients who received no treatment allowed the authors to rule out changes related only to subject rescanning or simply to the passage of time. Decreases in the activity of the amygdala were seen in both the CBT and the citalopram groups, supporting an important role for this region in the symptoms of SAD. The two treatment groups, however, differed with respect to neural changes outside the amygdala, though interpretation of these findings is hampered by the very small sample sizes (six subjects per group). Interestingly, the degree to which amygdala activity decreased as a result of therapy predicted patients' reduction in symptoms 1 year later. Along similar lines, though using resting brain metabolic imaging, Baxter et al. (1992) noted normalization of caudate hyperactivity in OCD after treatment with either fluoxetine or CBT (nine subjects per group).

Finally, Straube et al. (2006)examined subjects with spider phobia, and compared the effects of symptom provocation in a group randomized to receive brief, intensive CBT (two 4–5 h sessions) to a wait list control group. At baseline, spider phobics hyperactivated the insula and dorsal ACC in response to video clips of spiders. After treatment, the CBT group no longer showed these abnormalities, but they persisted in the wait list control group. Together, these studies demonstrate that the neural abnormalities associated with symptomatology in anxiety disorders (e.g., amygdala and insula hyperactivation) are corrected after successful clinical interventions. Much, however, remains unclear, including a more thorough understanding of which neural

abnormalities persist after treatment, whether they reflect trait or vulnerability markers, and by what neurobiological mechanisms treatment-related change comes about. It is likely that an understanding of the circuits mediating emotional reactivity and regulation, as outlined above, will be useful in this respect as well. It is interesting in this regard that another study of CBT for spider phobia noted an increase in ventromedial prefrontal activation during symptom provocation after therapy, but not in a wait list control group (Schienle et al. 2007).

Another important aspect of understanding the mechanisms of treatments for anxiety is an appreciation of which subjects are most likely to respond to treatment, whether they respond differentially to various treatments, and why. To this end, two studies have reported results of correlations of pretreatment brain activation during emotional processing with treatment outcome in two anxiety disorders. Whalen et al. (2007) reported that increased rostral ACC and decreased amygdala activation to fearful faces at baseline predicts a better response to venlafaxine. Meanwhile, Bryant et al. (2008a) reported that increased activation in both the rostral ACC and amygdala in response to unconsciously presented fearful faces at baseline was predictive of a favorable response to CBT. While these results are preliminary and have not yet been replicated, they raise several interesting possibilities. First, the same brain region (e.g., amygdala) may differentially predict treatment outcome, depending on either the diagnosis or treatment strategy. Second, a common brain region (e.g., rostral ACC) may be broadly predictive of the likelihood of a patient to respond to any treatment. Indeed, treatment outcome prediction studies in depression have consistently and similarly implicated the rostral ACC, across different treatments and imaging modalities (reviewed in Etkin et al. (2005)). Even more intriguing, these data suggest that individual differences in the aspect of implicit emotion regulation mediated by the medial PFC may be the ultimate predictor of treatment response, across varied treatments and disorders.

6 Conclusion

In this chapter I have outlined a limbic-medial prefrontal neural circuit involved in the reactivity to and regulation of negative emotional stimuli. Figure 1 presents an information flow conceptual model of this circuit in which it is argued that a set of core limbic regions (amygdala, insula, hypothalamus, PAG) perform the initial evaluation of an emotional stimulus, and are critical for generating a coordinated physiological and subjective response to that stimulus indicative of emotional activation. Information about the emotional stimulus is conveyed to ventral prefrontal regions including the rostral ACC and ventromedial PFC, as well as to dorsal prefrontal regions including the dorsal ACC and dorsomedial PFC. The latter structures may get information about the emotional nature of the stimulus either through the direct influence of limbic regions or indirectly through projections from ventral prefrontal structures. Activation of the dorsal ACC and dorsomedial PFC enables more extensive evaluation of the emotional stimulus, and may gate the

access of that information into conscious awareness. Recruitment of the rostral ACC and ventromedial PFC either through the direct influence of limbic regions or through information flow from the dorsal ACC or dorsomedial PFC then results in context-appropriate feedback regulation of limbic structures involved in the emotional response. This feedback may take the form of inhibition or activation, depending on the context and goal of the organism with regard to the emotional stimulus.

As described, the limbic-medial prefrontal circuit appears to play an important role in emotion regulation occurring in the absence of deliberate efforts at regulation, thus implying that it underlies certain forms of "implicit" emotion regulation. By contrast, explicit emotion regulation appears to consistently involve activation of lateral prefrontal structures important in nonemotional forms of cognitive control. Deliberate emotion regulation, however, may be achieved through access to the medial PFC by lateral prefrontal structures.

Strikingly, abnormalities in clinical anxiety disorders are frequently and consistently noted within elements of the limbic-medial prefrontal circuit. Across most or all anxiety disorders, with the exception of OCD, which involves alterations outside of the limbic-medial prefrontal circuit, patients have been found to activate the amygdala and/or insula more than controls (see summary in Fig. 4). The apparent overlap between these effects and activation in healthy subjects of the amygdala and insula during the processing of negative emotional stimuli, including during

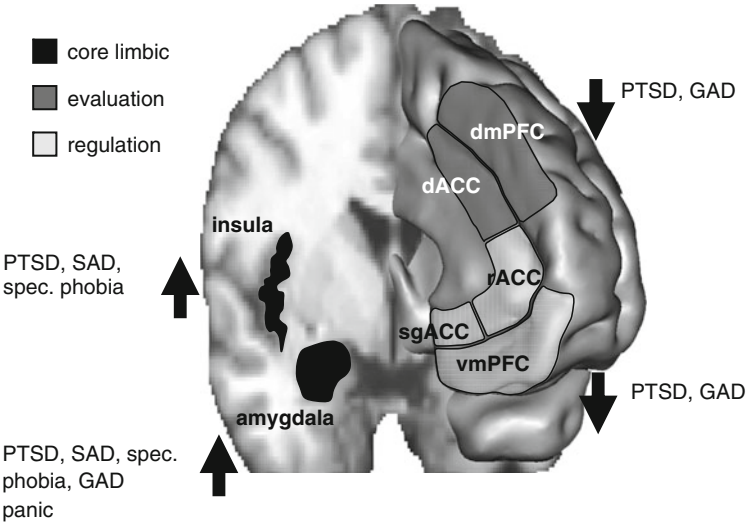

Fig. 4 Summary of functional alterations within nodes of the limbic-medial prefrontal circuit outlined in Fig. 1 for several anxiety disorders (PTSD, SAD), panic disorder, generalized anxiety disorder (GAD), and specific phobia). While all of these disorders appear to have in common abnormalities in the amygdala or the insula, only those in which anxiety is generalized and emotion is more widely dysregulated (PTSD and GAD), have prominent dysfunction in both medial prefrontal nodes of the circuit

fear learning, suggests that hyperactivation of these structures in anxiety disorder patients is related to symptoms of hyperarousal and hypervigilance – symptoms shared across anxiety disorders.

Disorders in which generalization of anxiety or profound dysregulation of emotion are prominent, however, appear to additionally be associated with hypoactivation in both the dorsal and ventral regions of the medial PFC (see Fig. 4). An understanding of how dorsal medial prefrontal structures evaluate emotional stimuli and recruit emotion regulation mechanisms in the ventral medial PFC is thus essential to better comprehend the neural bases of the generalized symptoms of PTSD and GAD. Moreover, preliminary indications are that the response of anxious individuals to treatment is positively predicted by pretreatment levels of activity in the medial PFC. These extremely preliminary findings suggest that some of the same emotion evaluation and regulation mechanisms that may underlie aspects of emotion dysregulation symptomatology in PTSD, and GAD may also determine the capacity of a patient to benefit from treatment.

The data discussed in this chapter, along with the information flow circuit model proposed, are intended to provide an integrated account of the functional neuroanatomy of normal negative emotional processing and its dysfunction in clinical anxiety disorders. While it is clear that certain domains have been well investigated and the literature now allows for the creation of specific neuroanatomical hypotheses for future experiments, it is also readily apparent that a great deal of work is needed in several of these disorders and in the study of treatment interventions. Importantly, the limbic-medial prefrontal neural circuit perspective outlined in this chapter may allow for exciting future directions in the development of novel therapeutics, identification of genetic or environmental vulnerability factors through the use of an endophenotype approach, improvements in diagnosis and disorder classification, and in the prognostication and tracking of the success of treatment.

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Pharmacological Enhancement of Behavioral Therapy: Focus on Posttraumatic Stress Disorder

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Contents

Abstract Improved efficacy in the treatment of posttraumatic stress disorder (PTSD) and other anxiety disorders is urgently needed. Traditional anxiety treatments of hypnosis and psychodynamic therapy may be of some help, but uncontrolled studies lead to inconclusive results on the efficacy of these treatment techniques. There is a larger literature supporting the efficacy of cognitive-behavioral procedures with PTSD, including prolonged exposure therapy, eye movement desensitization and reprocessing, and anxiety management techniques. The cutting-edge technology of virtual reality-based exposure therapy for PTSD is particularly exciting. To further build on effective psychosocial treatments, current pharmacological augmentation

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approaches to emotional learning are being combined with psychotherapy. In particular, D-cycloserine, a partial NMDA agonist, has shown to be effective in facilitating the exposure/extinction therapy to improve the efficacy of treating anxiety disorders, and may guide the way for new pharmacological enhancements of behavioral therapy.

Keywords Anxiety · D-cycloserine · Fear · Psychotherapy · PTSD

Abbreviations

AMT	Anxiety management training
CBGT	Cognitive-behavioral group therapy
CBT	Cognitive-behavioral therapy
CS	Conditioned stimulus
DCS	D-cycloserine
EMDR	Eye movement desensitization and reprocessing
ERP	Exposure and ritual prevention
OCD	Obsessive-compulsive disorder
PCT	Present-centered therapy
PE	Prolonged imaginal exposure
PTSD	Posttraumatic stress disorder
SAD	Social anxiety disorder
SD	Systematic desensitization
SIT	Stress inoculation training
SSRI	Selective serotonin reuptake inhibitor
SUDS	Subjective units of discomfort
TBI	Traumatic brain injury
TF-CBT	Trauma-focused cognitive-behavioral therapy
UCS	Unconditioned stimulus
VRE	Virtual reality exposure

1 Introduction

A large percentage of war veterans who are returning each year from the Middle East are being diagnosed with posttraumatic stress disorder (PTSD) and other various symptoms of depression, stress, and anxiety-related disorders. This places an utmost importance on advancing the efficacy of treatments for PTSD and anxiety disorders. Impressive advances in treating PTSD have been made in the past decade. Notably the Institute of Medicine (2008) only confirmed the efficacy for

exposure-based psychotherapy treatment for PTSD, while finding that the current data are inadequate to determine the efficacy of medication treatment or any other intervention for PTSD (Institutes of Medicine 2008). Currently, neuroscience research which underlies the psychotherapy for treating fear-based disorders is rapidly progressing. It is now being translated to the clinic in the form of pharmacological augmentation of emotional learning. This chapter covers the literature on psychosocial interventions for PTSD and other anxiety-related disorders, beginning with a brief review of traditional therapies. We then examine the larger literature on the efficacy of cognitive-behavioral procedures with PTSD, along with cutting-edge technology of virtual reality-based treatment for PTSD. Finally, we examine the current progress of pharmacological augmentation of emotional learning combined with psychotherapy to improve on the efficacy of treating anxiety disorders.

2 Traditional Hypnotherapy and Psychodynamic Treatments

Hypnosis has been advocated in the treatment of trauma since it was introduced by Freud to attain the abreaction and catharsis he deemed necessary to resolve a psychic conflict (reviewed in Spiegel 1989) and continues to be used to treat trauma survivors. Spiegel noted that hypnosis may be useful in treating PTSD because hypnotic phenomena such as dissociation are common in coping with trauma as it occurs and in its sequelae. Hypnosis may facilitate the recall of traumatic events that were encoded in a dissociative state and are therefore not available to conscious recollection.

A number of case studies have reported that hypnosis was useful in treating posttrauma disturbances following a variety of traumas, but most of these lack methodological rigor and thus cannot allow strong conclusions to be drawn. In the one controlled study (Brom et al. 1989) of 112 trauma victims, results indicated that participants were more improved than those in the waiting list control condition. The results suggest that hypnotherapy, as well as desensitization and psychodynamic therapy, may offer some help for posttrauma suffering. Overall, the Institutes of Medicine (2008) and the Cochrane Database review (Bisson and Andrew 2007) found that hypnosis treatment is inconclusive regarding the efficacy for treating PTSD.

Treatment by dynamic psychotherapy has often been advocated as a final component of crisis intervention (Burgess and Holmstrom 1974; Evans 1978; Fox and Scherl 1972). However, empirical investigations of its efficacy are scarce, and those that do exist are not usually well controlled. In an attempt to account for posttrauma reactions, psychodynamic theorists (e.g., Horowitz 1976) emphasize concepts such as denial, abreaction, catharsis, and stages of recovery from trauma. The target of Horowitz's brief psychodynamic therapy is the resolution of intrapsychic conflict arising from a traumatic experience, rather than specific symptom reduction. Other psychodynamic theorists focus largely on group process (Yalom 1995). In summary, most studies of hypnotherapy and psychodynamic therapy have been lacking in controls and assessments, and technically flawed in description and design of the treatment. Similar to the data with hypnotherapy, the Institutes of Medicine (2008)

and the Cochrane Database review (Bisson and Andrew 2007) found that hypnosis and psychodynamic psychotherapy approaches to PTSD remain understudied, and the efficacy of psychodynamic psychotherapy remains inconclusive. Thus, the information about the efficacy of traditional interventions with PTSD from these studies is quite limited and is open to various interpretations.

3 Cognitive-Behavioral Therapy

Cognitive-behavioral therapy (CBT) includes a variety of treatment programs, including exposure procedures, cognitive restructuring procedures, anxiety management programs, and their combinations. Reviews of the extant literature on the treatment of PTSD are quite positive regarding CBT (Bisson and Andrew 2007; Bradley et al. 2005; Institutes of Medicine 2008; Rothbaum et al. 2000; Solomon et al. 1992; Van Etten and Taylor 1998). A meta-analysis found the largest treatment effects for cognitive-behavioral techniques and selective serotonin reuptake inhibitor (SSRI) medications (Van Etten and Taylor 1998). Combined treatment approaches with medication and CBT together will be discussed below. One form of CBT employed with PTSD sufferers is exposure treatment, which assists patients in confronting their feared memories and situations. The exposure treatment that has been developed by Foa and Rothbaum (1998) typically incorporates imaginal exposure that makes the patient recall the traumatic memories to relive them in their imagination in the therapist's office in order for the trauma to be emotionally processed, or digested, so that it can become less painful over repeated sessions (Foa et al. 1989; Foa and Kozak 1986). Other forms of exposure involve repeatedly confronting realistically safe situations, places, or objects that are reminders of the trauma (called in vivo, or in real life, exposure) until they no longer elicit such strong emotions. Some therapists have patients write repeatedly about the trauma as a form of exposure (Resick and Schnicke 1993).

Another CBT approach, anxiety management training (AMT), involves teaching patients skills to control their anxiety, and has also been helpful with PTSD. Stress inoculation training (SIT), the AMT program that has received the most attention, was developed for victims who remained highly fearful 3 months after being raped (Veronen and Kilpatrick 1983). SIT typically consists of education and training of coping skills. These skills include deep muscle relaxation training, breathing control, role-playing, covert modeling, thought-stopping, and guided self-dialog following SIT. Sufferers of PTSD experience a great deal of anxiety in their lives because they are frequently reminded of the trauma. SIT aims to teach skills to help decrease this anxiety in many different situations.

3.1 Exposure Therapy

Exposure treatment for PTSD was first demonstrated with several case reports on war veterans (Fairbank et al. 1983; Johnson et al. 1982; Keane and Kaloupek 1982),

where both imaginal flooding (repeated presentation of traumatic event to the imagination) (Keane et al. 1989) and flooding in vivo to trauma related events (Johnson et al. 1982) appeared to be efficacious. There are several published reports of successful treatment of PTSD in veterans with exposure therapy. Three controlled studies have examined the utility of prolonged imaginal exposure (PE) for reducing PTSD and related pathology in Vietnam veterans. Treatment was conducted over 6–16 sessions. In one study, all patients received the "standard" PTSD treatment (weekly individual and group therapies) in addition to exposure (Cooper and Clum 1989). In the second study (Keane et al. 1989), PE was compared to a waiting list control group. During each session, patients were initially instructed to relax. The patients subsequently received 45 min of imaginal flooding, followed by relaxation. In the third study, all patients received a group treatment milieu program; one-half received additional PE and the remaining patients received weekly individual traditional psychotherapy (Boudewyns and Hyer 1990; Boudewyns et al. 1990). All three studies found some benefit from the PE compared to the control groups, but the effects were small. In the largest and best controlled study to date of exposure therapy with veterans (Schnurr et al. 2007), 277 female veterans and active duty personnel ($n = 7$) with PTSD were randomly assigned to receive 10 weekly sessions of present-centered therapy (PCT), which provides supportive intervention addressing current/daily problems in the present manifested with PTSD, or PE therapy. Women who received PE experienced greater reduction in PTSD symptoms, were more likely to no longer meet PTSD criteria and to achieve total remission than those who received PCT. It is also notable that PE was delivered by Veterans Administration therapists, not CBT experts.

Exposure therapy has not been as consistently effective when delivered in a group format in a VA setting. In a large VA cooperative study of male Vietnam veterans ($n = 360$), trauma-focused group psychotherapy was not significantly more effective than a present-centered comparison group treatment (Schnurr et al. 2003). However, recent uncontrolled pilot trials at the Atlanta VA of exposure therapy delivered in a combination of group and individual sessions have been more effective. An open trial of 102 veterans treated with Group Based Exposure Therapy found clinically significant and lasting reductions in the symptoms of war-related PTSD with large effect sizes on treating clinicians' assessments and moderate to large effect sizes on self-report PTSD scales (Ready et al. 2008). Another pilot study with 37 participants using a hybrid treatment model combining individually recorded audiotapes of three traumatic memories and daily exposure practice nested in a 10-week behavioral group therapy format has been effective in treating OEF/OIF and Persian Gulf combat veterans. There were no significant differences between those with (30%) and without traumatic brain injury (TBI) on pretreatment measures; nor was TBI status a predictor of significantly different treatment response (Crowe et al. 2008).

The first controlled study of the treatment of PTSD in rape victims randomly assigned PTSD rape victims to one of four conditions: SIT, prolonged imaginal exposure (PE), supportive counseling, or waiting list control. All conditions produced improvement on all measures immediately post treatment and at follow-up. SIT

produced significantly more improvement on PTSD symptoms than waiting list victim controls immediately following treatment. At follow-up, PE produced superior outcome on PTSD symptoms. Clients who received PE continued to improve after treatment termination, whereas clients in the SIT and supportive counseling conditions evidenced no change between post treatment and follow-up (Foa et al. 1991).

Another study compared PE, SIT, the combination of SIT and PE, and a waiting list control group (Foa et al. 1999). All three active treatments showed significant improvement in PTSD symptoms and depressive symptoms at posttest, and the waiting list group did not improve. These treatment effects were maintained at 6-month follow-up. Overall, combined SIT and PE treatment was not better than individual treatments. Versions of the PE program have been helpful in preventing the development of chronic PTSD following rape (Foa et al. 1995) and in treating PTSD in abused children (Deblinger et al. 1990).

Additional studies provide support for the efficacy of exposure treatment for PTSD in samples that are heterogeneous with regard to their traumas. Exposure therapy was compared to cognitive therapy in a mixed sample of trauma survivors (Tarrier et al. 1999). Type of trauma included crime (52%), accident (34%), and other (15%). There was a significant improvement on all measures post treatment, which was maintained at follow-up for both treatments, with no significant differences between the two treatments. Richards et al. (1994) treated 14 participants with PTSD with either four sessions of imaginal exposure followed by four sessions of in vivo exposure or in vivo followed by imaginal exposure. Patients in both treatment conditions improved considerably.

Note that the most recent large meta-analytic reviews have concluded that among all the types of psychosocial therapies, the best and largest randomized controlled trials exist for exposure-based models. The recent Cochrane Database review concluded that both individual and group trauma-focused CBT (TF-CBT)/exposure therapy were effective in the treatment of PTSD (Bisson and Andrew 2007). Additionally, the Institute of Medicine concluded that evidence is sufficient to conclude the efficacy of exposure therapies in the treatment of PTSD (Institutes of Medicine 2008).

3.2 Virtual Reality Exposure Therapy

A new medium for conducting exposure therapy has been introduced recently. Virtual reality exposure (VRE) presents the user with a computer-generated view of a virtual world that changes in a natural way with head motion. During VRE sessions patients wear the head-mounted display with stereo earphones that provide visual and audio cues consistent with being in the virtual environment that is used to reactivate their traumatic cues.

For treatment of PTSD, Vietnam veterans in one investigation were exposed to two virtual environments, a virtual helicopter flying over Vietnam and a clearing surrounded by a jungle, allowing patients to be repeatedly exposed to their most

traumatic memories but immersed in Vietnam stimuli. In this open clinical trial, scores on all measures decreased from pre- to post treatment, with significant decreases in all three symptom clusters (Rothbaum et al. 2001). A recent study by Difede and colleagues examined VRE for the treatment of PTSD following the September 11, 2001 attacks on the World Trade Center in New York (Difede et al. 2007). This pilot study examined 13 VRE treatment subjects compared to 8 waitlist control subjects and determined that the VRE group showed a significant decline on CAPS scores compared to the waitlist.

The results from these early studies consistently support the efficacy of imaginal and in vivo exposure for the treatment of PTSD resulting from a variety of traumas. These results are even more impressive given the methodological precision that was applied to many of these studies. Notably, there are now very good VRE environments for the Iraq war, the Vietnam war, the World Trade Center bombings, Terrorist attacks in Israel, and a variety of other nontrauma-based environments. However, despite the fact that almost 100 papers discussing VRE therapy for anxiety-related disorders are now in the literature, many are not optimally designed (Parsons and Rizzo 2007). Additional randomized clinical controlled trials and trials of increased size will likely lead to exciting new uses and validation for virtual reality approaches in the future.

3.3 Anxiety Management Techniques

The efficacy of SIT, (Kilpatrick et al. 1982) developed for rape victims with chronic fear and anxiety, has been supported by several reports (Foa et al. 1991; Resick et al. 1988). In an uncontrolled investigation, a clear treatment effect emerged on rape-related fear, anxiety, phobic anxiety, tension, and depression in female rape victims who showed elevated fear and avoidance to phobic stimuli 3 months post rape (Veronen and Kilpatrick 1983). In a controlled study, the efficacy of six 2-h sessions of three types of group therapy for rape-related fear and anxiety were compared to a naturally occurring waiting list control group, including SIT, assertion training, or supportive psychotherapy plus information (Resick et al. 1988). Results indicated that all three treatments were highly effective in reducing rape-related fears, intrusion, and avoidance symptoms, with no group differences evident. Improvement was maintained at 6-month follow-up on rape-related fear measures, but not on depression, self-esteem, and social fears.

A controlled study compared three different forms of relaxation for 90 Vietnam veterans (Watson et al. 1997). Relaxation, relaxation plus deep breathing exercises, and relaxation plus deep breathing plus biofeedback were equally, but only mildly, effective in leading to improvement. In summary, among anxiety management approaches, SIT has received the most support for PTSD. Other AMTs such as relaxation or cognitive therapy are best viewed as treatment components of a comprehensive treatment package.

3.4 Systematic Desensitization

In systematic desensitization (SD), the patient is taught how to relax, and then presented with reminders of the trauma gradually, working up a hierarchy from the least disturbing to the most disturbing. If they become very anxious or upset, they stop the trauma imagery, relax themselves, and then go back to the material for exposure, until they can encounter all memories or situations without becoming upset. Earlier behavioral treatments for PTSD adopted the SD technique pioneered by Wolpe (1988), however there were methodological problems rendering these studies inconclusive. The successful outcome of SD compared to a no-treatment control group was demonstrated in two studies with war veterans using psychophysiological measures (Bowen and Lambert 1986; Peniston 1986) but the treatment required a large number of sessions over an extended period of time and PTSD was not assessed. Thirteen to 18 sessions of SD with the last two sessions spent in in vivo exposure were used successfully with three automobile accident victims (Muse 1986). Several uncontrolled studies demonstrated that SD was effective with rape victims in reducing fear, anxiety, depression, and social maladjustment (Frank et al. 1988; Frank and Stewart 1983; Turner 1979).

In summary, several studies show some beneficial results of SD with a variety of trauma victims, but the lack of adequate control conditions and/or the absence of PTSD diagnoses limit the conclusions that can be drawn from them. With the empirical finding that relaxation during confrontation with feared material was not necessary, and with evidence for the inferiority of SD to flooding in most anxiety disorders, the use of SD for anxiety disorders including PTSD was largely abandoned for a variety of imaginal and in vivo exposure techniques.

3.5 Eye Movement Desensitization and Reprocessing

Eye movement desensitization and reprocessing (EMDR) (Shapiro 1996) is a form of exposure (desensitization) accompanied by saccadic eye movements. Briefly, the technique involves the patient's imagining a scene from the trauma, focusing on the accompanying cognition and arousal, while the therapist waves two fingers across the client's visual field and instructs the client to track the fingers. The sequence is repeated until anxiety decreases, at which point the patient is instructed to generate a more adaptive thought and to associate it with the scene while moving his/her eyes. After each session patients indicate their subjective units of discomfort (SUDS) level and their degree of belief in a positive cognition.

A number of case studies have reported positive findings with EMDR (review in Lohr et al. 1998). In the first study, (Shapiro 1989) randomly assigned trauma victims to either one session of EMDR or an exposure control condition (EC; EMDR without the eye movements) showed that clients who received EMDR reported lower SUDS ratings after one session of EMDR than did clients in the exposure control condition, but lack of methodological rigor makes this finding

difficult to interpret. Combat veterans were randomly assigned to two 90-min EMDR sessions, an EC, both as an adjunct to standard milieu treatment for veterans with PTSD, or standard milieu treatment alone (Boudewyns et al. 1993). SUDS ratings to traumatic stimuli were lower in the EMDR group and therapists rated more patients as responders in the EMDR versus EC group. However, the three groups did not differ in their lack of response as seen on standardized self-report measures, interviews of PTSD, or on physiological responses.

Jensen and Silver et al. (1995) examined EMDR in veterans with PTSD with mixed results between SUDS measures and PTSD severity measures. Pitman et al. (1996) addressed the role of eye movement comparing EMDR with and without the eye movement component in a random crossover design with 17 male veterans diagnosed with PTSD. The results indicated that both treatments modestly improved symptoms, but there was slightly more improvement in the eyes-fixed condition than from EMDR. A well-controlled randomized study on the efficacy of EMDR by Rothbaum (1997) examined 21 female rape victims assigned to either EMDR or a waiting list control group. Four weekly sessions of EMDR led to improvement on PTSD symptoms and gains were maintained at a 3-month follow-up. In one of the best-controlled studies involving EMDR to date, a course of nine sessions of EMDR was compared to nine sessions of a CBT (Devilly and Spence 1999). The results indicated that CBT was superior to EMDR at post treatment and 1-year follow-up. Another well-controlled study evaluated the relative efficacy of prolonged exposure (PE) and EMDR compared to a no-treatment waitlist control in the treatment of PTSD in adult female rape victims ($n = 74$). Improvement in PTSD as assessed by blind independent assessors, depression, dissociation, and state anxiety was significantly greater in both PE and EMDR group than the waitlist control group (20 completers per group). PE and EMDR did not differ significantly for a change from baseline to either post treatment or 6-month follow-up measurement for any quantitative scale (Rothbaum et al. 2005), but on a measure of good end-state functioning at 6 months post treatment, participants who had received PE were doing better than participants who had received EMDR.

In summary, several studies report the beneficial effects of EMDR, although other studies report equivocal findings with EMDR not resulting in significant improvements over control conditions or comparison treatments, especially on blind standardized PTSD measures. The recent Cochrane Database review concluded that EMDR was more effective than traditional therapies or no therapy, but not different from CBT and stress management (Bisson and Andrew 2007). The Institute of Medicine, however found that evidence is inadequate to determine efficacy, and that future well-designed studies are critical (Institutes of Medicine 2008).

4 Traditional Combinations of Psychotherapy with Medication

Although combining treatments with apparently different modes of action, such as SSRI antidepressant therapy with psychotherapy for anxiety would seem straight-forward, numerous large trials and reviews have been disappointing. These studies have

suggested that standard treatment for anxiety or depression with combined chronic pharmacotherapy and psychotherapy provide little benefit over either treatment alone (Barlow et al. 2000; Foa et al. 2002, 2005; Otto et al. 2007). There are a variety of studies examining non-PTSD anxiety disorders treated with combined pharmacology (typically antidepressants) and psychotherapy that will be briefly discussed below.

4.1 Panic Disorder

Three studies evaluated combined approaches for panic disorder. Marks and colleagues compared alprazolam, exposure therapy, and alprazolam plus exposure therapy in the treatment of panic disorder with agoraphobia (Marks et al. 1993). 154 patients were randomized to one of four conditions: exposure +/− alprazolam and relaxation +/− alprazolam. Although exposure conditions produced greater improvement at post treatment, by 5 months follow-up, exposure treatment plus placebo outcome was superior to all other conditions. The second study included 77 patients with a diagnosis of panic disorder with agoraphobia randomized to 16 weeks of either CBT plus buspirone or CBT plus placebo (Cottraux et al. 1995). Results indicated no-treatment differences between groups, suggesting that the main effect in this study was the CBT and not the medication augmentation. The third study examined CBT, imipramine, or the combination in 312 randomly assigned patients (Barlow et al. 2000). CBT plus imipramine was found to be superior to CBT alone during treatment over 9 months. However, 6 months following treatment discontinuation, CBT groups without imipramine evidenced a superior outcome compared with the combined treatment.

Research on combined treatment for panic disorder suggests that the combination of traditionally used antianxiety and antidepressant medication and CBT may interfere with long-term gains. Necessary cognitive changes may be impeded by medication if gains are attributed to medication, or if medication hampers CBT disconfirmation of erroneous beliefs associated with anxiety-related physical responses (Foa et al. 2002).

4.2 Obsessive-Compulsive Disorder

Here we discuss several randomized controlled trials of combined pharmacologic and psychotherapeutic treatment for obsessive-compulsive disorder (OCD). Cottraux and colleagues compared exposure and ritual prevention (ERP) plus fluvoxamine, ERP plus placebo, and fluvoxamine alone, in a 24 week treatment of 60 patients (Cottraux et al. 1990). No group differences were detected as measured by the reduction of daily rituals greater than or equal to 30% at either post treatment or follow-up. Hohagen and colleagues compared ERP plus fluvoxamine to ERP plus placebo in the treatment of 58 patients (Hohagen et al. 1998).

Both groups improved significantly from pre- to post treatment, with no significant differences between groups with an advantage in percent responders for the combined treatment. Cognitive therapy, ERP, fluvoxamine plus cognitive therapy, fluvoxamine plus ERP and waitlist were compared in 117 patients (van Balkom et al. 1998). Using the Y-BOCS as the outcome measure, all four active treatments were superior to the waitlist at both midtreatment and post treatment, with no significant differences among active treatments. Recently, a double-blind randomized trial examined a pill placebo versus clomipramine, ERP, and clomipramine plus ERP in the treatment of 122 adult outpatients (Foa et al. 2005). Results as measured by Y-BOCS total score and the Clinical Global Impression indicated that all active treatments were superior to placebo. ERP did not differ from ERP plus clomipramine, and both were superior to clomipramine alone. Overall, the reviewed studies indicate no clear advantage for combined treatment over CBT alone in the treatment of OCD.

4.3 Social Phobia

Blomhoff and colleagues examined sertraline, exposure therapy plus sertraline, exposure therapy plus placebo, and placebo in the treatment of 387 patients (Blomhoff et al. 2001). Response to treatment, based on Clinician Global Impression–Improvement scale self and investigator ratings, indicated that patients in all active treatment groups were significantly more improved than placebo-treated patients at week 12, but at weeks 16 and 24, only the sertraline groups remained significantly superior to placebo. However, no significant differences between the active treatment groups were evidenced. A randomized placebo-controlled trial treating 60 patients diagnosed with generalized social phobia was conducted with cognitive therapy, fluoxetine plus self-exposure, or placebo plus self-exposure (Clark et al. 2003). Significant improvements were evidenced in all three treatment conditions, with cognitive therapy superior to fluoxetine plus self-exposure. Fluoxetine plus self-exposure and placebo plus self-exposure did not differ significantly. Davidson and colleagues performed a randomized, double-blind, placebo-controlled trial comparing fluoxetine, comprehensive cognitive-behavioral group therapy (CBGT), placebo, and combined fluoxetine and CBGT in the treatment of 295 outpatients (Davidson et al. 2004). Results indicated that by the final visit, all active treatments were significantly better than placebo but did not differ from each other. The authors conclude that there was no evidence for greater benefit of combined treatment over monotherapy, which seems to be the case for social phobia in general.

4.4 Posttraumatic Stress Disorder

There is evidence for the effectiveness of both pharmacologic and psychotherapeutic treatment interventions for PTSD. Rothbaum and colleagues (Rothbaum et al. 2006)

conducted a multicenter investigation of augmentation of sertraline with prolonged exposure in the treatment of PTSD. Eighty-eight male and female outpatients diagnosed with PTSD were treated with open label sertraline (up to 200 mg or the maximum tolerated by week 6) for 10 weeks in phase I, and then were randomly assigned to receive continuation with sertraline alone or to receive augmentation with PE (10 twice weekly sessions of 90–120 min) in phase II. PE included psychoeducation about trauma reactions, breathing exercises, in vivo exposure, prolonged imaginal exposure, and homework which involved listening to a tape of the imaginal exposure recorded in session. Results indicated that five additional weeks of treatment with sertraline alone did not result in further improvement on measures of PTSD severity, depression, and general anxiety. Augmentation with PE did result in further reduction of PTSD severity as measured by the structured interview for PTSD (Davidson et al. 1997), but not in depression as measured by the Beck Depression Inventory (Beck et al. 1961) or general anxiety as measured by the state portion of the State-Trait Anxiety Inventory (Spielberger et al. 1970). The beneficial effect of PE augmentation was observed among medication partial (weaker) responders. There were no significant differences at the end of treatment between groups on PTSD severity. This study of combined treatment for PTSD indicates some advantage for medication partial responders in adding CBT, but did not compare CBT alone to the combined approach.

A recent study examined the potential benefits of adding sertraline versus placebo to TF-CBT for improving PTSD and related psychological symptoms in children who have experienced sexual abuse (Cohen et al. 2007). Twenty-four 10- to 17-year-old girls and their primary caretakers were randomly assigned to receive TF-CBT + sertraline or TF-CBT + placebo for 12 weeks. Both groups experienced significant improvement in PTSD and other clinical outcomes from pre- to post treatment with no significant group × time differences between groups except in Child Global Assessment Scale ratings, which favored the TF-CBT + sertraline group. These results only minimally suggest a benefit to adding sertraline to TF-CBT. A drawback of adding sertraline was determining whether TF-CBT or sertraline caused clinical improvement for children with comorbid depression.

Another method to combine treatment is to use antidepressants to augment psychotherapy for patients who are refractory to initial treatment. Simon and colleagues prospectively examined the relative efficacy of augmentation of continued prolonged exposure therapy (PE) with paroxetine controlled release (CR) versus placebo for individuals remaining symptomatic despite a course of PE (Simon et al. 2008). Adult outpatients meeting DSM-IV criteria for PTSD underwent eight sessions of individual PE over a 4- to 6-week period. Participants who remained symptomatic were randomly assigned to the addition of paroxetine CR or matched placebo for an additional five sessions of PE. Consistent with prior studies, the 44 phase I completers improved significantly with initial PE. However, they found no additive benefit of augmentation of continued PE with paroxetine CR compared to pill placebo for the 23 randomly assigned patients, with relatively minimal further gains overall in the second phase. These data do not support the addition of an SSRI to continued PE for individuals with PTSD who remain

symptomatic after initial PE, suggesting the need for development of novel treatment approaches for PTSD refractory to PE.

5 Novel Approach: Pharmacotherapy Augmentation of Psychotherapeutic Learning

An alternative approach to both standard chronic pharmacological treatments, which tend to target symptom clusters but not necessarily the root cause of the disorder, is the possibility of enhancing the specific new learning that occurs with psychotherapy. The pathways mediating this learning are on the one hand very complex, but on the other hand, specific learning processes that utilize brain regions such as amygdala and prefrontal cortex that are involved with extinction of fear are well understood. The sections below will discuss evidence that disorders of aversive emotion respond to behavioral therapy and utilize new emotional learning to compete with or inhibit the aversive memories – a process known as extinction – and that this process may be enhanced with specific pharmacotherapy. Together these approaches offer tantalizing future ways in which fear, aversive, and traumatic memories may be modulated to alleviate suffering due to negative memories.

The inhibition of fear acquired by associative learning has been studied in both animals and humans. Following the pairing of an aversive unconditioned stimulus (UCS) to a neutral conditioned stimulus (CS), a conditioned fear response is established. If the neutral CS is then repeatedly presented in the absence of the UCS, a procedure known as extinction training, the result is an inhibition of the conditioned fear response to the neutral CS. From an operational perspective, extinction may thus be defined as "a reduction in the strength or probability of a conditioned fear response as a consequence of repeated presentation of the CS in the absence of the UCS" (Rothbaum and Davis 2003).

A variety of behavioral observations support the hypothesis that extinction is a form of learning and not "unlearning" or the forgetting of a conditioned association (reviewed in Davis et al. 2006; Myers and Davis 2002). Thus, Davis and colleagues tested the hypothesis that enhancing neurotransmission at NMDA receptors would facilitate extinction (Walker et al. 2002). D-cycloserine (DCS) is a partial NMDA agonist, acting at the strychnine-insensitive glycine recognition site of the NMDA receptor complex to enhance NMDA receptor activity (Hood et al. 1989; Monahan et al. 1989). The central findings of this study were that both systemic and amygdala-specific administration of DCS dose-dependently enhanced extinction of previously conditioned fear but did not influence fear in rats that had not received extinction training. The general findings of this study have been replicated by numerous groups with extinction of fear with startle and freezing and with extinction of appetitive cues, such as cocaine-conditioned place preference (Ledgerwood et al. 2003, 2004, 2005; Richardson et al. 2004; Yang et al. 2006; Yang and Lu 2005). Collectively, data from rodent studies suggest that DCS, a drug already

shown to be safe for use in humans for treating tuberculosis, may have potential use in the facilitation of extinction-based therapies for human anxiety disorders.

From a therapeutic standpoint, the behavioral therapies for different anxiety disorders generally involve some form of extinction training (Rothbaum and Davis 2003). This involves graded exposure to the feared object or event in the absence of any likely actual harm. This exposure may be *imaginary* in nature wherein a narrative is read or listened to by the patient or in vivo where the feared stimulus is directly encountered by the patient. Considering the similarity between extinction training in rodents and exposure therapy for anxiety disorders in humans, novel ways to integrate pharmacotherapy with psychotherapy seemed plausible. Historically, there has been hope to combine these two approaches into a treatment more effective than either alone but unfortunately this has not generally been achieved (Foa et al. 2002; Otto 2002). In fact, sometimes combining pharmacotherapy with psychotherapy can make a bad situation worse (Barlow et al. 2000; Marks et al. 1993). However, extinction-based therapies for anxiety may be an exception to this trend.

To determine whether DCS would also improve extinction of fear in human patients with fear-related disorders, a double-blind, placebo-controlled trial in a controlled exposure paradigm was performed (Ressler et al. 2004). Twenty-eight subjects with fear of heights (acrophobia) were treated with two sessions of behavioral exposure therapy using VRE to heights within a virtual glass elevator. Single doses of placebo or DCS were taken prior to each of the two sessions of VRE therapy. Exposure therapy combined with DCS resulted in significantly larger reductions of acrophobia symptoms on all main outcome measures, including fear within the virtual environment, acrophobia symptoms outside of the environment, and acrophobia-related anxiety. Additionally, subjects receiving DCS showed significantly greater decreases in a physiological measure of anxiety during the test exposure. These pilot data provided initial support for the use of acute dosing of DCS as an adjunct to exposure-based psychotherapy to accelerate the associative learning processes that contribute to correcting psychopathology.

However, the underlying brain mechanisms mediating this fear reduction have yet to be completely understood. Recently, Britton and colleagues tested the effects of orally administered DCS on amygdala activity during the processing of repeated facial expressions in a double-blind study (Britton et al. 2007). Fourteen healthy males (30.0 $\pm$ 8.7 years of age) randomly received DCS or placebo prior to fMRI imaging. All participants viewed four separate runs, consisting of a single block of a repeated facial expression (happy or fearful) bracketed by fixation blocks. Anatomic region-of-interest analyses showed that the placebo group exhibited amygdala activation and response habituation, while the DCS group displayed blunted amygdala responses to emotional faces across the experiment, whereby habituation was not detected. This finding may have relevance for testing treatments of anxiety and depression through functional imaging.

DCS has also been used in a double-blind, placebo controlled trial for the treatment of social phobia or social anxiety disorder (SAD), utilizing exposure therapy as well. In this study, 27 subjects received four exposure therapy sessions combined with DCS or placebo. Those receiving DCS in addition to exposure

therapy reported significantly less social anxiety compared with patients receiving exposure therapy plus placebo. Controlled effect sizes were in the medium to large range (Hofmann et al. 2006a, b; reviewed in Hofmann 2007). Guastella et al. (2008) have recently extended on these findings in another similarly larger designed study of 56 participants who met primary diagnosis for SAD. Subjects administered DCS prior to exposure therapy reported greater improvement on measures of symptom severity, dysfunctional cognitions, and life-impairment from SAD in comparison with placebo-treated participants. Additionally, the results also provide the first preliminary evidence to suggest that DCS moderates the relationship between a reduction in negative appraisals about one's speech performance and improvement in overall SAD symptoms.

DCS has also been used to accelerate obsession-related distress reduction in patients with OCD undergoing extinction-based exposure therapy. DCS given prior to the therapy was found to decrease both the number of exposure sessions required to achieve clinical milestones and the rate of therapy dropout. After four exposure sessions, patients in the DCS group reported significantly greater decreases in obsession-related distress compared to the placebo group (Kushner et al. 2007). Most recently, a separate study of 23 OCD patients also found DCS enhances the efficacy of behavior therapy for OCD versus placebo augmentation of 10 behavior therapy sessions. Relative to the placebo group, the DCS group's OCD symptoms were significantly more improved at midtreatment, and the DCS group's depressive symptoms were significantly more improved at post treatment (Wilhelm et al. 2008).

There are currently two negative findings of DCS use in the augmentation of extinction in humans. However, in both studies, one utilizing exposure therapy for the treatment of spider phobia (Guastella et al. 2007a) and the second utilizing extinction for a conditioned cue in healthy human subjects (Guastella et al. 2007b), the placebo-controlled groups had good recovery as well. This suggests, as has been found in the animal studies, that if a "floor effect" of complete extinction is accomplished, then DCS may not augment extinction any further. Rather it appears that DCS may be most useful in disorders in which either a large number of therapy sessions are normally needed for a response, or in which the nature of the disorder is quite refractory to normal exposure-based therapy approaches.

There are currently several ongoing randomized clinical trials of DCS augmentation of treatment for PTSD. Since these studies have yet to be published, there is a great deal of excitement as to whether these new approaches will lead to advances in psychosocial treatment methodologies. It is hoped that with progress in the neurobiology and neuropharmacology of extinction of fear, that previously undiscovered approaches to pharmacotherapy will significantly enhance treatment for refractory mood and anxiety disorders, including PTSD.

Most recently, Norberg and colleagues (Norberg et al. 2008) performed a meta-analysis providing a quantitative review of DCS, which nicely summarized the role of DCS in augmenting fear extinction and exposure therapy in the preclinical and clinical literature. Data were extracted for DCS studies from 1998 to 2007. The analyzed results indicated that DCS enhances fear extinction/exposure therapy in both animals and anxiety-disordered humans, while some lessening of efficacy

occurs over the length of the treatment time. D-cycloserine was more effective when administered a limited number of times and when given immediately before or after extinction training/exposure. The major contribution of DCS to exposure-based therapy might be to increase its speed or efficiency, because the effects of DCS seem to decrease over repeated sessions. Overall, DCS continues to be promising as a pharmacological enhancement of psychotherapy, and may guide translational research towards future potential agents that effectively enhance behavioral therapy.

A recent review examined critical parameters for D-cycloserine enhancement of CBT (Rothbaum 2008). As stated in that review, there are several different strategies for combining pharmacotherapy and psychotherapy and challenges and advantages to each. For the antidepressant medications, if they are added at the start of CBT, there is the problem of time to response and the extant literature does not suggest any advantage to combining these medications with CBT for anxiety disorders. For anxiolytic medications other than the antidepressants, generally the onset of action is much quicker, and they can be combined with psychotherapy from the onset. However, there is some evidence that they may impede CBT, especially exposure therapy. For the more novel medication approaches such as D-cycloserine, the drug is not expected to afford any benefits in and of itself; it is only in combination with the exposure therapy, so they should be on board at the same time. In contrast to the more traditional medications (e.g., antidepressants and anxiolytics) combined with CBT for anxiety, these novel medication approaches do seem to afford some advantages when combined with CBT. The new approaches aim to combine a cognitive enhancer medication that will specifically enhance the efficacy of the emotional learning process that takes place in psychotherapy and hopefully make these new memories more robust, stable, and long-lasting. What is particularly promising about this approach is that it was developed rationally, based on the neuroscience of fear and anxiety and extinction.

6 Conclusion

Overall, the most controlled studies of psychosocial treatments for PTSD have been conducted on cognitive-behavioral treatments. These studies demonstrate that techniques such as prolonged exposure procedures are effective in reducing symptoms of PTSD. SD has largely been abandoned in favor of pure exposure techniques. Relaxation and cognitive therapy are best viewed as treatment components rather than stand-alone treatments. Contrary to clinical intuition, there is no evidence indicating the superiority of programs that combine different cognitive-behavioral techniques. In view of the fact that these interventions are widely employed with trauma victims, it is imperative that their efficacy will be examined in well-controlled studies. Furthermore, the majority of studies combining antidepressant and other antianxiety medication with behavioral therapies have not been successful or have been mixed at best. New approaches with pharmacotherapy of D-cycloserine, and potentially other medications aimed at specifically enhancing

the emotional learning process that occurs with psychotherapy, may be quite effective and hold promise for improving the treatments of PTSD and other anxiety disorders in the near future.

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The Pharmacology of Anxiety

C. Durant, D. Christmas, and D. Nutt

Contents

Abstract Understanding the neurochemistry of anxiety is of fundamental importance in the development and use of novel anxiolytics. Through measuring peripheral markers of brain biochemistry, direct pharmacological challenges and brain neuroimaging techniques our understanding of this field has increased substantially in the past few decades. We review the four most studied neurotransmitter systems with respect to in anxiety disorders: gamma amino-butyric acid, serotonin, noradrenaline and dopamine. We have focussed upon clinical studies to highlight the

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current techniques used to determine brain neurochemistry in vivo. Future research in this field will greatly benefit from recent advances in neuroimaging techniques and the discovery of novel ligands targeting specific receptors.

Keywords Anxiety disorders · Pharmacology · GABA · Serotonin · Noradrenaline · Dopamine · Glutamate

1 Introduction

Anxiety is an emotion experienced as a part of everyday life and can be viewed as a continuum. At one end of the scale, mild anxiety can improve motivation and productivity; at the other end intense anxiety with the 'fight or flight' response promotes survival in response to danger. However, when occurring at inappropriate times or to an excessive degree, anxiety can become pathological. Anxiety disorders are the most common psychiatric illnesses (Pillay and Stein 2007) with over 10% (3.6 million) of individuals experiencing an anxiety disorder at some point in their life time (Wittchen and Jacobi 2005). These disorders have profoundly disabling consequences, leading to significant suffering and a reduced quality of life (Rapaport et al. 2005). For many of these individuals, leading a normal life is no longer possible, with a high prevalence of divorce and unemployment and a huge socioeconomic cost, estimated at £5 billion per year cross-nationally (WHO 2000). Anxiety disorders are the most chronic of all mental illnesses with the youngest age of onset (15 years; WHO 2000), making them a devastating and often lifelong disorder.

Despite the recognition of pathological anxiety as a serious clinical problem, it was not until the publication of the DSM-III in 1980 that systematic study of 'anxiety disorders' was undertaken. There are now six clinically recognised anxiety disorders listed by the DSM-IV allowing more specific study of different 'anxiety syndromes'. However, at present, understanding of the underlying pathological processes of specific anxiety disorders remains limited.

Understanding of the neural basis of anxiety, specifically the role of different neurotransmitters, is of fundamental importance for the development of novel anxiolytics. However, study of the neurochemical basis of anxiety is difficult, with initial research being driven by the chance discovery of drugs with anxiety reducing properties. As such, focus on gamma-aminobutyric acid (GABA) and the monoamine neurotransmitters, noradrenaline, serotonin and dopamine, has dominated the field, based on evidence that effective anxiolytics interact with these systems. Direct manipulation of these neurotransmitters and their receptors in animal models has resulted in the expression of 'anxiety-like syndromes', through which it has been possible to investigate the underlying circuitry and receptors involved.

Study of the neurochemistry of anxiety in humans is somewhat more complicated, with a number of different approaches developed to explore underlying

abnormalities in anxiety disorders. Peripheral measures of neurotransmitters in the blood and urine of patients with anxiety disorders have been unable to demonstrate any dramatic changes, with similar results for measures of cerebrospinal fluid (CSF). A different approach has been to use challenge tests in which anxiety is induced experimentally and the consequences, physiological and psychological are investigated. Challenge tests in both healthy volunteers and anxiety patients have been well developed and are now used extensively in research for development of novel anxiolytics. Indeed, the use of agonists and antagonists directed at specific neurotransmitter receptors in challenge paradigms has highlighted important abnormalities in the responses of anxiety patients, which are discussed in more detail below. Advances in brain imaging techniques have also allowed direct study of the neurocircuitry and receptors involved in the living human brain. These are now being combined with challenge tests to assess via more direct means the functional integrity of various systems. Neuroimaging technologies have already highlighted important abnormalities in GABAergic and monoaminegic systems associated with anxiety disorders and represent a powerful tool for the future study of neurochemical aspects of anxiety. This chapter will present an overview of the GABAeric and monoaminergic systems in anxiety disorders, with emphasis on clinical studies, as pre-clinical data will be covered elsewhere.

1.1 The GABAergic System and Anxiety

Much evidence implicates the GABAergic system in human anxiety, though most direct evidence is currently provided by pre-clinical data (see Kalueff and Nutt 1996). However, indirect manipulation and brain imaging of GABA in humans provides a strong case for its involvement in human anxiety disorders.

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system. Released at an estimated 20–50% of central synapses, GABAergic neurotransmission plays a crucial role in controlling the excitability of neuronal activity in the brain (Sieghart 1995). The majority of GABAergic neurones are found in the CNS where they are widely distributed, with very few found in the peripheral nervous system. Reflecting their extensive presence in the brain, GABAergic neurones help to set the ongoing level of neuronal activity by opposing ongoing excitatory input (mainly glutamatergic, Nutt 2006).

GABA is synthesised from glutamate by L-glutamic acid decarboxylase, after which GABA is stored in synaptic vesicles. Neuronal activation results in the release of GABA into the synaptic cleft, activating clusters of post-synaptic receptors, resulting in rapid inhibitory 'phasic' neurotransmission. Released GABA can also 'spillover' into the extra-synaptic space, activating a range of extra-synaptic receptors found both pre- and post-synaptically on adjacent neurones so providing a 'tonic' inhibition that controls background levels of neuronal excitation (reviewed by Farrant and Nusser 2005). Uptake transporters located on presynaptic neurones

and supporting glial cells terminate GABAergic neurotransmission by removing GABA from the synapse and extra-cellular space (Nutt 2006).

GABA acts through two main types of receptor: the fast-acting ion gated or 'ionotropic' receptors of which two, $GABA_A$ and $GABA_C$, have been identified and the slower acting G-coupled 'metabotropic' $GABA_B$ receptor (Bormann 2000).

The $GABA_A$ receptor is the most prominent type of GABA receptor in the brain. It plays a key role in regulating the excitatory tone of many other types of neurones including dopaminergic, cholinergic and serotonergic (Fritschy et al. 1992; Gao et al. 1995). Abnormalities of $GABA_A$ receptor expression and function have been the focus of intense research in anxiety as the majority of anxiolytics target this receptor complex.

Less is currently known about the $GABA_B$ receptor which is widely expressed in both the periphery (including non-neuronal tissue) and in many brain areas including cortical, thalamic, cerebellar and limbic regions (Billinton et al. 2000). $GABA_B$ receptors exert their action via activation of G-protein linked intracellular messengers. Structurally, the $GABA_B$ receptor is made up of two subunits: B1, responsible for ligand binding and B2, coupled to intracellular signalling proteins. When located in the pre-synaptic neurone $GABA_B$ receptors reduce conductance though voltage sensitive calcium channels, so inhibiting neurotransmitter release. Post-synaptically $GABA_B$ receptors increase potassium influx, leading to hyperpolarisation (see Bettler et al. 2004).

Of interest for anxiety is the finding that $GABA_B$ expression is particularly high in the limbic system, a region linked to the processing of emotional material (Cryan and Kaupmann 2005). However, current understanding of the role of these receptors in human anxiety is poor. Studies administering the specific $GABA_B$ agonist baclofen in attempts to produce anxiolytic effects have shown highly variable results, in both pre-clinical and clinical studies (reviewed in Kalueff and Nutt 1996). More recently, the finding of increased anxiety in $GABA_{B(1)}$ knockout mice over a range of standard anxiotylic tasks has renewed interest in these receptors (Cryan and Kaupmann 2005). The potential of the $GABA_B$ receptor as a target for novel anxiolytics will no doubt be of continued interest as research uncovers a more detailed picture of this receptor in the human brain.

1.2 Early Evidence for the Role of the GABAergic System in Anxiety

The behavioural results of altering GABAergic signalling provides strong support for the role of this system in anxiety. Enhancing the GABAergic signal, e.g., using $GABA_A$ agonists, is anxiolytic as well as anti-convulsant, sedative, amnestic and ataxia-producing. Attenuating GABA function, e.g., with the inverse agonist FG7142, causes arousal, anxiety, restlessness, insomnia and seizures. Ways of enhancing GABAergic transmission for its anxiolytic effects have been sought by

humans for centuries, with the use of alcohol providing courage in the face of stressful situations reported throughout history.

Preliminary evidence that reducing GABAergic transmission induces anxiety came from the early use of pentylenetetrazol (PTZ) a convulsant drug used to induce seizures in the treatment of severe psychiatric disorders before the discovery of ECT. During its use dose titration was difficult, in many cases too little was given and no seizure was caused. This, however, produced a severely anxious state, leading to patients feeling 'as if they were going to die', and trying (often successfully) to escape from the clinic. Memory of this anxiety was extremely strong resulting in resistance to return to therapy and it was later shown that PTZ acts as an antagonist $GABA_A$ receptor. Reduced transmission through the $GABA_A$ receptor, therefore, causes some of the main symptoms associated with anxiety disorders, excessive experiencing of anxious emotion, escape behaviour and avoidance of associated situations (Nutt 2001).

A brief look at the early history of anxiolytic drugs also points towards a relationship between the GABAergic system and anxiety. The first class of drug developed specifically to target anxious symptoms was the barbiturates in 1903, which were highly effective and became widely used until the 1950s. Unfortunately, the barbiturates can cause respiratory arrest and have a narrow therapeutic index, which resulted in many accidental deaths. The benzodiazepines (BDZs) were developed as a safer alternative in the 1960s, quickly becoming the most widely prescribed drugs worldwide to treat anxiety. There are now a number of BDZs in clinical use for anxiety, including diazepam, lorazepam, clonazepam and alprazolam (Pillay and Stein 2007). It is now known that both barbiturates and BDZs potentiate GABA receptor function. It is understandable, therefore, that there is considerable interest in the $GABA_A$ receptor and its manipulation in attempts to treat and understand the underlying neurochemistry of human anxiety disorders.

1.3 The $GABA_A$ Receptor and Anxiety

Following the discovery of BDZs, interest focused on determining their mechanism of action. Evidence indicated BDZs caused specific GABA potentiation through binding to a particular site in the CNS, later isolated as a subunit of the $GABA_A$ receptor complex (reviewed by Nutt and Malizia 2001). With the use of electron microscopy it has been possible to visualise the $GABA_A$ receptor (Nayeem et al. 1994) which consists of five protein subunits arranged like a rosette around a central pore permeable to chloride and other anions (Fig. 1). The $GABA_A$ receptor exhibits significant heterogeneity with 19 different subunits identified to date, each coded for by a different gene. These subunits have been grouped into six main families based on sequence homology: $\alpha(1–6)$, $\beta(1–3)$, $\gamma(1–3)$, δ, ε and $\sigma(1–3)$. The generic stoichiometric composition of the $GABA_A$ receptor comprises 2α, 2β and 1γ subunits, with $\alpha1\beta2\gamma2$ representing the most abundant form in the human CNS (Korpi

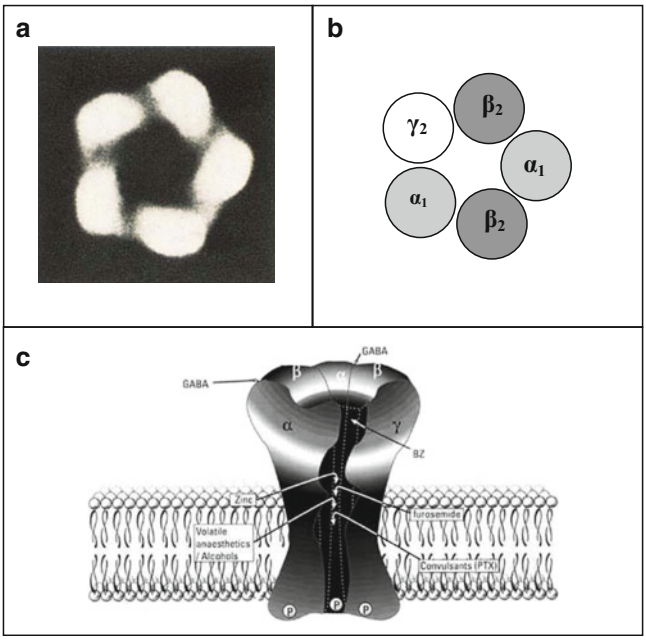

Fig. 1 (**a**) Electron micrograph of $GABA_A$ receptor. Reproduced with permission from (Nayeem et al. 1994). (**b**) $GABA_A$ receptor subunits, indicating the most prevalent form, $\alpha1\beta2\gamma2$ with given 2:2:1 stoichiometery (Nutt 2006). (**c**) Schematic of the $GABA_A$ receptor channel with agonist and antagonist binding sites highlighted, with permission from (Whiting et al. 1999)

and Sinkkonen 2006). Importantly, different subunit isoforms have been shown to confer specific distributions and receptor properties within the human brain.

Based on rodent immunocytochemistry the $GABA_A$ receptor subunits exhibiting the highest expression levels include the $\alpha1$, $\beta1$–3 and $\gamma2$ receptor subunits, although these show differences in regional distribution. The $\alpha1$ and $\gamma2$ subunits exhibit the widest expression throughout all brain regions; in contrast, the remaining $\alpha2$–6 subunits show more restriction to certain brain regions. For example, the $\alpha2$ subunit is confined mainly to forebrain and cerebellar areas, whereas $\alpha5$ expression is localised to the hippocampus, hypothalamus and olfactory bulb (Pirker et al. 2000). There is also evidence of co-distribution of certain subunits, e.g., both δ and $\alpha4$ expression was observed in the hippocampus, thalamus, striatum and outer cortical areas (Pirker et al. 2000).

In addition, subunits also show subcellular localisation. Whereas the $\alpha1$, $\alpha2$, $\alpha3$, $\alpha6$, $\beta2/3$ and $\gamma2$ subunits are mainly found post-synaptically, the δ and $\alpha5$ subunits are predominantly extra-synaptic in location (Farrant and Nusser 2005). Importantly, $GABA_A$ subunit composition and localisation relates to the role of the receptor. Extra-synaptic receptors, required to respond continuously to lower neurotransmitter levels, contain subunits that confer slower desensitisation rates and higher affinities for GABA, providing 'tonic' inhibition compared with synaptic receptors (Orser 2006).

GABA acts as an agonist at the receptor complex, binding to the receptor at the interface between the α and β subunit; it induces a conformational change that enhances the permeability of the central pore to chloride ions. The resulting influx of anions into the post-synaptic neurone results in a state of hyperpolarisation, reducing neuronal excitability (Nutt 2006). Excessive activity of GABA receptors will result in profound sedation and respiratory depression. In addition to GABA, a number of other substances can directly open the chloride channel at high concentrations, including barbiturates, chloral hydrate and alcohol, explaining their fatality in overdose.

BDZs have a different mechanism of action, binding to a specific site on the $GABA_A$ receptor complex between α and γ subunits (Olsen et al. 2004), reducing the concentration of GABA needed to cause channel opening. This positive 'allosteric' binding augments the response of the receptor to endogenous GABA, therefore, enhancing GABAergic transmission (Nutt and Malizia 2001). This explains why BDZs, which are limited to enhancing the normal inhibitory signal in the brain, are relatively safe in overdose compared to other GABAergic drugs which can act directly on the ionophore itself.

1.4 How the BDZ-$GABA_A$ Receptor Site Has Contributed to the Understanding of Anxiety

Studies of the evolution of the $GABA_A$ subtypes singled out the BDZ site as the most evolutionarily advanced region of the receptor (Nielsen et al. 1978). Indeed, further pharmacological investigation of this site revealed a unique ability not only to reduce anxiety, as already exploited by BDZs (acting as agonists), but also induce anxiety (when acted on by inverse agonists) in humans. This property of the BDZ site has created much interest surrounding the role of this receptor in both the treatment and understanding of anxiety disorders (Fig. 2).

Paradoxically, it was through the search for anxiolytic compounds that the potential of the BDZ to show a bidirectional anxiogenic response came to light. It was thought that the β-carboline compound FG7142, a putative endogenous ligand, might prove a useful anxiolytic due to its affinity for the BDZ receptor. However, during experiments in healthy volunteers it quickly became apparent that the reverse was true; high doses produced severe anxiety and panic in two participants (Dorow et al. 1983). It is now known that FG7142 acts as an 'inverse agonist' at the BDZ site, reducing the effects of GABA and so eliciting an anxiogenic response. Supporting this finding is Ro15-3505 which also acts as an inverse agonist at the BDZ site and has anxiogenic responses (Gentil et al. 1990).

Naturally occurring 'endozepines' thought to activate the BDZ site, have been isolated in the human brain. Increased endozepine-4 levels have been linked to stuporous attacks in patients, in whom flumazenil infusion can increase arousal, supporting the existence of endogenous BDZ ligands and their potential role in CNS diseases (Cortelli et al. 2005). However, the role of endozepines in anxiety

disorders remains poorly understood. Other candidates for endogenous $GABA_A$ ligands are the neurosteroids, such as progesterone metabolites, which act as potent allosteric agonists at sites distinct from the BDZ region on $GABA_A$ receptor. There is growing evidence that locally produced neuroactive steroids may be involved in 'fine tuning' of inhibitory signalling (Longone et al. 2008), and that altered levels may be involved in the pathogenesis of anxiety (Kalueff and Nutt 2007). However, exactly how the vast array of neurosteroids may interact during anxiety disorders is not yet understood. Importantly, with advances in molecular technology beginning to identify specific neuroactive steroid binding pockets on the $GABA_A$ receptor (Longone et al. 2008), it may be possible to design 'steroid based modulators' as fast-acting anxiolytics for the future treatment of anxiety disorders (Kalueff and Nutt 2007).

An alternative hypothesis suggests that an abnormality may lie in the GABA BDZ receptor itself. Such that a shift in the 'set-point' of the BDZ receptor spectrum of function may underlie anxiety in panic patients (Fig. 2). Evidence for this is that in these patients the usually 'silent' antagonist flumazenil behaves like a partial inverse agonist, being anxiogenic like FG1472 mentioned above (Nutt and Lawson 1992). Further clinical observations and findings support the notion of a shifted set point in the inverse agonist direction, which would render typical agonists less effective. Panic disorder patients often require higher doses of BDZs to elicit sufficient anxiolytic response and the physiological responses to BDZs such

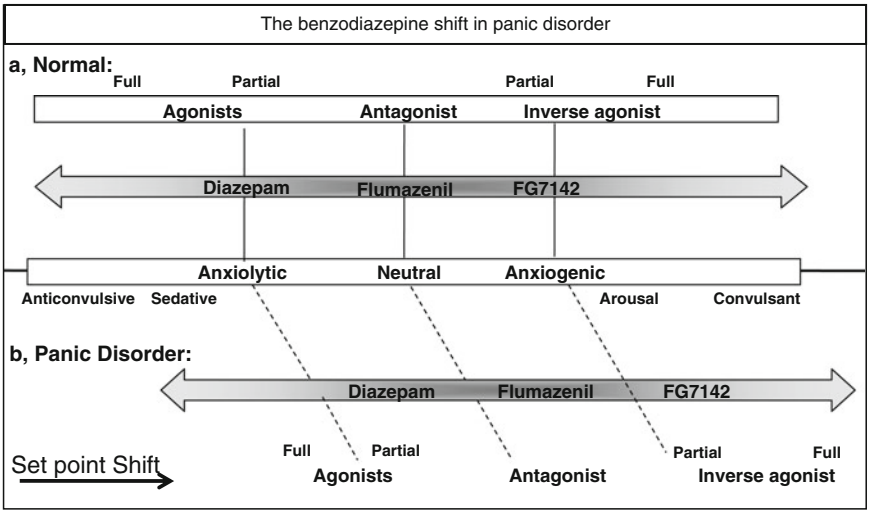

Fig. 2 (**a**) Number of compounds interact with the $GABA_A$ BDZ site to elicit a range of behavioural responses. (**b**) Evidence suggests that the intrinsic set point of the receptor may change in panic disorder shifting the response of the receptor to these compounds. Agonists, such as diazepam have an attenuated action, antagonists; once neutral are now slightly anxiogenic acting as partial inverse agonists and the action of inverse agonists themselves are enhanced (Adapted from Nutt and Malizia 2001; Sandford et al. 2000)

as saccadic eye movements are reduced in these individuals (Roy-Byrne et al. 1990). Shifts in the BDZ set point could also explain why tolerance can occur over time with chronic BDZ use, and the subsequent difficulties associated with withdrawal (Nutt 2003).

Further flumazenil challenge studies have failed to exhibit anxiogenic effects in patients with anxiety disorders other than panic (Argyropoulos and Nutt 2003), suggesting this 'receptor shift' may be particularly marked or specific to panic disorder and is not an underlying phenomenon of anxiety disorders as a whole (Sandford et al. 2000). It is possible that this shift is observable only in acute episodes of anxiety, such as exhibited by panic patients and that the degree of shift may produce a continuum of anxiety type symptoms contributing to different anxious phenotypes. Although the contribution of a potential BDZ receptor shift to the pathophysiology of anxiety is still unknown, the evidence supporting its existence again implicates abnormalities of the GABAergic system in human anxiety. In addition, the mechanism of a set point shift altering the threshold of the $GABA_A$ receptor complex is an important one, representing further avenues both for treatment evolution and scientific understanding in anxiety disorders.

1.5 Evidence of a GABA Receptor Abnormality in Brain Imaging Studies

Powerful brain scanning techniques such as PET and SPECT have provided further evidence for $GABA_A$ BDZ receptor abnormalities in panic and other anxiety disorders (reviewed by Malizia 2002). With the development of radiolabelled tracers that directly interact with the $GABA_A$ BDZ receptor site, such as carbon labelled flumazenil ([^{11}C] flumazenil, used for PET) and iodine labelled Iomazenil ([^{123}I] Iomazenil, used for SPECT), it has been possible to visualise the binding properties of the BDZ site in specific regions of the living human brain (Fig. 3).

Most imaging studies point towards a reduction in BDZ binding associated with panic disorder (Malizia 2002). With the use of SPECT imaging and [^{123}I] Iomazenil, Schlegel et al. (1994) first reported a decrease in the BDZ-receptor binding in occipital and frontal and temporal cortical regions. A similar pattern of reduced binding in the inferior fronto-temporal, as well as left hippocampal caudate and precuneus areas has been reported by subsequent SPECT studies (Kaschka et al. 1995; Bremner et al. 2000b). Interestingly, in one such study decreased binding in the frontal regions was specifically related to the experience of a panic attack during the scan (Bremner et al. 2000b). Other SPECT studies have provided more contradictory results, with reports of increased binding density in temporal and occipital regions (Bremner et al. 2000b; Kuikka et al. 1995).

A pivotal PET study by Malizia et al. (1998) indicated 20% global decrease of [^{11}C] flumazenil binding, in panic disorder patients, with prominent reductions in the orbito-frontal, anterior insular and anterior temporal cortex, compared to controls.

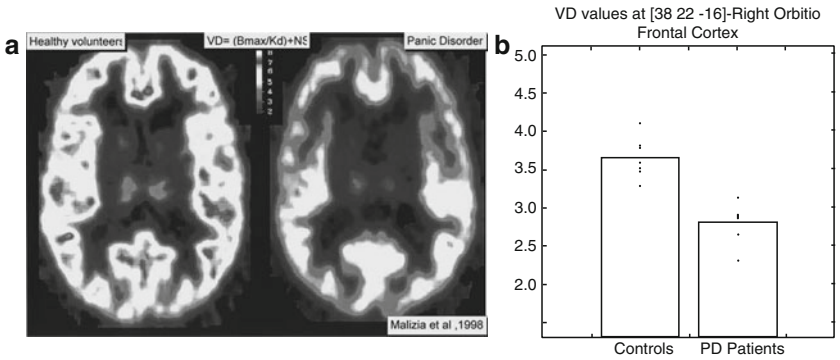

Fig. 3 (**a**) Images showing VD (volume of distribution) of [^{11}C] flumazenil binding in panic patients (*right*) compared to healthy volunteers (*left*), showing a global decrease in binding. (**b**) Benzodiazepine receptor binding in the orbito-frontal cortex shows the maximal separation of binding with no overlap in values between controls and panic patients. Images provided by Dr. A. Malizia (personal communication)

A more recent PET study of panic patients also found a bilateral reduction of [^{11}C] flumazenil binding in the insular cortex especially in patients with comorbid depression (Cameron et al. 2007). Decreased BDZ binding has also been shown in the frontal cortices of Vietnam veterans with PTSD (Bremner et al. 2000a; Geuze et al. 2008) and temporal regions in generalised anxiety disorder (GAD, Tiihonen et al. 1997b).

These studies support the notion that abnormal $GABA_A$ BDZ function is an important feature of human anxiety. Strikingly, areas reported as showing the most prominent decreases in receptor binding are those related to emotional experiencing of anxiety, namely frontal and temporal lobes and the insular cortex. If indeed reduced BDZ binding is indicative of defective GABAergic signalling, this could clearly contribute to the underlying pathophysiology of human anxiety.

It is still unclear, for instance, whether a decrease in BDZ binding represents down regulation of the whole $GABA_A$ receptor complex, or a reduction in a particular part of the BDZ site within the receptor complex. Equally, there could be a change in affinity of the receptor for BDZ ligands or an increase in an endogenous BDZ ligand reducing available BDZ sites for binding.

1.6 A Glimpse at the Future: How Individual Subunits of the $GABA_A$ Receptor Could Contribute to Anxiety

More recently, sophisticated genetic manipulation of the $GABA_A$ receptor has aimed to address some of the intriguing findings presented above. Using cloning techniques and genetically modified animals, it has been possible to start depicting

how individual $GABA_A$ subunits alter the intrinsic properties of the receptor complex itself.

Indeed, although six α subunits have been identified, only those receptors containing α1, α2, α3 and α5 subunits show BDZ sensitivity, with α4 and α6 subtypes having very low BDZ binding affinity. Further analysis revealed that the anxiolytic effects of BDZs appear to be mediated in part via the α2 subunit. Animal models in which the α2 subunit has been mutated or knocked out fail to show an anxiolytic response to diazepam, while the sedative, anti-convulsant and motor impairing effects remain. A similar picture for the γ subunit exists, with the presence of the γ2 subunit found to confer BDZ sensitivity. Interestingly, heterozygous γ2 knock out mice exhibit spontaneous anxiety behaviour, hyperarousal and decreased flumazenil binding in a similar manner to panic patients. In addition, δKO mice show an enhanced learning and consolidation of contextual fear (Nutt 2006) with insensitivity to anxiolytic neuroactive steroids (Wu et al. 2008), a combination that may have important consequences for the development of anxiety.

Subunit alterations in anxiety disorders may help explain the changes BDZ binding presented above, with clear implications for abnormalities in response to endogenous signalling molecules in the pathophysiology of anxiety. Subunit selective compounds have the potential to elicit anxiolytic effects without the sedative or amnesic qualities of higher dose BDZs and are already in the early stages of development (Rupprecht et al. 2006). One such compound, MK-0343, a $GABA_A$ agonist has been developed as a novel anxiolytic with hopes that its specific activity at the α2 and α3 but not α1 subunits will reduce sedative side effects. In early trials using healthy volunteers, MK-0343 was only slightly less sedative in comparison to the full agonist lorazepam, although reduced effects on memory and postural stability indicate a better tolerability profile for this compound as it moves into clinical trials (de Haas et al. 2008).

1.7 Impaired GABAergic Metabolism in Anxiety Disorders: Additional Approaches to Targeting the GABA System

In addition to GABA receptor abnormalities, there is emerging evidence that impaired GABAergic metabolism may play a role in anxiety disorders. Targeting the range of GABAergic uptake proteins and enzymes to indirectly alter GABAergic levels is already in practise (Pillay and Stein 2007) and could represent an important area for development of novel anxiolytics.

Evidence using a relatively new imaging technique to measure GABA levels in the living human brain suggests impaired GABAergic metabolism in some anxiety disorders. Proton magnetic resonance spectroscopy (pMRS) uses differences in chemical structure to ascertain metabolite concentration based on specific resonance frequencies (Chang et al. 2003). Studies using ^{1}H-MRS have highlighted a 22% reduction in total occipital cortical GABA concentration in panic disorder

patients compared with controls. Further studies using more advanced MRS techniques indicated that these patients showed a blunted neural response to BDZ (clonazepam) administration compared with controls, and consistently lower GABA levels in occipital regions following chronic BDZ treatment (Goddard et al. 2004). It has been suggested that these abnormalities could represent a 'trait-like disturbance' in GABA metabolism in panic disorder, and that dysfunction of the GAD_{65} enzyme may be part of this (Goddard et al. 2004).

A more recent ^{1}H-MRS study in social anxiety disorder patients indicated significantly lower thalamic GABA levels at baseline, which increased in response to levetiracetam (a novel GABAergic enhancing anti-convulsant, Pollack et al. 2008). Collective emerging evidence from pMRS studies clearly implicates GABAergic abnormalities in anxiety disorders, although it is important to remember pMRS represents a relatively new technology.

Indeed, compounds targeting GABAergic metabolism to enhance endogenous GABAergic function represent an important avenue for the treatment of anxiety. Pregabalin, a GABA analogue, has recently been licensed for the treatment of GAD (Feltner et al. 2008). Pregabalin, similar to its predecessor gabapentin, is thought to increase total brain levels of GABA through interaction with a specific calcium channel subunit (Rupprecht et al. 2006). Vigabatrin, developed as anti-epileptic, inhibits GABAergic degradation though inhibition of GABA transaminase (GABA-T), increasing endogenous GABAergic levels (Nemeroff 2003). Although vigabatrin has shown promise for the treatment of PD, investigations have been terminated due to reports of visual field disturbances associated with long-term use. Finally, tiagabine, another anti-convulsant, acts as a selective reuptake inhibitor, blocking the GAT-1 transporter, inhibiting uptake of GABA from the synapse (Nemeroff 2003). Tiagabine has shown promise for the treatment of GAD and PTSD in ongoing clinical trials, and also as a combination therapy with other anxiolytics for treatment resistant anxiety (Pillay and Stein 2007).

2 Serotonin

Serotonergic neurones arise from the median and dorsal raphe nuclei in the brainstem and project throughout the forebrain. Serotonin (5HT) is synthesised from the dietary essential amino acid tryptophan, with tryptophan hydroxylase being the rate limiting enzyme. Following release it is actively removed from the synaptic cleft by serotonin transporters back into the neurone. The degradation product of serotonin is 5-hydroxyindolacetic acid (5-HIAA, Ruddick et al. 2006).

There have been 13 serotonin receptors identified. All of these are g-protein linked except for the 5HT-3 receptor, which is coupled to a sodium ion channel. The 5HT-1A receptor has particularly been linked to anxiety disorders. It is negatively linked to the second messenger cAMP and is both post-synaptic and a pre-synaptic auto-receptor in the dorsal raphe, regulating raphe firing. Serotonin coordinates many functions including: appetite, sleep, neuroendocrine regulation (such as

prolactin release) and impulse control. This diversity of effects may be explained by the extensive distribution of serotonergic neurone projections and multiple receptors.

Serotonin has been implicated in anxiety disorders for a long time. Since it was found that drugs acting upon the serotonergic system were effective treatments for anxiety disorders, interest in this area has increased substantially. However, identifying the precise relationship of the serotonin system to anxiety disorders has been very difficult. There is currently no direct way to measure levels of serotonin or serotonergic neuronal firing in humans. Therefore, clinical observation, indirect measures, challenge tests and more recently functional neuroimaging have been utilised to characterise serotonin functioning in anxiety.

Serotonergic agents are effective in treating anxiety disorders and the selective serotonin reuptake inhibitors (SSRIs) are now recommended as first line treatments for these, although this therapeutic effect is delayed several weeks (Baldwin and Polkinghorn 2005). The SSRIs are also effective in blocking the anxiogenic effect of various challenge tests in humans including: flumazenil, cholecystokinin tetrapeptide (CCK-4) and 35% carbon dioxide in panic disorder (Bell et al. 2002; Schruers and Griez 2004; Shlik et al. 1997) and social scripts or pramipexole challenge in social anxiety disorder (Argyropoulos et al. 2004; Hood et al. 2006). However, despite this it remains unclear whether the mechanism of action of the SSRIs is to effectively increase or attenuate 5HT transmission. This is exemplified by the contradictory evidence of whether a 5HT deficiency or excess is associated by anxiety disorders as shown in Table 1.

The theory that there is an excess of 5HT in anxiety disorders is supported by studies into the effects of fenfluramine (a 5HT releasing agent) and *m*-chlorophenylpiperazine (mCPP, a 5HT agonist), both of which cause an acute increase in anxiety in both anxiety disorders and healthy controls (Charney et al. 1987; Tancer et al. 1994; Targum 1990). Moreover, intravenous administration of clomipramine, a tricyclic with serotonin reuptake inhibition, is also acutely anxiogenic (George et al. 1995). This is also compatible with the clinical picture seen when SSRIs are taken; some patients with anxiety disorders suffer an acute increase in anxiety, or jitteriness, when these medications are first taken before the delayed therapeutic effect begins (Sinclair et al. in press). The 5HT excess theory postulates that supersensitive post-synaptic 5HT receptors are responsible for anxiety symptoms. An SSRI would increase the 5HT availability acutely and

Table 1 Conflicting evidence for both serotonin excess and deficiency theories

Serotonin excess theory	Serotonin deficiency theory
Acute SSRI/clomipramine are anxiogenic	ATD – lowering brain 5HT is anxiogenic in anxiety disorders
Acute mCPP (5HT agonist) is anxiogenic	Microdialysis (pre-clinical) experiments – brain 5HT increases with clinical response to SSRIs
Acute fenfluramine (5HT releaser) is anxiogenic	Chronic SSRI is efficacious treatment for all anxiety disorders
Increased central 5HT turnover in PD	
Elevated urinary 5HT-IAA in GAD	

thus worsen symptoms in the short term. However, when the post-synaptic receptors become downregulated an anxiolytic effect is seen, albeit delayed. This theory is supported by jugular venous sampling showing a fourfold increase in brain 5HT turnover in panic patients compared with controls. Moreover, this normalises with successful SSRI treatment (Eser et al. 2007). Patients with GAD also have elevated urinary levels of 5-HIAA again suggesting an excess of serotonin in this disorder (Garvey et al. 1995).

However, this theory does not sit well with the acute tryptophan depletion (ATD) challenges in anxiety disorders (Fig. 4). ATD utilises the fact that brain availability of tryptophan (and thus 5HT) can be manipulated by altering the ratio of tryptophan to other large neutral amino acids (LNAAs) in the blood. All LNAAs, including tryptophan, are competitively transported across the blood–brain barrier by transport proteins. The levels of LNAAs can easily be manipulated over hours by dietary means; to deplete brain tryptophan a low tryptophan diet is combined with a bolus of other LNAAs. This reduces the tryptophan to LNAA ratio over 2–4 h, consequently reducing the brain availability of tryptophan by up to 80% (Hood et al. 2005). Tryptophan can either be included or excluded from the amino acid drink to produce double-blind experiments. ATD has been found to reverse the therapeutic effect of SSRIs in panic disorder and social anxiety disorder when patients have been challenged by 35% carbon dioxide (Schruers and Griez 2004), flumazenil (Bell et al. 2002), CCK-4 (Shlik et al. 1997) or social scripts (Argyropoulos et al. 2004). Therefore, this suggests that maintaining a high level of serotonin availability is important to the therapeutic effects of SSRIs. This is supported by animal microdialysis experiments some of which suggest there is no acute increase in synaptic 5HT, but instead a delayed increase which would mirror the delayed therapeutic effect of the SSRIs (Fig. 4).

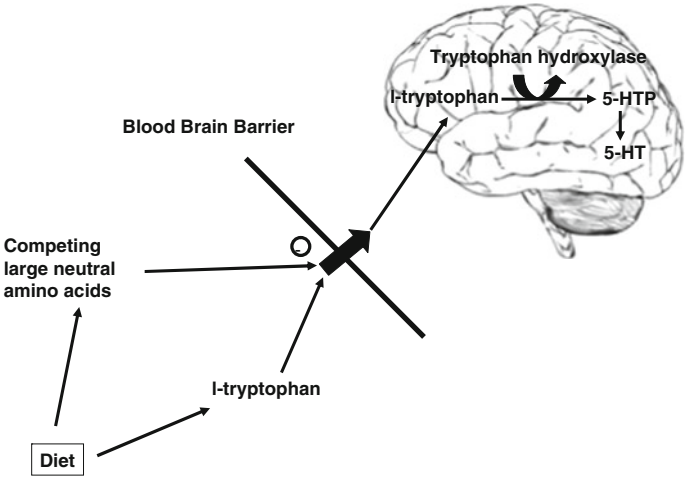

Fig. 4 Acute tryptophan depletion works by dietary manipulation of the tryptophan vs. large neutral amino-acid ratio. By altering this ratio the brain level of tryptophan, and thus serotonin, can be reduced by up to 80%

This has lead to a competing theory of serotonin deficiency in anxiety disorders. In this theory, SSRIs do indeed increase serotonin levels as part of their therapeutic effect. However, this effect is delayed due to the initial increased stimulation of 5HT-1A autoreceptors – which decrease raphe firing. It is only when the 5HT-1A receptors are downregulated that the raphe firing rate recovers. This, combined with the SSRI blocking the removal of 5HT from the synapses, increases 5HT throughput to produce a therapeutic effect (Blier et al. 1990). It should be noted that the only two trials of ATD in treated obsessive compulsive disorder showed no worsening of symptoms (Rasmusson et al. 1997). This may be because no challenge (such as flumazenil in panic or social scripts in social anxiety) was undertaken during the study, or may reflect a fundamental difference in the disorder.

Deakin and Graeff (Graeff et al. 1996) proposed that different serotonergic pathways could have different effects upon anxiety behaviours (i.e., anxiogenic in one, anxiolytic in another), thus explaining the apparent contradictory information regarding the effect of serotonin in anxiety. In this model, the dorsal raphe pathway to the amygdala and cortex increases conditioned fear (as seen in phobias, etc.) and the pathway from the dorsal raphe to the periaqueductal grey attenuates fight/flight responses (as seen in panic). More recently a specific subset of serotonergic neurones arising in the ventrolateral dorsal raphe nucleus has been found to project to the rostral ventrolateral medulla (where it inhibits stress related sympathetic activation) and the dorsolateral periaqueductal grey (where it inhibits behavioural fight/flight responses; Johnson et al. 2004).

Imaging studies have implicated 5HT-1A receptor abnormalities in anxiety disorders. Reduced 5HT-1A receptor binding has been found in panic disorder (Nash et al. 2008; Neumeister et al. 2004), and also social anxiety disorder (Nash et al. 2008; Lanzenberger et al. 2007). Moreover, in the Nash study a reduction of 5HT-1A receptors was found in the orbito-frontal cortex, dorsal raphe, amygdala and temporal lobes – undoubtedly part of the brain anxiety circuit. This reduction was found to recover in some areas, notably the prefrontal cortex, with successful SSRI treatment, but not in the dorsal raphe. It could be postulated that this continuing deficit may account for the continued high rates of relapse in panic disorder, even after several years of successful remission with SSRIs (Choy et al. 2007). A PET study in healthy controls found the fewer 5HT-1A receptors, the greater anxiety scores (Tauscher et al. 2001). Clinically, the 5HT-1A partial agonist buspirone is effective in the treatment of GAD, but has failed trials in panic disorder (Sheehan et al. 1990). Other 5HT-1A agonists have also failed to prove helpful in panic disorder (van et al. 1996), suggesting that although abnormal levels of this receptor may be seen in panic, targeting this receptor alone is insufficient to alleviate the disorder.

Post-synaptic 5HT-2C receptors are downregulated following successful treatment with SSRIs which may explain the progressive attenuation of the jitteriness syndrome with SSRIs (Millan 2005). 5HT-2C antagonists are effective in alleviating anxiety in animal experiments (Griebel et al. 1997). The mixed melatonin agonist and 5HT-2C antagonist agomelatine is efficacious in treating major depression (Eser et al. 2007), and is currently under investigation in anxiety.

3 Noradrenaline

The catecholamine noradrenaline is produced from hydroxylation of dopamine by dopamine β-hydroxylase. Although it can then be converted to adrenaline (epinephrine) by phenylethanolamine *N*-methyltransferase, the major catecholamine transmitter in the brain is noradrenaline. After release noradrenaline is actively transported back into neurones via specific transporters. In neurones, it is catabolised by monoamine oxidase (MAO) to form 3-methoxy-4-hydroxyphenylglycol (MHPG). Noradrenaline can also be catabolised in the glial cell by catechol-*O*-methyltransferase (COMT) to form normetanephrine. Most noradrenergic neurones arise in the locus coeruleus in the brainstem and project extensively throughout the forebrain.

There are two main types of adrenoreceptors: α and β receptors. The α come as subtypes, e.g., α1 or α2, each of which have further subtypes. α2 are the most implicated in anxiety disorders. These receptors are both pre-synaptic and post-synaptic. The pre-synaptic α2 receptors act as autoreceptors (Hein et al. 1999), inhibiting further noradrenaline release. They are also present pre-synaptically on serotonergic (Trendelenburg et al. 1994b) and dopaminergic (Trendelenburg et al. 1994a) neurones where they act as inhibitory heteroreceptors. Stimulation of post-synaptic α2 receptors releases growth hormone from the pituitary, an action that has been used as a proxy for the function of the central noradrenaline system in mood and anxiety disorders. α1 receptors are post-synaptic and are involved in blood pressure regulation and arousal. Peripheral β receptors are well known to mediate the peripheral autonomic effects of anxiety (such as increased heart rate, tremor and sweatiness), but are not thought to directly relate to the conscious experience of anxiety. β receptors also exist in the brain, but it remains unclear as to their function in humans, although a recent experiment with mice has implicated them in cocaine induced anxiety (Schank et al. 2008).

From the clinical symptoms of anxiety (such as tremor, sweatiness, palpitations, etc.) it is clear that the peripheral autonomic nervous system is activated in anxiety. These symptoms can be blocked by β blocking drugs, such as propranolol. However, β blockers do not alleviate the mental symptoms of anxiety (Maes et al. 2002; Munjack et al. 1989). Direct measurement of the human brain sympathetic activity is not possible. Therefore, indirect evidence, such as challenge tests and peripheral markers, have been used to assess whether the brain sympathetic system is also overactive in anxiety, as summarised in Table 2.

Blood noradrenaline samples have been used as a proxy measure of brain sympathetic activity. This is because noradrenaline is not produced (to any large extent) by the adrenal glands (Brown et al. 1981) and blood noradrenaline levels correlate with CSF levels (Ziegler et al. 1977). Therefore, circulating noradrenaline to some extent reflects central activity. Studies have not shown any difference in circulating noradrenaline in anxiety patients under normal conditions (Maes et al. 2002; Munjack et al. 1990). However, in panic disorder increased cardiac noradrenaline spillover is found (Alvarenga et al. 2006). This is associated with increased

Table 2 Challenge tests of the sympathetic system in anxiety disorders

Test	Rationale	Result	Interpretation
Blood NA levels	Proxy of brain NA levels	No differences in resting state between anxiety disorders and controls	Need to challenge NA system to detect functional abnormalities
Clonidine – growth hormone (GH) response	Measure of post-synaptic α2 receptors	Blunted GH response in panic (unstimulated GH levels normal in panic)	Subsensitive post-synaptic α2 receptors in panic. Normal GH levels at rest indicate abnormal reactivity of nAdr system
Clonidine – MHPG or blood pressure (BP)	Measure of pre-synaptic α2 receptors	Exaggerated drop in MHPG and BP in panic	Supersensitive pre-synaptic α2 receptors
Yohimbine	Measure of pre-synaptic α2 receptors	Exaggerated MHPG in panic	Supersensitive pre-synaptic α2 receptors
Measure locus coeruleus (LC) firing	Main source of NA neurones/ measure of sympathetic tone	Many classes of anti-depressant attenuate LC firing	Possible common anxiolytic effect of anti-depressants is reducing LC firing

sympathetic neuronal activity and overall it appears that excess noradrenaline activity in the heart is linked to panic disorder (Lambert et al. 2006).

However, these measures only reflect the resting state and does not give any information about the reactivity (or 'functional integrity') of the sympathetic system. For this to be assessed patients are challenged with a stressor (either pharmacological such as carbon dioxide, or flumazenil – depending upon the mechanisms of interest, or contextual, such as presenting tasks in social anxiety disorder), and then monitored for abnormal reactions.

In challenge tests with drugs that increase brain noradrenaline levels, such as yohimbine, the level of anxiety corresponds to measures of sympathetic activity, i.e., the more anxious the higher the sympathetic drive (Charney et al. 1984). Moreover, illicit drugs which increase noradrenaline levels have long been known to cause anxiety (Louie et al. 1989). The converse of this is also true; lowering the sympathetic drive with drugs such as clonidine or imipramine reduces anxiety in tandem with reductions of sympathetic outflow (Ko et al. 1983). Many classes of drugs which are efficacious in anxiety disorders have been shown to reduce locus coeruleus firing, the main source of noradrenergic neurones. These drugs include SSRIs, tricyclic anti-depressants, MAO inhibitors and selective noradrenergic reuptake inhibitors (Szabo and Blier 2001). It is interesting to note that mirtazapine, an α2 receptor antagonist which increases locus coeruleus firing (Millan et al. 2000), has yet to have robust evidence of efficacy in anxiety disorders despite having a pharmacologic profile which should otherwise prove anxiolytic

(such as 5HT-2C receptor antagonism). It could be postulated that the increase in locus coeruleus firing counteracts any further anxiolytic effects, but this hypothesis has yet to be confirmed.

Most studies of sympathetic functioning have revolved around the α2 receptors. Post-synaptic α2 receptor functioning can be assessed by the level of growth hormone response to drugs acting upon α2 receptors. Clonidine, due to its α2 partial agonist properties, stimulates the release of growth hormone from the pituitary. This response is blunted in panic disorder, indicating a decreased sensitivity of the post-synaptic α2 receptors (Nutt 1986). However, 24-h growth hormone release in unstimulated conditions is normal in panic disorder, indicating a deficiency in functional reactivity of the sympathetic system (Abelson et al. 2005). The growth hormone response to clonidine in patients with other anxiety disorders has been less well studied. There is one study showing a decreased response in patients suffering GAD (Abelson et al. 1991). However, the evidence is mixed in both social anxiety disorder (Tancer et al. 1994, 1993) and obsessive compulsive disorder (Habib et al. 2000; Hollander et al. 1991).

Clonidine can also be used to assess the pre-synaptic α2 receptors. As noted above, clonidine decreases the firing rate of the locus coeruleus (Ko et al. 1983). It also reduces the frequency of panic attacks in panic disorder, but unfortunately this effect is not sustained due to the development of tolerance (Uhde et al. 1989). Panic disorder patients also show exaggerated responses to clonidine as measured by a larger decrease in blood levels of MHPG (Charney and Heninger 1986) and hypotension (Nutt 1986). Further assessment of pre-synaptic α2 receptors functioning has been performed with the α2 antagonist Yohimbine; it increases anxiety in healthy controls (Goldberg et al. 1983) and gives a disproportionately large increase in MHPG in panic disorder patients (Charney et al. 1984). The responses to clonidine and yohimbine suggest, in panic disorder at least, that there are supersensitive pre-synaptic α2 receptors to both their agonists and antagonists. This will make control over locus coeruleus firing increasingly brittle and unstable which may pre-dispose to anxiety. This may result in exaggerated responses to minimal triggers and possibly a pre-disposition to panic (Nutt 1989). The reduced sensitivity of post-synaptic α2 receptors seen also fits this model; overstimulation resulting in eventual downregulation and subsequent blunted growth hormone responses. α2 receptors are also found upon blood platelets. The expression of these receptors have also been measured as a proxy for the expression of brain α2 receptors and they have been found to increase after stress in humans (Maes et al. 2002). This again correlates with animal models which show central α2 receptors may be altered by stress (Stanford and Salmon 1989). If confirmed in humans this provides an explanation for the association between life events and the development of panic attacks (Roy-Byrne et al. 1986). The α2 receptors are split into three subtypes, α2A, α2B and α2C, which may play different roles in anxiety. However, because at present there are no clinically available subtype selective drugs, the relevance of these subtypes in the clinic remains limited.

4 Dopamine

Dopamine is synthesised from the dietary essential amino acid tyrosine. Tyrosine hydroxylase converts tyrosine to L-Dopa, which in turn is converted to dopamine by dopa decarboxylase. As mentioned before dopamine can then be further metabolised to noradrenaline in noradrenergic neurones. However, it is commonly catabolised into homovanillic acid (HVA) by pathways involving either MAO or COMT. Like noradrenaline and serotonin, dopamine is actively transported back into the neurone by specific transporters following release into the synaptic cleft. The main dopaminergic pathways are the nigrostriatal (involved in motor control), tuberoinfundibular (inhibitory to prolactin release by the pituitary), mesolimbic (associated with reward behaviours) and mesocortical pathways (abnormalities of which are implicated in schizophrenia). Dopamine acts upon five different receptors (D1–D5), which are clustered into two families. The D1-like (D1+D5) family are excitatory and g-protein linked to adenylyl cyclase. The D2-like family (D2, D3+D4) are inhibitory and linked via g-proteins to phosphodiesterase.

A number of clinical observations have linked dopamine to social anxiety disorder. First, social anxiety is commonly seen as an early symptom of Parkinson's disease, which is the archetypal neurological disease of dopamine deficiency (Berrios et al. 1995). Second, chronic stimulant abusers may also be vulnerable to the development of social anxiety disorder; presumably the stimulant abuse has damaged the dopaminergic terminals resulting in a functional deficiency (Williams et al. 2000). Third, treatment with dopamine blocking drugs, such as the neuroleptics, has also produced reports of social phobia as a side effect (Scahill et al. 2003). Fourth, there are also case reports of successful treatment of social anxiety disorder with phenelzine or bupropion which increase dopamine availability (although they both also have actions on other monoamines too; Liebowitz et al. 1992). From this it may be tempting to hypothesise that dopamine deficiency is fundamental to social anxiety. However, this evidence is indirect, or preliminary, and must be interpreted with care.

HVA has been used as a proxy measure of dopamine turnover. In one study, there were no differences between social phobia, panic disorder and normal controls with respect to blood HVA levels. However, a small subgroup of this cohort also had CSF tested for HVA; this showed a significant decrease in CSF HVA in the patients with social anxiety + panic disorders (there was no social anxiety alone group) compared to either panic disorder patients or controls (Johnson et al. 1994).

Imaging studies have produced further evidence of an association between dopamine and social anxiety. A SPECT study has shown reduced striatal dopamine reuptake sites, a result which mirrors that seen in early Parkinson's disease (Tiihonen et al. 1997a). Further imaging studies have also shown reduced D2 receptor binding in social anxiety disorder (Schneier et al. 2000, 2008).

Social anxiety patients challenged with L-Dopa did not differ from controls on either endocrine (prolactin levels) or behavioural (eye-blinking) measures (Tancer et al. 1994). A more recent study challenged social anxiety patients with both a

dopamine agonist (pramipexole) and antagonist (sulpiride) before and after treatment with an SSRI. This study found both the agonist and antagonist increased anxiety in untreated patients, but that treatment with an SSRI reduced the anxiety associated with the dopamine agonist, but not the antagonist (Hood et al. 2006). The authors postulated that the SSRIs effect was via post-synaptic D3 receptor desensitisation.

There is less evidence of dopamine dysfunction in other anxiety disorders. In obsessive compulsive disorder, there is clinical evidence that some patients respond to D2 blockers (Goodman et al. 1992). However, imaging studies have been mixed showing both lower (Denys et al. 2004) and no change (Schneier et al. 2008) in numbers of D2 receptors in obsessive compulsive disorder. In panic disorder, levels of HVA in the CSF are unchanged from controls (Eriksson et al. 1991).

5 Glutamate

Glutamate is the major excitatory neurotransmitter in the brain which acts upon both metabotropic and ionotropic receptors. It is thought to mediate effects upon memory, learning, performance and anxiety. There are eight metabotropic receptors which can be classified into three groups. The group 1 receptors comprise mGlu1 and mGlu5. This group is excitatory, post-synaptic and is positively coupled to phospholipase C (Shigemoto et al. 1997). Group 2 comprises mGlu2 and mGlu3 receptors. These receptors are located either pre-synaptically (mGlu2) or post-synaptically and on glial cells (mGlu3; Ohishi et al. 1993). This group of receptors are inhibitory and negatively coupled to Adenylate cyclase (Shigemoto et al. 1997). Group 3 receptors (mGlu4, mGlu6, mGlu7, mGlu8) are also inhibitory, are negatively coupled to adenylate cyclase and are pre-synaptic (Cartmell and Schoepp 2000).

The ionotropic receptors effect ion channels, and therefore directly mediate synaptic excitability. They consist of *N*-methyl-*D*-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainite receptors. NMDA receptors comprise two NR1 subunits and two or three NR2 subunits. There are eight different NR1 and four different NR2 subtypes available, thus allowing for significant heterogeneity between different NMDA receptors. When activated, NMDA channels open, allowing an influx of sodium and calcium ions into the cell causing cell depolarisation. The NMDA receptor itself contains several modulatory sites. The inhibitory sites include phencyclidine and magnesium, which in the resting state 'blocks' the ion channel preventing any sodium and calcium influx. The excitatory sites include polyamide, zinc and glycine – a necessary co-agonist for activation. AMPA and kainite receptors are frequently co-localised with NMDA receptors and are synergistic to NMDA mediated transmission. Indeed, AMPA activation may be necessary to depolarise the cell before Ca^{2+} can enter the cell upon NMDA activation (Javitt 2004).

A wealth of pre-clinical evidence has shown anxiolytic effects of blocking NMDA glutamatergic receptors. MK 801 (a non-competitive NMDA antagonist) and AP-7 (competitive NMDA antagonist) both decrease anxiety behaviours in rats (Plaznik et al. 1994). Glycine site antagonists (such as L-701,324) have also been found to be anxiolytic in some animal models (Kotlinska and Liljequist 1998). However, when transferred to human studies, direct antagonists of NMDA receptors tend to cause serious side effects such as sedation, psychosis and memory impairment. (Bergink et al. 2004).

The problem of major side effects with direct NMDA antagonism has caused a shift away from this approach to more subtle ways of decreasing glutamatergic tone as a way of decreasing anxiety. This will hopefully have the effect of minimising the prohibitive side effects of direct NMDA antagonism, whilst retaining the potential for efficacy. From the information above, potential targets for this can be deduced: antagonism of AMPA or kainite receptors and regulation of metabotropic glutamatergic receptors (such as group I mGlu antagonists or group II agonists). To date, most potential compounds (of which many have been identified – especially metabotropic modulators) are still at the pre-clinical stage. Here we will describe only those which have been tested in human subjects.

Fenobam was a drug developed in the 1980s as a non-BDZ anxiolytic. Its development was ceased after it was thought to have a psychostimulant effect. Recently, it has been discovered that fenobam is an mGlu5 receptor antagonist, which has reawakened interest in it. The non-selective group II mGlu agonist LY354740 has shown reduced anxiety in humans during carbon dioxide challenges and fear-potentiated startle tests (Grillon et al. 2003; Schoepp et al. 2003). However, it has not proven efficacious in panic disorder, with poor bioavailability being a potential reason (Bergink and Westenberg 2005). To counter these bioavailability problems a prodrug of LY354740 was developed with improved bioavailability, LY544344. Unfortunately, testing has been halted to the development of seizures in pre-clinical studies (Dunayevich et al. 2008).

6 Conclusions

We have reviewed the abnormalities detected thus far in the major neurotransmitter systems implicated in anxiety disorders (GABA, serotonin, noradrenaline and dopamine). In recent years, there has been an exponential increase in research of and subsequent understanding of the neurochemistry of anxiety. In part, this has been driven by a greater acknowledgement of the degree of morbidity and frequency of these disorders, but is also aided by the acquisition of new tools, such as imaging techniques, which give us the potential to directly assess brain abnormalities in vivo for the first time. However, at this stage, there are more questions than answers; even the mechanism of action of the SSRIs on mood states is under debate (such as the possibility that they alter the neurosteroid levels as their primary action (Uzunova et al. 1998). There are other brain systems implicated in anxiety disorders

we have not reviewed here (metabotropic glutamate receptors, cannabinoids, neuropeptides to name a few), mainly due to a lack of clinical evidence at this time. However, we expect this to change dramatically in the coming years as bioavailable ligands for specific receptors become more readily available. No single approach (pharmacological challenge testing, imaging studies, pre-clinical studies, etc.) will provide a comprehensive guide to brain dysfunction in anxiety disorders. Therefore, it is important to continue to develop and utilise the range of tools we have available to enable more effective diagnosis and treatment of these common and disabling conditions.

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$GABA_A$ Receptor α2/α3 Subtype-Selective Modulators as Potential Nonsedating Anxiolytics

John R. Atack

Contents

Abstract Nonselective benzodiazepines exert their pharmacological effects via $GABA_A$ receptors containing either an α1, α2, α3, or α5 subunit. The use of subtype-selective tool compounds along with transgenic mice has formed the conceptual framework for defining the requirements of subtype-selective compounds with potentially novel pharmacological profiles. More specifically, compounds

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which allosterically modulate the α2 and/or α3 subtypes but are devoid of, or have much reduced, effects at the α1 subtype are hypothesized to be anxioselective (i.e., anxiolytic but devoid of sedation). Accordingly, three compounds, MRK-409, TPA023 and TPA023B, which selectively potentiated the effects of GABA at the α2 and α3 compared to α1 subtypes were progressed into man. All three compounds behaved as nonsedating anxiolytics in preclinical (rodent and primate) species but, surprisingly, MRK-409 produced sedation in man at relatively low levels of occupancy (<10%). This sedation liability of MRK-409 in man was attributed to its weak partial agonist efficacy at the α1 subtype since both TPA023 and TPA023B lacked any α1 efficacy and did not produce overt sedation even at relatively high levels of occupancy (≥50%). The anxiolytic efficacy of TPA023 was evaluated in Generalized Anxiety Disorder and although these clinical trials were terminated early due to preclinical toxicity issues, the combined data from these incomplete studies demonstrated an anxiolytic-like effect of TPA023. This compound also showed a trend to increase cognitive performance in a small group of schizophrenic subjects and is currently under further evaluation of its cognition-enhancing effects in schizophrenia as part of the TURNS initiative. In contrast, the fate of the back-up clinical candidate TPA023B has not been publicly disclosed. At the very least, these data indicate that the pharmacological profile of compounds that differentially modulate specific populations of $GABA_A$ receptors is distinct from classical benzodiazepines and should encourage further preclinical and clinical investigation of such compounds, with the caveat that, as exemplified by MRK-409, the preclinical profile might not necessarily translate into man.

Keywords GABAA receptor · Benzodiazepine · Subtype-selective · Anxiolytic · Selective efficacy

1 Introduction

It is now approaching 50 years since benzodiazepines entered clinical practice in the form of chlordiazepoxide (Librium®) and shortly thereafter diazepam (Valium®; Sternbach 1979). Subsequently, a variety of analogues based upon the same 1,4-benzodiazepine core structure were also introduced (Fig. 1), including the so-called high-potency (high-affinity) benzodiazepines such as lorazepam, clonazepam, and alprazolam (Moroz 2004). Benzodiazepines rapidly gained acceptance based upon their efficacy as anxiolytics and hypnotics as well as their greatly improved safety profile compared to the barbiturates that they superseded (Ator 2005). The popularity of benzodiazepines and their use as lifestyle drugs is exemplified by the Rolling Stones who included on their 1966 album *Aftermath* a track about benzodiazepines titled "Mothers Little Helper" and which described how "She [mother] goes running for the shelter; Of a mother's little helper; And it helps her on her way; Gets her through her busy day."

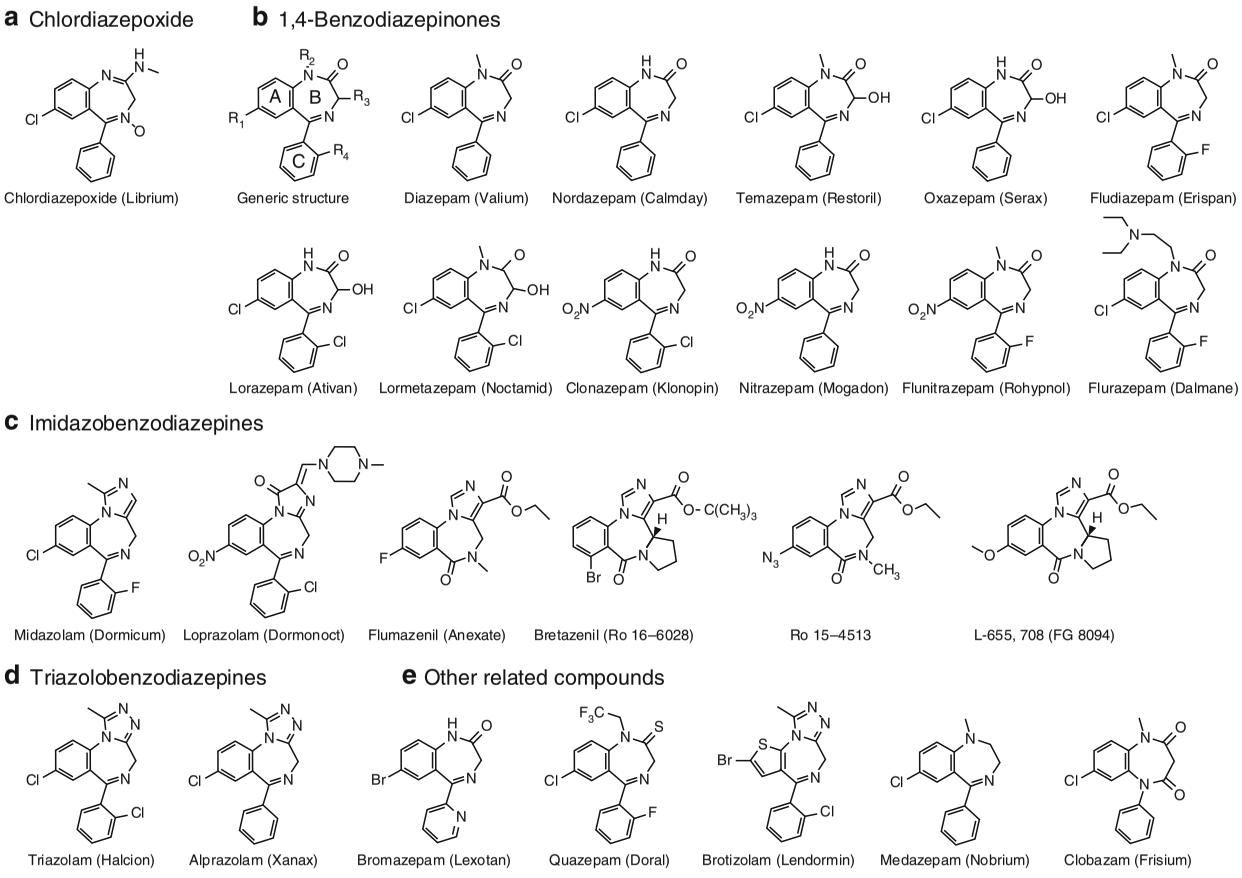


Fig. 1 Structures of members of the benzodiazepine family of GABA$_A$ modulators. (**a**) The first marketed benzodiazepine was chlordiazepoxide (Librium), which was introduced in 1960 (Sternbach 1979). (**b**) Subsequent studies showed that the *N*-oxide and methylamino groups were not required for

There is little doubt that the use of benzodiazepines represented a major advance in psychopharmacology, in terms of both safety and efficacy (Gorman 2005). However, these beneficial effects began to be overshadowed by the emergence of reports of physical and psychological dependence, such as those described in Barbara Gordon's 1979 book *I'm Dancing As Fast As I Can*. Issues associated with dependence and the cessation of benzodiazepine use are detailed on the internet at sites such as www.benzo.org.uk, a location that includes Heather Ashton's *Benzodiazepines: How They Work and How to Withdraw*. The problem of physical dependence was exacerbated by the large scale overprescribing of benzodiazepines as a result of either inappropriate prescribing to people that did not have a real medical need or a continuation of prescribing for much longer than actually required. In the 1980s, this situation was highlighted in the UK by the popular BBC consumer program *That's Life* and the backlash against benzodiazepines arguably reached its nadir in the mid to late 1980s with, for example, the introduction in 1989 of regulations imposed by the NewYork State requiring benzodiazepines to be prescribed on the State's triplicate-copy prescription forms (Woods 1998); an unfortunate consequence of which was that benzodiazepines were often replaced by older, less effective, and less safe drugs (Weintraub et al. 1991).

It is now generally accepted that the use of benzodiazepines is associated with a variety of risks (Woods et al. 1992; O'Brien 2005). These include sedation in subjects taking them as anxiolytics, resulting in, for example, reduced performance while driving (O'Hanlon et al. 1982); increased falls and associated hip fractures in the elderly (Cumming and Le Couteur 2003); interaction with ethanol, with flunitrazepam (Rohypnol) being an archetypal date-rape drug (Woods

Fig. 1 (continued) pharmacological activity and a variety of 1,4-benzodiazepinone compounds could be generated by varying the substituent at the R1 position in the A-ring, the R2 and R3 positions in the B-ring and the R4 position in the C-ring. Within this series of 1,4-benzodiazepinones, the prototypic compound is diazepam, which was introduced under the trade name of Librium in 1963 (Sternbach 1979). Diazepam is extensively metabolized in man and a number of these metabolites, including nordazepam (*N*-desmethyldiazepam), temazepam and oxazepam have been marketed separately. (**c**) Introduction of an imidazo group into the benzodiazepine core results in structures typified by the clinically used drugs midazolam, loprazolam, and flumazenil, the latter of which is the prototypic benzodiazepine antagonist (Hunkeler, et al. 1981). In addition, bretazenil (Ro 16-6028), which progressed into man but never reached the market (Van Steveninck et al. 1996), Ro 15-4513 (Suzdak et al. 1986, 1988) and L-655,708 (Quirk et al. 1996; Atack et al. 2006a) remain pharmacological tools. (**d**) Introduction of an extra nitrogen into the imidazobenzodiazepine core results in triazolobenzodiazepines, such as triazolam and alprazolam. (**e**) Additional compounds that are structurally related to the 1,4-benzodiazepinones include: bromazepam (in which the C-ring phenyl group is replaced by a pyridyl), quazepam (a 1,4-benzodiazepine-2-thione), brotizolam (a thienotriazolodiazepine), medazepam (which lacks a carbonyl substituent on the benzodiazepine core and has diazepam as one of its metabolites, de Silva and Puglisi 1970), and clobazam, which is a 1,5- rather than 1,4-benzodiazepine and possesses two rather than the more usual one carbonyl groups and is reported to have a reduced sedation liability (Wildin et al. 1990). The generic names are shown with representative trade or brand names within parentheses. However, trade names can differ from country to country and a more complete listing is provided elsewhere (http://www.non-benzodiazepines.org.uk/benzodiazepine-names.html)

and Winger 1997); withdrawal upon abrupt cessation (Ashton 2005; Lader et al. 2009), although "tapering" can be used as a mitigation (Shader and Greenblatt 1993); or abuse, which seems preferentially associated with habitual drug abusers (Woods and Winger 1995; Ator 2005). However, all these risks need to be balanced against the clinical efficacy of benzodiazepines, particularly as regards anxiolytic activity (Nutt 2005), and the generally safe clinical profile of such drugs.

Since the multiple clinical effects of benzodiazepines are associated with four distinct populations of the $GABA_A$ receptor (discussed later), it has been hypothesized that by selectively targeting certain subtypes it might be possible to retain the anxiolytic activity seen with the nonselective benzodiazepines (i.e., benzodiazepines which interact equally with all four subtypes) yet avoid the sedation, physical dependence, abuse, and ethanol interaction liabilities (Atack 2003). Accordingly, efforts at pharmaceutical companies such as the Danish company NeuroSearch A/S and Merck Sharp & Dohme (the European subsidiary of Merck & Co., Inc.) have focused on developing $GABA_A$ receptor subtype selective compounds that are anxioselective (i.e., nonsedating anxiolytics). The purpose of the present chapter is to review the properties of those compounds emerging from the Merck Sharp & Dohme laboratories and that progressed into man, namely MRK-409, TPA023, and TPA023B.

2 Benzodiazepines and the $GABA_A$ Receptor

Although benzodiazepines were introduced at the start of the 1960s, it was only in the mid-1970s that their GABA-mediated mechanism of action began to be understood (Costa et al. 1975; Haefely et al. 1975). With the delineation of the effects of GABA within the CNS to $GABA_A$ and $GABA_B$ receptor subtypes (Bowery et al. 1980, 1987), it became apparent that benzodiazepines modulated the effects of $GABA_A$ receptors. Furthermore, based upon their differential affinity for nonbenzodiazepine compounds, such as the triazolopyridazine CL-218872, two subtypes of the $GABA_A$ receptor benzodiazepine recognition site, designated BZ_1 and BZ_2, were identified (Klepner et al. 1979).

The molecular basis for the $GABA_A$ receptor BZ_1 and BZ_2 subtypes was clarified with the elucidation of the subunit composition of $GABA_A$ receptors. Hence, the purification and sequencing of native $GABA_A$ receptors (Sigel and Barnard 1984) triggered the cloning of a family of $GABA_A$ subunit genes which initially comprised just single α and β subunits (Schofield et al. 1987), but was subsequently extended to include a total of 19 mammalian genes (α1–6, β1–3, γ1–3, δ, θ, π, ρ1–3) with additional subunits (β4 and γ4) being found in birds (Simon et al. 2004; Olsen and Sieghart 2008).

$GABA_A$ receptors are members of the Cys-loop super-family of ligand-gated ion channels that also include nicotinic acetylcholine, glycine, and serotonin 5HT3 receptors. The structure of subunits comprising this superfamily is relatively well described, being based upon comparisons with the freshwater snail *Lymnaea*

stagnalis acetylcholine binding protein (Brejc et al. 2001) as well as the nicotinic acetylcholine receptor of *Torpedo marmorata* (Sine and Engel 2006), the collective data from which have permitted homology models of the $GABA_A$ receptor to be generated (Ernst et al. 2005). Hence, each $GABA_A$ subunit consists of a large extracellular hydrophilic N-terminal domain followed by four transmembrane (TM) α-helices and a relatively short intracellular C-terminal domain, with TM2 lining the pore of the ion channel. Native $GABA_A$ receptors are heteropentameric structures and despite the multiple possible permutations of 19 different subunits into a pentameric assembly, only in the region of 20–30 subtypes probably exist in the brain (Olsen and Sieghart 2008), with the majority containing two α, two β, and a single γ, δ or ε subunit (Fig. 2a: Sieghart and Sperk 2002; Sigel et al. 2006).

The benzodiazepine recognition site is formed by a γ2 subunit in conjunction with either an α1, α2, α3, or α5 subunit (Fig. 2b; Sieghart 1995). The sequences of the α4 and α6 subunits differs from those of α1, α2, α3, and α5 in that a histidine residue in the latter subunits is replaced by an arginine in α4 and α6 (Wieland et al. 1992). This single amino acid replacement is sufficient to render α4βγ2 and α6βγ2 receptors diazepam-insensitive, although this binding site is recognized by certain imidazobenzodiazepines, such as Ro 15-4513 (Fig. 2c; Pym et al. 2005).

The α subunit histidine to arginine difference that confers diazepam insensitivity (Wieland et al. 1992) has been used as the basis for generating point-mutated mice in which specific $GABA_A$ receptor populations (i.e., either α1-, α2-, α3-, or α5-containing receptors) are rendered insensitive to the pharmacological effects of diazepam (Benson et al. 1998). Using this approach Rudolph, Mohler, and colleagues performed a series of elegant studies that have begun to delineate which $GABA_A$ subtypes mediate particular aspects of the pharmacology of diazepam and other benzodiazepine site modulators (Rudolph et al. 1999; Löw et al. 2000; Rudolph and Möhler 2006; Knabl et al. 2008, 2009). This approach has been

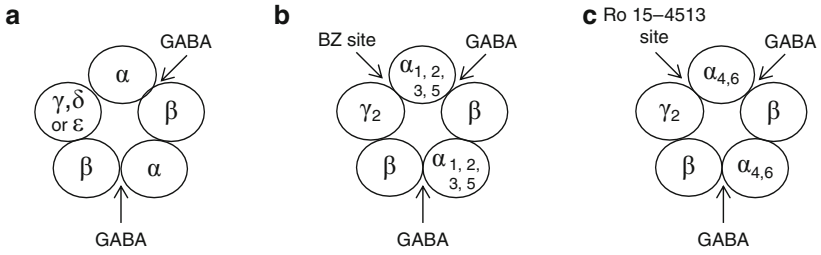


Fig. 2 Schematic representations of the structure of $GABA_A$ receptors. (**a**) The arrangement of subunits in the major subtypes of native $GABA_A$ receptors as viewed from the synapse (Sieghart and Sperk 2002; Sigel et al. 2006; Olsen and Sieghart 2008) with the GABA binding site being formed at the interface of the α and β subunits. (**b**) The benzodiazepine recognition site is formed at the interface of the α and γ2 subunits, with the so-called "classical" benzodiazepine site being defined by either an α1, α2, α3, or α5 subunit. (**c**) Diazepam-insensitive sites, which are recognized by the imidazobenzodiazepine Ro 15-4513, are associated with either an α4 or α6 subunit along with a γ2 subunit

complemented by the use of subtype-selective $GABA_A$ modulators, such as L-838417 (McKernan et al. 2000), data from which is generally in good agreement with transgenic mouse studies. More specifically, it would appear that the α1 subtype is associated with sedation, the α2 and/or α3 subtypes are the "anxiolytic" subtypes (Rudolph et al. 1999; Löw et al. 2000; Atack et al. 2005; Dias et al. 2005) whereas α5-containing $GABA_A$ receptors are associated with aspects of cognition (Dawson et al. 2006; Maubach, 2006). Accordingly, it is hypothesized that a compound that preferentially modulates the α2 and α3 subtypes but has reduced or, preferably, no activity at the α1 subtype should have anxiolytic-like activity but a much reduced sedation liability, i.e., it should be anxioselective (Atack 2003).

3 Identification of MRK-409 and TPA023

Based upon the hypothesis that a compound which preferentially activates α2- and/or α3-rather than α1-containing $GABA_A$ receptors should be anxioselective, initial attempts were directed towards identifying a compound with higher affinity for the α2 and α3 versus α1 subtypes. However, such attempts were unsuccessful with the maximum selectivity achieved being 12-fold (Atack 2009a).

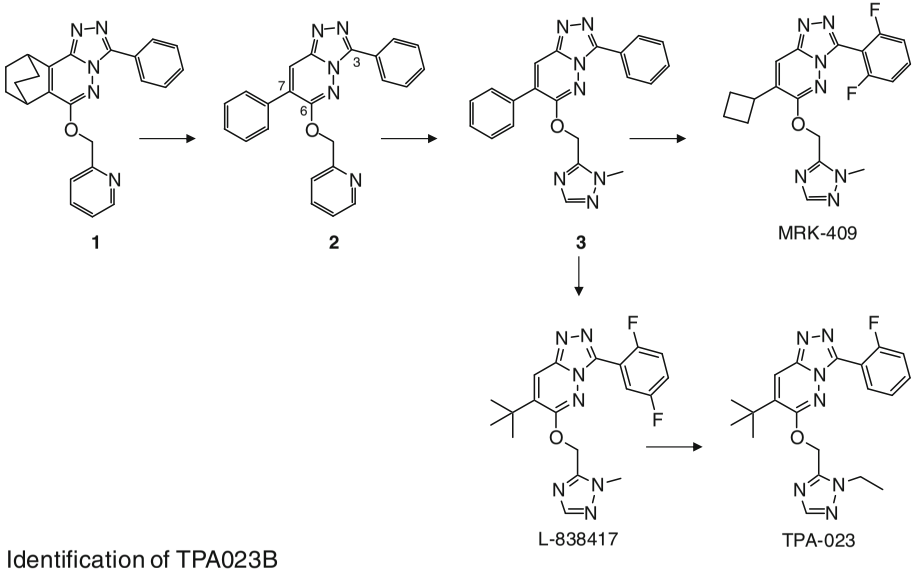
The strategy was then switched to identifying a compound that would bind to each of the four $GABA_A$ subtypes but would have higher efficacy for the α2 and α3 compared to α1 subtypes. The starting point for this selective-efficacy approach was **1** (Fig. 3), which had a slight (3- to 6-fold) α2/α3 versus α1 binding selectivity. Replacement of the [2.2.2] bicyclic ring system with a pendant phenyl group at the C-7 position resulted in a compound, **2**, which had increased affinity and efficacy relative to **1**, but no difference between the efficacy at the α1 and α3 subtypes. Replacement of the 2-pyridyl group at the C-6 position with a 1,2,4-triazole in **3** did not affect affinity but did introduce a modest degree of α3 versus α1 efficacy selectivity (albeit that the efficacy at both subtypes was still high). Efficacy at both subtypes could be lowered appreciably by reducing the hydrophobicity at the C-7 position by replacing the phenyl group with either a cyclobutyl or *t*-butyl moiety and this also increased α3 efficacy selectivity.

The unsubstituted phenyl ring at the C-3 position was a metabolic liability and this could be reduced by fluorination, which in the C-7 cyclobutyl series resulted in MRK-409 and in the C-7 *t*-butyl series produced the prototypic efficacy selective compound L-838417 (McKernan et al. 2000) and its structural analogue TPA023 (Atack et al. 2006b).

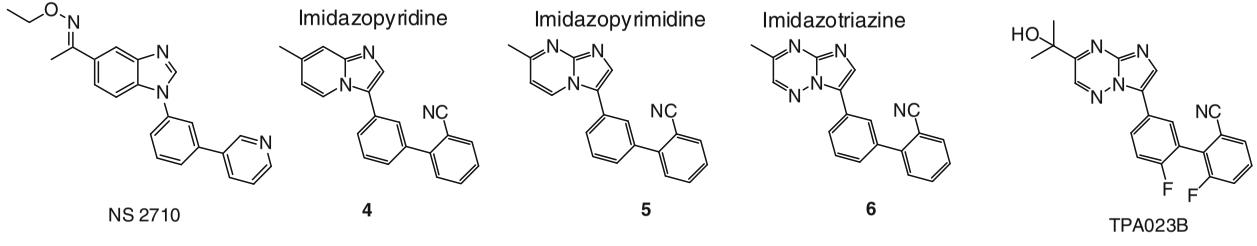
4 Identification of TPA023B

NS 2710 is a benzimidazole that was being developed as a novel anxiolytic by NeuroSearch A/S (Mirza et al. 2003). However, this compound caused skin rashes in Phase II studies and development was terminated. By combining features of NS

a Identification of MRK-409 and TPA023



b Identification of TPA023B



2710 with L-838417, a series of imidazopyridines (e.g. **4**) was described (Fig. 3b; Goodacre et al. 2006) in which the α3 efficacy was consistently greater than that at the α1 subtype. Unfortunately, however, the imidazopyridines had generally poor pharmacokinetic properties in rat. The introduction of an addition nitrogen into the imidazopyridine core resulted in an imidazopyrimidine series (e.g. **5**) in which the α3 versus α1 efficacy-selective structure–activity relationship observed with the imidazopyridines was generally retained (Goodacre et al. 2006; Blackaby et al. 2006). The addition of a further nitrogen gave imidazotriazine compounds (e.g. **6**) that had similar affinities but generally lower efficacy relative to their imidazopyrimidine counterparts whilst, importantly, retaining the α3 versus α1 efficacy selectivity (Jennings et al. 2006; Russell et al. 2006). Within the imidazotriazine series, TPA023B was selected for progression into man (Van Laere et al. 2008).

5 Note on Nomenclature

The nomenclature for the compounds described in the present manuscript can be confusing and merits further discussion. The prototypic efficacy-selective compound originating from the Merck labs, L-838417, was known simply by its unique, internal Merck identifier (or "L-number"). Unfortunately, this unambiguous convention was revised for subsequent compounds. For example, the prefix "MRK" was added to the last three digits of the L-number to describe MRK-409 but elsewhere this compound has been described as MK-0343 (de Haas et al. 2008). As regards TPA023, the "TP" refers to Terlings Park (the Merck site from where the compound originated), the "A" refers to agonist (to differentiate such compounds

Fig. 3 Summary of the identification of the clinical candidates MRK-409, TPA023, and TPA023B. (**a**) MRK-409 and TPA023 are triazolopyridazines originating from **1**. Introduction of a phenyl group at the C-7 position gave **2** and then replacement of the 2-pyridyl group with a 2-methyl-1,2,4-triazole at the C-6 position gave **3**. Replacement of the C-7 phenyl with a cyclobutyl and fluorination of the C-3 phenyl at the 2 and 6 positions gave MRK-409. Alternatively, replacement of the C-7 phenyl with a *t*-butyl and fluorination of the C-3 phenyl at the 2 and 5 positions gave the prototypic α2/α3 efficacy-selective partial agonist L-838417 (McKernan et al. 2000). TPA023 differs from L-838417 in that a 2-fluorophenyl replaces the 2,6-difluorophenyl at the C-3 position and an ethyl replaces the methyl at the 2 position of the 1,2,4-triazole at the C-6 position (Carling et al. 2005). (**b**) TPA023B was derived from the NeuroSearch compound NS 2710 (Mirza et al. 2003). Hence, the benzimidazole core structure of NS 2710 was changed to an imidazopyridine (e.g., **4**) which retained $GABA_A$ receptor affinity but this series had generally poor pharmacokinetic properties in rat. The introduction of an additional nitrogen gave an imidazopyrimidine core (e.g., **5**) that resulted in an approximately 10-fold higher binding affinity and good rat, but initially poor dog, pharmacokinetic properties (due to metabolic instability of the methyl group and the central phenyl ring, both of which were addressed separately; Goodacre et al. 2006). The introduction of an additional nitrogen into the core produced an imidazotriazine core, **6**, which resulted in only a relatively modest reduction in affinity (Russell et al. 2006). Replacement of the C-3 methyl group with a propan-2-ol and the introduction of a central 4-fluorophenyl ring along with a 2-cyano-6-fluorophenyl group resulted in the identification of TPA023B

from inverse agonists such as α3IA and α5IA, Atack et al. 2005; Dawson et al. 2006) and the "023" refers to zero agonism at the α1 but (partial) agonism at the α2 and α3 subtypes. However, this nomenclature has not been consistently employed with TPA023 also being referred to as L-830982 and MK-0777 (Lewis et al. 2008).

Having adopted the "TPA" naming convention meant that subsequent compounds with an efficacy profile similar to TPA023 (i.e., zero efficacy at α1 but agonism at α2 and α3) would also have to share this name and accordingly the back-up compound to TPA023 was given the name TPA023B, although elsewhere, this same compound has also been described as L-891190 (Anonymous 2006). Finally, the "A for agonism" rule has not been consistently applied since a compound with zero efficacy at the α1 and α2 subtypes but agonism at α3 was given the name TP003 (Dias et al. 2005).

6 Comparison of the In Vitro Properties of MRK-409, TPA023, and TPA023B

Figure 4 shows a graphical representation of the affinity and relative efficacy of MRK-409, TPA023, and TPA023B at recombinant human $GABA_A$ receptors, with data for the nonselective partial agonist bretazenil also being included for comparative purposes. The upper graphs illustrate how all four compounds have equivalent affinity at each of the four different subtypes. Moreover, the affinities at human recombinant $GABA_A$ receptors of MRK-409 (Ki values ranging from 0.21 to 0.40 nM), TPA023 (0.19–0.41 nM) and TPA023B (0.73–2.0 nM) are comparable to those observed against native, rat brain receptors (respective Ki values of ~0.3, ~0.3, and 0.3–1.0 nM). The fact that there is comparable affinity at the different subtypes suggests that each compound has analogous molecular interactions with α1-, α2-, α3-, and α5-containing $GABA_A$ receptors. Clearly, however, these interactions presumably produce allosteric changes that translate into differing efficacy at each subtype and although pharmacophore models exist for binding at the benzodiazepine recognition site (Clayton et al. 2007), there are no such models for efficacy.

7 Preclinical Pharmacokinetics of MRK-409, TPA023, and TPA023B

Figure 5 shows the pharmacokinetic profiles of single doses of MRK-409, TPA023, and TPA023B dosed to rats and dogs, with the parameters derived from these data being presented in Table 1. The triazolopyridazines MRK-409 and TPA023 had similar pharmacokinetic properties following i.v. dosing in rats and dogs, with the

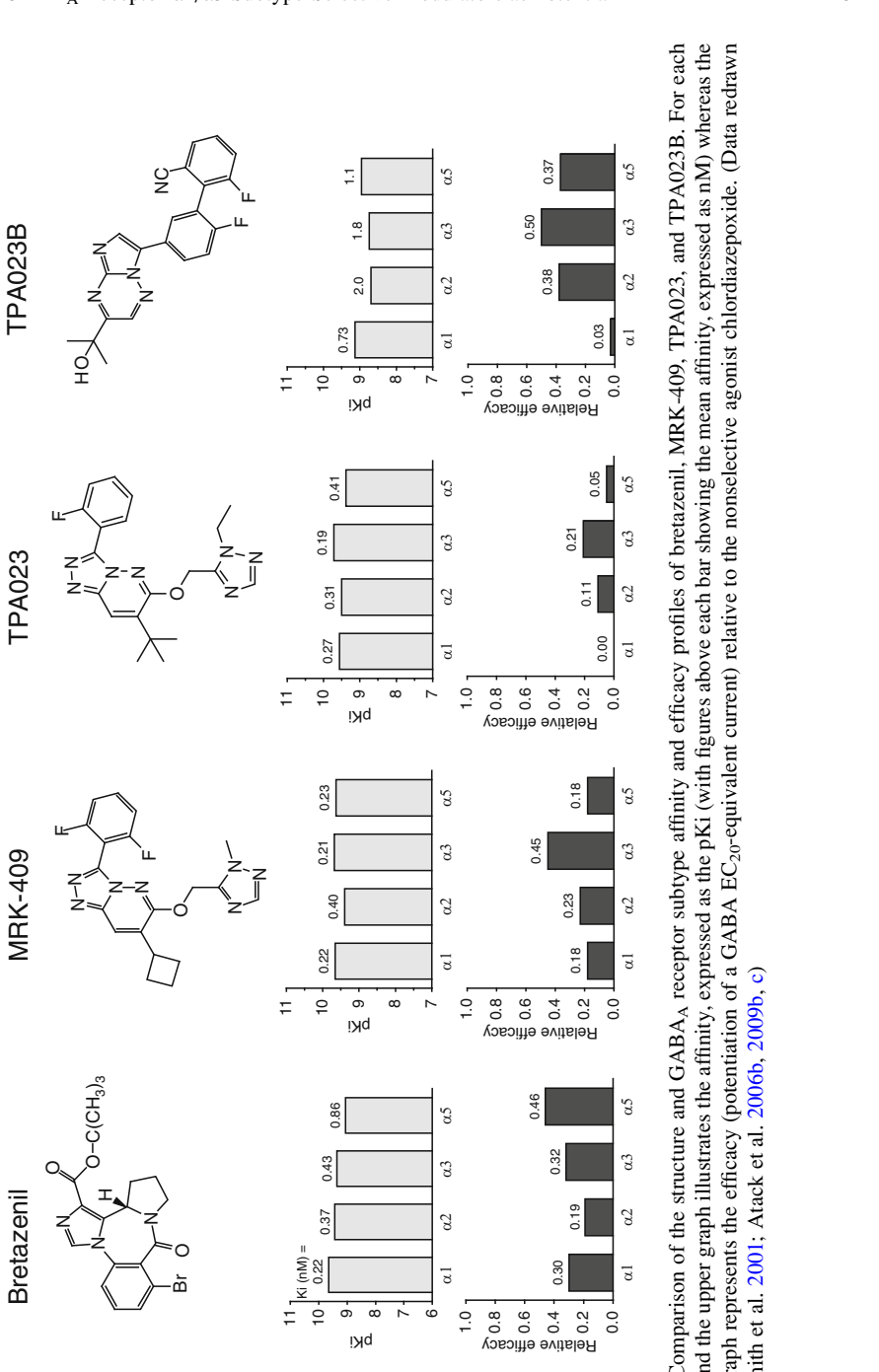


Fig. 4 Comparison of the structure and $GABA_A$ receptor subtype affinity and efficacy profiles of bretazenil, MRK-409, TPA023, and TPA023B. For each compound the upper graph illustrates the affinity, expressed as the pKi (with figures above each bar showing the mean affinity, expressed as nM) whereas the lower graph represents the efficacy (potentiation of a GABA EC_{20}-equivalent current) relative to the nonselective agonist chlordiazepoxide. (Data redrawn from Smith et al. 2001; Atack et al. 2006b, 2009b, c)

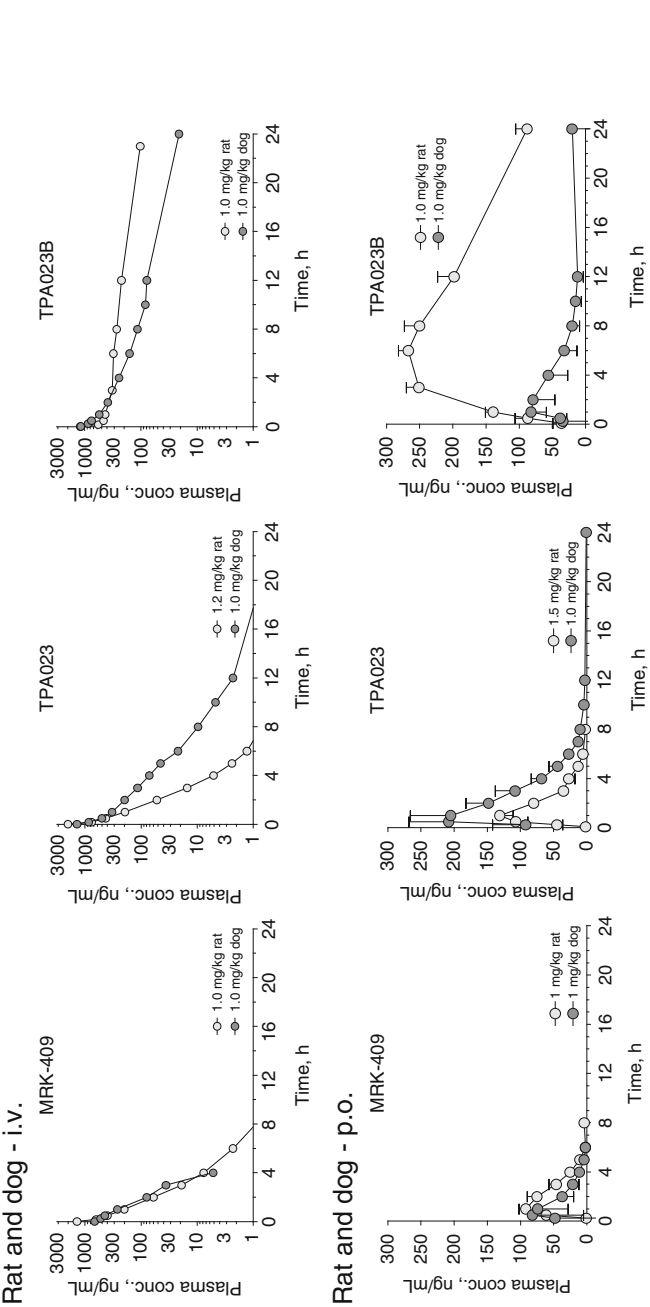


Fig. 5 Pharmacokinetics of MRK-409, TPA023, and TPA023B in rat and dog plasma. (**a**) Kinetics after i.v. dosing to rats and dogs (drug formulated in a 50% polyethylene glycol aqueous vehicle). (**b**) Kinetics after p.o. dosing in a 0.5% methyl cellulose suspension in rats and dogs. Values shown represent mean ± S.D. ($n = 3$/group, except MRK-409 dog p.o., where $n = 6$)

Table 1 Summary of rat and dog plasma pharmacokinetic parameters for MRK-409, TPA023, and TPA023B

		MRK-409		TPA023		TPA023B	
		Rat	Dog	Rat	Dog	Rat	Dog
i.v. dose	(mg kg^{-1})	1.0	1.0	1.2	1.0	1.0	1.0
AUC i.v. (0–∞)	(ng.h mL^{-1})	690 ± 149	704 ± 85	767 ± 33	1293 ± 167	7013 ± 309	3781 ± 990
$t_{1/2}$	(h)	1.2 ± 0.1[a]	0.6 ± 0.0[b]	1.4[c]	1.5[d]	10.7[e]	6.5[f]
CL	(mL min^{-1} kg^{-1})	25 ± 5	24 ± 3	26	13	2.4 ± 0.1	4.6 ± 1.4
Vd_{ss}	(L kg^{-1})	1.1 ± 0.1	1.2 ± 0.1	1.0	1.4	2.1 ± 0.1	1.9 ± 0.2
p.o. dose	(mg kg^{-1})	1.0	1.0	1.5	1.0	1.0	1.0
AUC p.o. (0–∞)	(ng.h mL^{-1})	204 ± 26	177 ± 81	343 ± 81	714 ± 181	5416 ± 669	843 ± 618
C_{max}	(ng mL^{-1})	92 ± 10	94 ± 76	163	208	267 ± 15	88 ± 26
T_{max}	(h)	0.5	0.5–3	0.5	0.5	6	1.3 ± 0.6
Oral bioavailability		30 ± 7%	25 ± 12%	36%	54%	77 ± 10%	22 ± 17%

Results given above are mean ± SD, $n = 3$ except for MRK-409 dog p.o., where $n = 6$

i.v. doses were administered in solution in a 50% polyethylene glycol vehicle at a drug concentration of 1 mg mL^{-1}

p.o. doses were administered as a 0.5% methyl cellulose suspension at a drug concentration of 1 mg mL^{-1} for rats or 0.2 mg mL^{-1} for dogs

AUC Area under the curve, $t_{½}$ Half-life, *CL* Clearance rate, Vd_{ss} volume of distribution at steady-state, C_{max} Maximum plasma concentration, T_{max} Time at which maximum concentration occurred

[a, b]The half life of MRK-409 in rat and dog was estimated from the data between 3–8 and 1–4 h, respectively. Calculation of the mean residence time gave values in rat and dog that were very similar (0.8 and 0.9 h, respectively)

[c, d]The half life of TPA023 in rat and dog was estimated from the data between 4–8 and 2–12 h, respectively. Calculation of the mean residence time gave values in rat and dog that differed by 3-fold (0.6 and 1.8 h, respectively).

[e, f]The half life of TPA023B in rat and dog was estimated from the data between 1–24 and 6–30 h, respectively. Calculation of the mean residence time gave values in rat and dog (15 and 7 h, respectively) that were similar to the half-life values.

relatively low volume of distribution of each compound (ranging from 1.0 to 1.4 L kg^{-1}) combining with moderate to high rates of clearance (13–26 mL min^{-1} kg^{-1}) to produce short half-lives (0.6–1.5 h). In contrast, in both species TPA023B had a slightly higher volume of distribution (1.9–2.1 L kg^{-1}) and reduced clearance (2.4–4.6 mL min^{-1} kg^{-1}) which resulted in much longer half-lives in rat and dog (10.7 and 6.5 h, respectively). Following oral dosing to rats and dogs, the kinetics of MRK-409 and TPA023 again resembled each other, with both compounds having a rapid absorption phase such that T_{max} was generally reached 0.5 h after dosing. However, the absorption of TPA023B was slower than MRK-409 and TPA023 with T_{max} in rat being achieved only 6 h after oral dosing.

For all three compounds, the i.v. kinetics in rhesus monkey were similar to those seen in rat and dog, but for MRK-409 and TPA023 the oral bioavailability values (10% and 1%, respectively) were lower than rat and dog (data not shown). In contrast, the oral bioavailability of TPA023B in rhesus monkey (29%) was within the range measured in rat and dog data (77% and 22%, respectively).

8 Receptor Occupancy in Rats

After oral dosing in rats, the pharmacodynamics (receptor occupancy) of MRK-409, TPA023, and TPA023B reflected the pharmacokinetics. Hence, whereas both TPA023 and MRK-409 showed rapid absorption with their respective T_{max} values for occupancy being 0.5 and 1 h (Atack et al. 2006b, 2009b), maximum occupancy of TPA023B was only achieved after 8 h (Atack et al. 2009b). Moreover, although the dose of TPA023B required to produce 50% occupancy (0.22 and 0.09 mg kg^{-1} 0.75 and 8 h post dose, respectively) was lower than that of TPA023 (0.42 mg kg^{-1}; Atack et al. 2006b), the plasma concentrations required for 50% occupancy were comparable (19 and 25 ng mL^{-1}; Table 2).

The Occ_{50} values of MRK-409, TPA023, and TPA023B (respective values of 2.2, 0.42, and 0.09 mg kg^{-1}) were not related to the affinity of these compounds since the most potent compound, TPA023B, had the lowest affinity, with Ki values for TPA023B ranging from 0.73 to 2.0 nM compared to Ki values of 0.19–0.41 nM and 0.21–0.40 nM for TPA023 and MRK-409, respectively. However, the potency as measured by the Occ_{50} is presumably related, in part, to the absorption characteristics of these compounds, and consistent with its higher potency, TPA023B

Table 2 Summary of potency of MRK-409, TPA023, and TPA023B to inhibit rat brain in vivo [^{3}H]flumazenil binding

Parameter	MRK-409	TPA023	TPA023B
Occ_{50}[a] (mg kg−1 p.o.)	2.2	0.42	0.09
Plasma EC_{50}[b] (ng mL^{-1})	115	25	19

[a]Occ_{50} = dose required to produce 50% occupancy. Compounds were dosed as suspensions in 0.5% methyl cellulose vehicle, using a dose volume of 10 mL kg^{-1}

[b]Plasma EC_{50} = plasma concentration of drug required to produce 50% occupancy

had higher rat oral bioavailability (77%) compared to either MRK-409 or TPA023 (30% and 36%, respectively).

The EC_{50} value is independent of dose and primarily reflects the ability of compound to cross the blood–brain barrier and access its target. If these factors are similar across compounds, then the plasma EC_{50} values should correlate with the affinity of the compounds. However, once again this does not appear to be the case since TPA023 and MRK-409 have essentially identical affinities yet TPA023 is 4–5 times more potent than MRK-409 (respective EC_{50} values of 25 and 115 ng mL^{-1}; Table 2) whereas TPA023B has the lowest affinity of the three compounds (0.73–2.0 nM) but was the most potent with respect to its plasma EC_{50} (19 ng mL^{-1}). Taken together these data demonstrate that potency in an in vivo occupancy assay is not solely related to the in vitro affinity of a compound and is consistent with observations in baboon using [^{11}C]flumazenil PET (Brouillet et al. 1991).

9 Preclinical Nonsedating Anxiolytic Profiles of MRK-409, TPA023, and TPA023B

MRK-409, TPA023, and TPA023B were evaluated in a variety of rodent and primate animal models to assess their anxiolytic- and sedative-like properties. As regards anxiolytic-like effects, all three compounds demonstrated efficacy in the rat elevated plus maze, fear-potentiated startle, and conditioned suppression of drinking assays (Table 3). Similarly, each compound produced dose-dependent anxiolytic-like effects in the squirrel monkey conditioned emotional response, which is analogous to the rat conditioned suppression of drinking. Overall, there was a tendency for MRK-409 to require lower levels of occupancy to produce anxiolysis compared to either TPA023 or TPA023B.

None of the compounds produced overt sedation with performance being unaffected in the mouse rotarod, rat chain-pulling and squirrel monkey lever-pressing assays, even at doses corresponding to levels of occupancy >90%. With respect to ethanol interaction studies, neither TPA023 nor TPA023B produced any appreciable effects as judged using the mouse rotarod assay. On the other hand, MRK-409 did have a marked interaction with ethanol, with the minimum effective dose (3 mg kg^{-1} p.o.) corresponding to 32% occupancy.

10 Additional Preclinical Pharmacology

Since nonselective full agonist benzodiazepines produce physical dependence and have an abuse liability, it is important to assess these aspects in subtype-selective compounds (Ator 2005). In the drug discrimination paradigm, animals are trained to associate the interoceptive cues produced by a training drug with pressing one of

Table 3 Summary of the preclinical nonsedating anxiolytic properties of MRK-409, TPA023, and TPA023B in rodents and primates

Behavior	Assay	Observation	MRK-409		TPA023		TPA023B	
			Dose (mg kg^{-1} p.o.)	Occupancy[b]	Dose (mg kg^{-1} p.o.)	Occupancy[b]	Dose (mg kg^{-1} p.o.)	Occupancy[b]
Anxiety assays								
Rat	EPM	Anxiolysis	2[a]	43%	1	70%	1	88%
Rat	CSD	Anxiolysis	3	63%	1	70%	1	87%
Rat	FPS	Anxiolysis	3	63%	3	88%	0.3	61%
Primate	CER	Anxiolysis	0.1	35%	0.3	65%	0.3	80%
Sedation assays								
Mouse	Rotarod	No effect	10	79%	30	>90%	10	99%
Rat	Chain-pulling	No effect	10	93%	30	99%	10	100%
Primate	Lever-pressing	No effect	10	95%	10	>99%	10	>98%
Ethanol interaction assay								
Mouse	Rotarod	Potentiation of EtOH effects	3	32%	10 (modest)	83	10 (no effect)	100%

[a]Doses refer to either the minimum effective dose or, when no effect was observed, the maximum dose tested

[b]Receptor occupancy was measured either directly after completion of the behavioral assay (EPM and rotarod) or was extrapolated from either the occupancy dose-response curve for rats or, in the case of squirrel monkey, from the plasma drug concentration measured in a separate squirrel monkey pharmacokinetic study and by extrapolation from the rat plasma drug concentration–occupancy relationship

EPM Elevated plus maze, *CSD* Conditioned suppression of drinking, *FPS* Fear-potentiated startle, *CER* Conditioned emotional response

two levers (the "drug" lever) which in turn elicits a food reward. Trained animals are next given the test compound and if this "feels" like the training drug, the animal presses the lever associated with the training drug; if the test compound does not "feel" like the training drug, or produces no interoceptive cues, then the "nondrug" lever is pressed.

The principle behind the self-administration procedure is that drugs which are subject to abuse by humans will also be self-administered by rodents and/or nonhuman primates. Finally, physical dependence refers to the fact that following repeated dosing with a compound, discontinuation of the drug produces a withdrawal syndrome which may include seizure activity, increased aggression, and/or reduced food intake (Ator 2005).

10.1 Drug Discrimination

Although TPA023 does not appear to produce positive reinforcement in the baboon self-administration model (discussed later), it does produce interoceptive cues that allow rats to discriminate it from vehicle, with an ED_{50} of 0.42 mg kg^{-1} i.p. (Kohut and Ator 2008). Interestingly, however, rats could not be trained to discriminate TPA023B (Kohut and Ator 2008), despite that fact that TPA023B has greater intrinsic efficacy at the α2, α3, and α5 subtypes relative to TPA023 (Fig. 4). Nevertheless, TPA023B does apparently produce interoceptive cues which although insufficient to produce discrimination per se, did permit TPA023B to generalize to TPA023 (but not zolpidem or lorazepam; Kohut and Ator 2008). As regards TPA023, it does not generalize to the nonselective full agonist benzodiazepine lorazepam (Ator 2005; but it is not clear if this refers to rats or baboons). Although MRK-409 was not tested, an additional compound, TPA123 (also known as MRK-067), which has an efficacy profile very similar to MRK-409 (Atack 2009a), did demonstrate a partial generalization to lorazepam (Ator 2005). This partial generalization of TPA123 to lorazepam was attributed to its weak α1 agonism (Ator 2005).

10.2 Self Administration

In rhesus monkeys trained to i.v. self-administer methohexital, L-838417 produced a degree of self-administration, albeit to a much reduced extent compared to either zolpidem, midazolam, or diazepam (Rowlett et al. 2005). Consistent with this the rewarding properties of L-838417 were much reduced relative to zolpidem, midazolam, or diazepam as judged by the much lower break-point for L-838417. In other words, animals were willing to work much harder (i.e., press the lever more often) to obtain an injection of zolpidem, midazolam, or diazepam compared to L-838417.

In contrast to L-838417, TPA023 appears to have no rewarding properties in baboons trained on a cocaine baseline since it was not self-administered (Ator 2005), even at a dose (0.32 mg kg^{-1} i.v.) corresponding to essentially 100% $GABA_A$ receptor occupancy (Ator et al. 2009). In this same model, TPA123 maintained rates of self-injection greater than vehicle, but the peak rates of self-administration were less than those of lorazepam in the same animals (Ator 2005).

10.3 Physical Dependence

In mice, the nonselective partial inverse agonist FG 7142 did not precipitate withdrawal signs (seizure activity) after either 7 days of dosing with TPA023 (Atack et al. 2006b) or 4 days of dosing with L-838417 (Mirza and Nielsen 2006). Similarly, TPA023 did not produce pronounced spontaneous (i.e., not FG-7142-precipitated) withdrawal signs in baboons when intragastric dosing of TPA023 was terminated after a period of 1 month (Ator et al. 2009). Whether the lack of physical dependence seen with L-838417 and TPA023 in preclinical species is the result of a lack of α1 efficacy, reduced efficacy at the α2, α3, and/or α5 subtypes relative to nonselective full agonist benzodiazepines or is a consequence of the pharmacokinetics and pharmacodynamics of these compounds is difficult to determine, given the complex interactions between these various factors (Mirza and Nielsen 2006). However, these data suggest that modulation of $GABA_A$ receptors does not per se produce physical dependence.

In summary, it is clear that subtype-selective $GABA_A$ modulators are distinct from the nonselective benzodiazepines. Probably most striking is the fact that in baboons TPA023 was not self-administered nor did it produce a marked withdrawal syndrome (Ator 2005; Ator et al. 2009). It is uncertain if this is as a result of zero efficacy at the α1 subtype or reduced α2 and/or α3 efficacy relative to lorazepam, for example, but at the very least these collective data suggest that TPA023 (and presumably other compounds with a similar efficacy profile) will have a much reduced, if not negligible, abuse liability in man.

11 Clinical Pharmacokinetics of MRK-409, TPA023, and TPA023B

Data showing the human plasma pharmacokinetics following single doses of MRK-409 (1 mg), TPA023 (1.5 mg immediate-release (IR) and extended-release (ER)), and TPA023B (1 mg) are presented in Fig. 6. As in the rat and dog (Fig. 5), both MRK-409 and TPA023-IR were rapidly absorbed, with the C_{max} occurring 1–2 h after dosing. The pharmacokinetic profile of TPA023 could be "flattened" considerably by formulation into an extended-release capsule, which slowed absorption (T_{max} ~6 h) and, for a dose of 1.5 mg, reduced the C_{max} from approximately 16 down to 6 ng mL^{-1}. As in the rat and dog, TPA023B had a slow

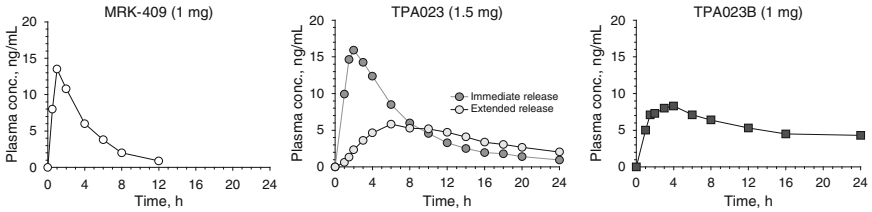


Fig. 6 Plasma concentrations of MRK-409, TPA023, and TPA023B in man following dosing as a tablet. In the case of TPA023, both an immediate-release as well as extended-release formulation was evaluated. Data for MRK-409 and TPA023B are part of Single Ascending Dose (SAD) safety and tolerability studies, whereas data for TPA023 are from a separate cross-over study comparing formulations. Values shown are mean ($n = 6$ for MRK-409 and TPA023B and $n = 16$ for TPA023)

absorption (T_{max} ~4 h) in man as well as a relatively long half-life (~25–40 h), presumably as a consequence of a low rate of clearance.

12 Tolerability of MRK-409, TPA023, and TPA023B in Man

Table 4 summarizes the tolerability of MRK-409, TPA023, and TPA023B in healthy, normal volunteer studies. For MRK-409, the maximum tolerated dose was 1 mg with marked sedation and somnolence being observed at higher doses (1.5 and 2 mg). This was clearly unexpected given that there were no signs of sedation in preclinical mouse, rat, or primate experiments (Table 3). Moreover, subsequent PET studies (Fig. 7) established that this sedation was observed at relatively low levels of occupancy. As a consequence, it was considered that there would have been no therapeutic window between doses causing sedation and those putatively producing anxiolysis and accordingly development of this compound was halted.

The experiences with MRK-409 demonstrated that, and for whatever reason, the lack of sedation seen in animal models does not necessarily translate into man. It was, therefore, reassuring to observe that TPA023 did not produce the same effects as MRK-409 in man. Hence, the maximum tolerated doses in either the SAD study (2 mg IR) or the MAD study (8 mg ER) occurred at plasma drug concentrations corresponding to occupancy levels well in excess of 50%. The dose-limiting adverse events were dizziness, altered perception, and motor incoordination occurring in the SAD study at a dose of 3 mg or drowsiness and motor incoordination at a dose of 12 mg in the MAD study.

For TPA023B, the maximum tolerated dose was 2 mg with fatigue and drowsiness being observed at a dose of 3 mg. Interestingly, although the maximum tolerated dose of TPA023B corresponded to an estimated occupancy level (67%) comparable to TPA023 in either the SAD or MAD study (66% and 73%, respectively), the dose-limiting adverse events for TPA023B were different from those seen with TPA023 and are presumably related to the greater intrinsic efficacy of TPA023B compared to TPA023 (Fig. 4).

Table 4 Summary of Safety and Tolerability of MRK-409, TPA023, and TPA023B in healthy, young normal volunteers

Drug	Formulation[a]	Study[b]	MTD[c] (mg)	C_{max} (ng mL^{-1})	Occupancy	Dose-limiting adverse events
MRK-409	Tablet, IR	SAD	1	14	≤10%	Sedation/somnolence
TPA023	Tablet, IR	SAD	2	18	66%[d]	Dizziness, altered perception, motor incoordination
TPA023	Tablet, ER	MAD	8	25	73%[d]	Drowsiness, motor incoordination
TPA023B	Tablet, IR	SAD	2	12	67%[e]	Fatigue, drowsiness

[a]*IR* Immediate release, *ER* Extended (or slow) release
[b]*SAD* Single ascending dose, *MAD* Multiple ascending dose
[c]*MTD* Maximum tolerated dose
[d]Calculated on the basis of a plasma EC_{50} value of 9.3 ng mL^{-1} and the Hill slope fixed at 1.0
[e]Calculated on the basis of a plasma EC_{50} value of 5.8 ng mL^{-1} and the Hill slope fixed at 1.0

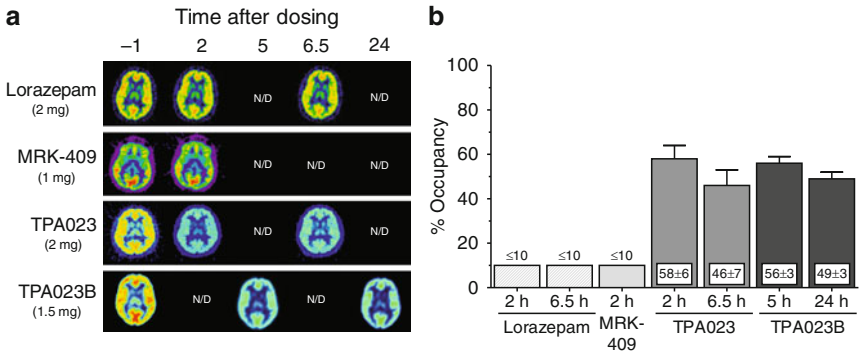


Fig. 7 Occupancy of human brain $GABA_A$ receptors by MRK-409, TPA023, and TPA023B and, by comparison, lorazepam. (**a**) Representative pseudocolour images showing the uptake of [^{11}C] flumazenil into human brain in individual subjects before (baseline) or at various time after oral administration of single doses of either lorazepam (2 mg), MRK-409 (1 mg), TPA023 (2 mg IR), or TPA023B (1.5 mg). (**b**) Graph showing the % reduction in [^{11}C]flumazenil uptake (i.e., % occupancy) produced by the different drugs. For MRK-409 and lorazepam, the levels of occupancy were below the limits of reliable detection and are therefore shown as ≤10%. N/D = not determined. Values shown are mean ± SEM ($n = 3$ per data point, except MRK-409 where $n = 2$)

13 [^{11}C]Flumazenil PET After Oral Dosing of MRK-409, TPA023, and TPA023B in Man

The ability of MRK-409, TPA023, and TPA023B to occupy the benzodiazepine site of human brain $GABA_A$ receptors was assessed using [^{11}C]flumazenil PET. Representative images of the extent to which [^{11}C]flumazenil binding was inhibited

following oral dosing with each drug plus, for comparative purposes, lorazepam, are shown in Fig. 7a. From these images it is clear that lorazepam (2 mg) and MRK-409 (1 mg) both produce very little inhibition of [^{11}C]flumazenil binding (i.e., low occupancy) with the level of occupancy of both drugs being at or below the level of sensitivity of the assay (Fig. 7b) as determined by the test–retest reliability measured in separate studies of ~10%. In contrast, both TPA023 (2 mg IR) and TPA023B (1.5 mg) produced marked inhibition of [^{11}C]flumazenil binding. More specifically, TPA023 gave comparable levels of occupancy (~50%) 2 and 6.5 h after dosing whereas TPA023B gave a level of occupancy (again, ~50%) that was similar – 5 and 24 h after dosing (Fig. 7b).

Blood samples were collected during the PET procedure and plasma drug concentrations determined. Although the low levels of occupancy produced by MRK-409 did not permit the estimation of a plasma EC_{50}, corresponding values of 9.3 ng mL^{-1} for TPA023 and 5.8 ng mL^{-1} for TPA023B were calculated (compared to respective values in rat of 25 and 19 ng mL^{-1} (Table 2) and in baboon of 19 and 10 ng mL^{-1} (data not shown)).

Due to logistical reasons, the number of time points for the PET studies (which were selected based upon the pharmacokinetics of each compound; Fig. 6) are limited and therefore insufficient to establish whether the time course of occupancy follows the plasma pharmacokinetics (as opposed to sustained occupancy due to a slow off-rate). However, more detailed analysis of the kinetics of rat brain occupancy of α5IA, a triazolophthalazine structurally related to both MRK-409 and TPA023, has shown that the time course of occupancy does indeed track plasma drug concentrations (Atack et al. 2009a).

14 Comparison of the Pharmacodynamic Responses of MRK-409 and TPA023 in Man

Table 5 shows a summary of the pharmacodynamic effects of MRK-409 and TPA023 in healthy, young male volunteers (de Haas et al. 2007, 2008). In the two separate studies, lorazepam (2 mg) produced similar effects in that it reduced alertness, as measured on a Visual Analogue Scale (VAS), reduced saccadic eye movement peak velocity while increasing the latency and inaccuracy, and increased body sway and impaired cognitive performance in word and picture recognition tests (de Haas et al. 2007, 2008). The effects produced by MRK-409 and TPA023 were not only different from lorazepam but also different from each other. For example, lorazepam markedly increased body sway with eyes open and increased saccadic eye movement inaccuracy, yet neither MRK-409 nor TPA023 affected these parameters. Comparing MRK-409 and TPA023, the highest dose of MRK-409 (0.75 mg) decreased VAS alertness, increased saccadic eye movement latency, increased body sway with eyes closed, and increased the reaction time in the word recognition test, whereas TPA023 did not significantly affect any of these parameters.

Table 5 Comparison of the pharmacodynamic effects of MRK-409 and TPA023 with lorazepam in healthy male volunteers

	MRK-409		Lorazepam	TPA023		Lorazepam
	0.25 mg	0.75 mg	2 mg	0.5 mg	1.5 mg	2 mg
VAS scales						
Alertness	↔	↓↓	↓↓↓	↔	↔	↓
Contentedness	↔	↔	↔	↔	↔	↔
Calmness	↔	↔	↔	↔	↓↓	↔
Saccadic eye movements						
Peak velocity	↔	↓↓↓	↓↓↓	↓↓	↓↓↓	↓↓↓
Latency	↔	↑↑	↑↑↑	↔	↔	↑↑↑
Inaccuracy	↔	↔	↑↑	↔	↔	↑↑↑
Posture						
Body sway						
Eyes open	↔	↔	↑↑↑	↔	↔	↑↑↑
Eyes closed	↑↑	↑	↑↑↑	↔	↔	↑↑↑
Cognitive performance						
Word recognition						
Correct answers	↔	↔	↓	↔	↔	↓
Reaction time	↔	↑	↑	↔	↔	↑
Picture recognition						
Correct answers	↔	↔	↔	↔	↔	↓
Reaction time	↔	↔	↑	↔	↔	↑

↓, ↓↓, ↓↓↓ = significantly decreased by $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively
↑, ↑↑, ↑↑↑ = significantly increased by $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

Although the peak plasma concentrations of the low and high doses of MRK-409 (~3 and ~9 ng mL^{-1} following 0.25 and 0.75 mg, respectively) are comparable to the low and high doses of TPA023 (~5 and ~13 ng mL^{-1} following 0.5 and 1.5 mg, respectively), these plasma concentrations correspond to very different levels of occupancy. Hence, a 1-mg dose of MRK-409 corresponds to a plasma concentration of ~14 ng mL^{-1} (Table 4), yet produced levels of occupancy that were below the limits of detection (i.e., $\leq$10%; Fig. 7). On the other hand, the plasma concentration of TPA023 required to give 50% occupancy in man was 9.3 ng mL^{-1}. Therefore, even at comparatively high levels of occupancy (50% or greater at the 1.5 mg dose) TPA023 did not affect VAS alertness, body sway, or cognitive performance whereas MRK-409 did affect these parameters at relatively low (<10%) levels of occupancy (Fig. 8).

15 Anxiolytic-Like Efficacy of TPA023

TPA023 was being evaluated in three separate Phase II studies (one using flexible doses of 1.5–4.5 mg, the other two using flexible dosing of 3–8 mg) in Generalized Anxiety Disorder, but these were terminated prior to completion due to preclinical toxicity issues. Since these studies ended early there were insufficient data from

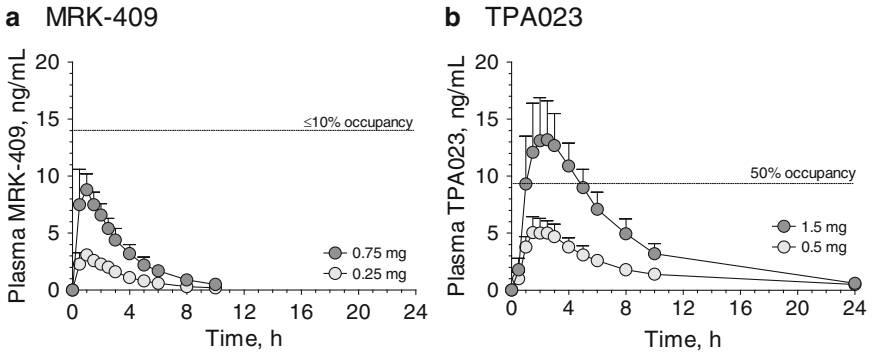


Fig. 8 Pharmacokinetic profiles of (**a**) MRK-409 and (**b**) TPA023 following single oral doses of 0.25 and 0.75 mg MRK-409 and 0.5 and 1.5 mg TPA023 IR to healthy, young male volunteers obtained as part of the assessment of the pharmacodynamic effects of these drugs. The dashed lines represent occupancy data extrapolated either from the rat plasma–occupancy relationship (MRK-409; Table 2) or from the human [^{11}C]flumazenil PET data (TPA023; Fig. 7). Values shown are mean ± SD (n = 12). (Data redrawn from de Haas et al. 2007, 2008)

Table 6 Comparison of the difference in the change in total HAM-A scores of TPA023 versus placebo treated patients

Week	Group size (placebo/TPA023)	Difference between TPA023 and placebo		
		Mean ± SE	95% confidence intervals	p value
1	60/61	−3.5 ± 1.0[a]	−5.5, −1.4	0.001
2	48/43	−2.9 ± 1.4	−5.7, −0.2	0.038
3	45/37	−3.0 ± 1.5	−5.9, −0.1	0.048
4	36/33	−1.1 ± 1.5	−4.1, 1.9	0.47

[a]Negative values indicate that the decrease in total HAM-A score was greater in the TPA023 compared to placebo group

any single study to make meaningful within-study comparisons. Nevertheless, by combining the data from these separate studies it was possible to show that compared to placebo TPA023 gave a significantly greater reduction in the HAM-A score relative to baseline (Table 6). Clearly, there are several caveats to consider in making such a post-hoc analysis of separate and incomplete clinical trials, but nevertheless an appropriately conservative interpretation of these data would be that TPA023 demonstrates anxiolytic-like activity.

16 Additional Clinical Data with TPA023

Based upon the involvement of the GABAergic system in the pathophysiology of schizophrenia (Lewis et al. 2005) a small, 4-week experimental study was carried out to assess the effects of TPA023 (MK-0777; 3–8 mg twice a day) on cognitive performance in schizophrenia patients. Although this may appear somewhat paradoxical insofar as benzodiazepines are well known to impair cognition (Stewart

2005), changes in the expression of α2 subunit-containing $GABA_A$ receptors on the axon initial segment of pyramidal neurons of subjects with schizophrenia suggest that enhancing the function of this subtype may actually improve cognitive function (Lewis et al. 2005). Despite being underpowered, this study was, nevertheless, able to demonstrate a tendency for TPA023 to improve cognitive performance (Lewis et al. 2008). This same compound is also currently under evaluation as part of the Treatment Units for Research on Neurocognition and Schizophrenia (TURNS) initiative.

17 Summary

MRK-409, TPA023, and TPA023B demonstrate that it is possible to develop compounds that modulate specific $GABA_A$ receptor populations based upon a selective-efficacy approach. Intuitively, it would seem that the best way to target specific $GABA_A$ receptor populations would be on the basis of selective affinity, i.e., a compound which binds with high affinity to certain subtypes but has much lower or no affinity for others. In the case of a nonsedating anxiolytic, such a compound would ideally have high affinity for the α2 and α3 but much lower affinity for the α1 and α5 subtypes. Unfortunately, however, this does not appear possible due to the commonality in the benzodiazepine recognition sites shared by each of the four subtypes (Atack 2009a). Using a selective-efficacy approach, a compound can bind with equivalent affinity for each of the four $GABA_A$ subtypes, but the levels of intrinsic efficacy differs between subtypes (Atack 2005). This may be relative (i.e., a compound has efficacy at all subtypes but is higher at certain subtypes relative to others) or maybe absolute (efficacy at some subtypes but no efficacy at others).

Compounds with selectivity for the α2/α3 compared to α1 subtypes, whether that be relative selectivity (MRK-409, which still retains a degree of α1 efficacy) or absolute selectivity (L-838417, NS11394, TPA023, and TPA023B, all of which have no intrinsic efficacy at the α1 subtype), behave as nonsedating anxiolytics in preclinical rodent and primate models (McKernan et al. 2000; Rowlett et al. 2005; Atack et al. 2006b; Mirza et al. 2008). Such compounds also have additional preclinical pharmacological characteristics that distinguish them from the classical nonselective benzodiazepines, such a diazepam or lorazepam. For example, they have reduced abuse liability as judged by self-administration (Ator 2005; Ator et al. 2009) or drug discrimination studies (Rowlett et al. 2005; McMahon and France 2006; Kohut and Ator 2008) and have a reduced withdrawal liability (Atack et al. 2006b; Ator et al. 2009).

MRK-409, TPA023 and TPA023B all progressed into man but the first compound, MRK-409, produced a marked sedation at relatively low levels of occupancy (~10%), which was unexpected given the lack of sedation seen in preclinical species but consistent with previous experiences with the nonselective partial agonist bretazenil which, like MRK-409 showed a clear separation between

anxiolysis and sedation in preclinical species but not in man (Van Steveninck et al. 1996). In retrospect, it could be argued that despite having higher α3 versus α1 efficacy (respective relative efficacy values of 0.45 and 0.18), the efficacy profile of MRK-409 is not too dissimilar from that of bretazenil, albeit that MRK-409 has slight lower α1 and α5 efficacy (0.18 and 0.18) compared to bretazenil (respective values of 0.30 and 0.46; Fig. 4). Consequently, the sedation observed with MRK-409 is consistent with that seen with bretazenil.

In contrast to MRK-409, neither TPA023 nor TPA023B caused overt sedation in man up to levels of occupancy exceeding 50%, raising the question of which feature of the in vitro efficacy profiles of TPA023 and TPA023B imparts the reduced sedation liability. Relative to MRK-409, the efficacy at the α2, α3, and α5 subtypes is lower for TPA023 but higher for TPA023B, and therefore the common feature of both compounds that is presumably responsible for the reduced sedation liability is the lack of efficacy at the α1 subtype (Fig. 4). Comparing the preclinical behavioral profiles of the three compounds (Table 3), the only appreciable difference between MRK-409, TPA023, and TPA023B is the fact that MRK-409 had a marked ethanol interaction on the rotarod whereas the others did not. Although relating the ethanol interaction of MRK-409 to its sedative properties in man is tenuous to say the least, it would nevertheless be interesting to examine the ethanol-interaction effects of subtype-selective compounds in the mouse beam-walking assay, since this may be more sensitive than the rotarod in detecting motor incoordination (Stanley et al. 2005).

Finally, despite the limitations of the retrospective analysis of incomplete Phase II trials, TPA023 appears to have anxiolytic-like activity in patients with Generalized Anxiety Disorder which, given the caveats, should therefore be viewed as a partial proof of concept. These data, along with the pharmacodynamic studies in healthy normal volunteers, collectively demonstrate that α2/α3 efficacy selective $GABA_A$ modulators have a pharmacological profile distinct from nonselective benzodiazepines such as diazepam or lorazepam and should encourage further investigations of these and other $GABA_A$ subtype-selective modulators, such as α5 selective inverse agonists (Dawson et al. 2006; Ballard et al. 2008; Atack 2009b).

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Antidepressant Treatment in Anxiety Disorders

Anton Y. Bespalov, Marcel M. van Gaalen, and Gerhard Gross

Contents

Abstract Antidepressant drug treatment is the clinical standard of care for all types of anxiety disorders. Broad efficacy of selective serotonin reuptake inhibitors suggests the importance of enhanced serotonergic function of the anxiolytic properties of current antidepressants. However, analysis of the preclinical evidence

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indicates that most conventional “anxiolytic” drug tests are not sensitive to antidepressants. Such dissociation is not surprising because of the traditional approach to validation of preclinical tests that is to a large extent based on establishing face validity as well as sensitivity to benzodiazepine anxiolytics. The present review argues for extending the cognitive model of antidepressant drug action to cover their anxiolytic properties as well. Such an approach is based on ambiguity or uncertainty in a broad sense as the hallmark of human stress that has different expressions ready for experimental modeling. These possibilities include schedule-induced behaviors that are directly based on intermittent reinforcement, conditioning to ambiguous stimuli, social stress where agonistic confrontations are possible but not predictable or controlled by the subject, and an even larger class of behaviors that are critically dependent on the inhibition of the prepotent responses in exchange for the ambiguous possibility of a later gain in reinforcement. Interestingly, in all these cases, antidepressant drug treatment is clearly effective in preclinical laboratory settings. One of the cognitive functions that appears to be affected by antidepressant drugs is inhibitory control. Inhibition of prepotent responding has beneficial effects in the “uncertainty” stress situations discussed above and therefore it is this cognitive function that may be critical for anxiolytic effects of antidepressants and novel anxiolytic drug development.

Keywords Antidepressant drugs · Anxiety · SSRI · Preclinical models

1 Introduction

In the last four decades of the last century, pharmacological therapy of anxiety disorders had been dominated by benzodiazepines. Due to their efficacy in acute and chronic treatment of a variety of anxiety states, they were classified as anxiolytic drugs, whereas the term “antidepressant drugs” was reserved for monoamine reuptake (e.g., tricyclics) and monoamine oxidase inhibitors (MAO-Is). As a consequence, GABAergic function was mainly linked to anxiety, whereas serotonergic and noradrenergic functions were linked to depression – in line with the respective monoamine theory. The development of animal models only responding to either anxiolytic or antidepressant drugs further contributed to this dichotomy. However, there is considerable overlap of symptoms in depression and anxiety disorders and high comorbidity, which is explained by shared genetic risk factors (Hettema 2008).

Benzodiazepines have proven efficacious in generalized anxiety disorder (GAD), panic disorder (PD) and social anxiety disorders (SADs). They are particularly appropriate in short-term treatment situations and thus still belong to the current therapeutic repertoire. However, their inability to treat comorbid depression, their adverse side-effect profile – including sedation, cognitive impairment and especially their liability to induce dependence and abuse – have limited their acceptance (Uhlenhuth et al. 1999a, b).

Early in the 1960s, before DSM-III or later DSM-IV criteria were established, first observations of anxiolytic effects of the prototypical tricyclic antidepressant imipramine and non-selective irreversible MAO-Is, like phenelzine, were published. This was followed later by similar reports on clomipramine. In contrast to benzodiazepines, the onset of anxiolytic action of these antidepressants was delayed as known for their antidepressant effect. While most tricyclic antidepressants inhibit neuronal reuptake of serotonin (5-hydroxytryptamine, 5-HT) as well as of noradrenaline (norepinephrine, NE), it is noticeable that anxiolytic efficacy was repeatedly demonstrated in controlled clinical studies only for non-selective NE/5-HT antidepressants and for clomipramine with its preference for the serotonin transporter. In contrast, antidepressants preferentially or selectively inhibiting NE uptake, like desipramine, nortriptyline, maprotiline, mianserin, and reboxetine, have not been shown to possess broad anxiolytic properties. This suggested that it is the serotonergic component that probably mediates the delayed anxiolytic response of tricyclic antidepressants and MAO-Is.

Despite the increasing evidence of efficacy in different anxiety disorders and the obvious lack of drug abuse liability, untoward effects like tyramine interaction for MAO-I, the many effects mediated by the autonomic nervous system, and antihistamine effects in tricyclic antidepressants clearly limited broader use in anxiety disorders. This situation changed with the introduction of selective serotonin reuptake inhibitors (SSRIs), which offered largely improved tolerability and efficacy proven in many controlled clinical studies. SSRIs are now recommended as the first-line medication for GAD, PD, SAD as well as for post-traumatic stress disorder (PTSD) and obsessive–compulsive disorder (OCD) in most recent European and US treatment guidelines (e.g., Bandelow et al. 2008).

The present review focuses on clinical and preclinical evidence, which suggests anxiolytic properties of antidepressant drugs and analyzes the mechanisms that are likely to be behind such properties.

2 Clinical Evidence on Anxiolytic Activity of Antidepressant Drugs

2.1 Generalized Anxiety Disorder

Antidepressants of different classes are in clinical use for the treatment of GAD (for review see Bandelow et al. 2008; Fricchione 2004; Hoffman and Mathew 2008). Due to the fact that major depression is the most common comorbid condition in GAD patients, tricyclic antidepressants like imipramine and amitriptyline drugs have long been used in this patient population, although initially it remained unclear to what extent antidepressant or genuine anxiolytic properties were responsible for treatment responses (Kahn et al. 1987).

In patients without major depression, Rickels et al. (1993) found that diazepam showed the most improvement in anxiety ratings during the first 2 weeks of treatment; in contrast, antidepressant drugs achieved comparable (trazodone) or even somewhat better efficacy (imipramine) after 3–8 weeks of treatment. Among completers, the most discernible improvements were seen with imipramine. Imipramine also significantly facilitates benzodiazepine discontinuation in patients with GAD (Rickels et al. 2000). Systematic clinical investigations in controlled and sufficiently powered clinical studies of other tricyclic antidepressants, selective or non-selective NE reuptake inhibitors, and selective or non-selective MAO-I are missing for GAD. The same is true for non-tricyclic agents with different pharmacologic profiles: bupropione (a mixed NE and dopamine reuptake inhibitor), mianserin and mirtazapine (α_2-adrenoceptor antagonists enhancing NE and 5-HT release and blocking 5-HT_{2A} and 5-HT_{2C} receptors), and nefazodone (a 5-HT_2 and histamine H_1 antagonist).

For many SSRIs, efficacy similar to that of imipramine was found in several randomized placebo-controlled studies in GAD (for review see Hoffman and Mathew 2008). For instance, it was shown that SSRIs led to reduction in clinical anxiety ratings after 8 weeks of treatment (Davidson et al. 2004; Rickels et al. 2003) and prevented relapse when treatment continued for 6 months (Allgulander et al. 2005; Stocchi et al. 2003). From existing clinical trials it remains unclear whether the therapeutic effect of paroxetine and escitalopram is a class effect and can be generalized to all other SSRIs. However, from available evidence, SSRIs like sertraline seem to exert a similar therapeutic effect in GAD (Allgulander et al. 2004; Dahl et al. 2005).

Venlafaxine and duloxetine are classified as selective serotonin and noradrenaline reuptake inhibitors (SNRIs). Due to the serotonergic component of their pharmacological profile it could be expected that their clinical profile would replicate that of SSRIs. Indeed, both medications were equally effective in placebo-controlled studies (e.g., Hartford et al. 2007) and are approved for the treatment of GAD. Like in depression, there is an impression that the onset of efficacy of SNRIs may be faster than that for other antidepressants. However, the extent to which NE reuptake inhibition contributes to the therapeutic effect remains unknown. In vitro, both SNRIs have more than tenfold higher affinity for the human 5-HT transporter compared to the human NE transporter (Bymaster et al. 2001). Blockade of the 5-HT transporter alone would be sufficient to explain anxiolytic effects and there are no clinical studies unequivocally demonstrating superior efficacy of SNRIs in comparison to SSRIs. Like in other anxiety disorders, treatment of GAD with SSRIs or SNRIs should usually be continued for 6–12 months, although objective clinical data are sparse (Fricchione 2004).

2.2 Panic Disorder

Up to 80% of patients with PD have experienced major stress events (Manfro et al. 1996) and 90% have at least one comorbid psychiatric disorder in their lives – often

depressive episodes (Kessler et al. 2005). These facts may explain why all classes of antidepressants have been investigated in patients with this disease. It is currently believed that tricyclic antidepressants and SSRIs are at least equivalent in their efficacy to benzodiazepines (for review see Bandelow et al. 2008; Hoffman and Mathew 2008; Katon 2006).

Among tricyclic antidepressants, imipramine and clomipramine were effective in placebo and comparator-controlled studies, reducing the number of panic attacks and the severity of anxiety. In comparison to benzodiazepines, their effect is delayed for about 4 weeks, with maximal efficacy taking up to 12 weeks. Although it has been suggested that all tricyclics may be similarly effective, both imipramine and clomipramine are potent 5-HT reuptake inhibitors, whereas desipramine, a preferential NE reuptake inhibitor, was not convincingly superior to placebo (Lydiard et al. 1993). Similarly, in a small non-placebo-controlled study, the non-tricyclic NE reuptake inhibitor maprotiline failed to reduce the frequency of panic attacks (den Boer and Westenberg 1988).

Non-selective irreversible MAO-I like phenelzine and tranylcypromine with their unfavorable side-effect profile are considered to have therapeutic potential for PD with or without agoraphobia, at least as second-line medication. However, only one phenelzine study showed superiority over placebo and equal efficacy to imipramine in the treatment of "phobic neurosis" (Sheehan et al. 1980). The impression that MAO-Is are more potent antipanic agents than tricyclics has never been proven in clinical studies. The efficacy of moclobemide, a reversible and MAO-A-selective inhibitor has never been unequivocally established in sufficiently large placebo-controlled trials.

SSRIs are by far the most prescribed class of antidepressants in PD. They are significantly more effective than placebo in reducing the number of panic attacks as well as in reducing global anxiety and a significant percentage of treated patients become panic-free (for review see Hoffman et al. 2008). Efficacy has been shown for all clinically used SSRIs (citalopram, escitalopram, fluvoxamine, fluoxetine, paroxetine, and sertraline) as well as for the SNRI venlafaxine. Its efficacy in placebo-controlled trials was equivalent to that of paroxetine; it reduced the frequency of attacks and prevented relapse (e.g., Pollack et al. 2007). As for GAD (see above), there is a doubt whether the noradrenergic component contributes significantly to venlafaxine's efficacy and superiority of venlafaxine over SSRIs is not established. For the selective NE reuptake inhibitor reboxetine, there are some hints of efficacy in PD (Versiani et al. 2002), but rigorous data in placebo-controlled sufficiently powered clinical studies are still missing for reboxetine just as for a number of other interesting drugs such as duloxetine, buproprion, nefazodone, and mirtazapine.

2.3 Social Anxiety Disorder

SAD, also known as social phobia, has a high lifetime prevalence of 12%, and comorbid psychiatric diseases, especially major depression, other anxiety disorders

and alcohol abuse are frequent (Kessler et al. 2005; Stein and Stein 2008). Benzodiazepines and beta-adrenoceptor antagonists, such as propranolol, are often used for non-generalized symptoms and in predictive situations, like public speaking, although evidence of efficacy is more limited than in other anxiety diseases (Schneier 2006). SSRIs and SNRIs are now commonly used as first-line pharmacological therapy (Bandelow et al. 2008; Hoffman and Mathew 2008; Schneier 2006; Stein and Stein 2008).

In contrast to GAD and PD, efficacy of tricyclic antidepressants has never been established. A small double-blind study comparing clomipramine with diazepam suggested efficacy of this preferential 5-HT reuptake inhibitor (Allsopp et al. 1984), but this has not been confirmed in a larger trial. An open trial with imipramine did not support its use in social anxiety (Simpson et al. 1998). In contrast, there is evidence from several studies that the irreversible MAO-Is such as phenelzine and tranylcypromine are efficacious in social anxiety (e.g., Stein et al. 2004; Versiani et al. 1988). The reversible MAO-A inhibitor moclobemide has been reported initially to be superior to placebo and comparable to phenelzine – a finding that has not been consistently confirmed in later studies (Stein et al. 2004).

The largest database concerning efficacy of pharmacological treatment of SAD exists for many SSRIs and venlafaxine as an SNRI. Many large studies proved higher response rates of 50–80% for citalopram, escitalopram, fluvoxamine, paroxetine, sertraline, and venlafaxine (for review see Hoffman and Mathew 2008; Schneier 2006; Stein and Stein 2008). Most studies investigated efficacy within 12 weeks of treatment, but relapse prevention trials with escitalopram, paroxetine, and sertraline proved their superiority over placebo also in an extended time frame of 24 weeks (e.g., Montgomery et al. 2005). Although several head-to-head comparison studies have been carried out among SSRIs and with venlafaxine, there is little reason to assume clear superiority of one compound over the other within this pharmacologic class (Stein and Stein 2008). The fact that venlafaxine shows comparable efficacy at lower doses, which preferentially inhibit 5-HT reuptake, and at higher doses, which in addition may block NE uptake, suggests that the influence on the serotonergic system is more relevant for efficacy than an effect on the noradrenergic system (Stein et al. 2005). This is in line with the fact that no studies reporting efficacy of preferential or selective NE reuptake inhibitors in SAD have been published.

2.4 Post-Traumatic Stress Disorder

Among all anxiety disorders, PTSD is the least studied and is characterized by the smallest effect sizes for currently available antidepressants. Only paroxetine and sertraline are approved for this indication, but Expert Consensus Guidelines on the treatment of PTSD consider switching to nefazodone or venlafaxine, when these medications fail (Foa et al. 1999). The general finding is that paroxetine and sertraline are superior over placebo and patients improve in all three symptom

Table 1 Recommended use of antidepressant drugs for the treatment of anxiety disorders (based on Bandelow et al. 2008)

Anxiety disorder	TCA	MAOI	SSRI	SNRI	NRI
GAD	++		++	++	
Panic attacks	++	+	++	+	+
Social phobia		+	++		
PTSD	+		++		
OCD	++		++		

++ first-line treatment; + second-line or supportive treatment

clusters (hyperarousal, re-experiencing, avoidance/numbing) (e.g., Marshall et al. 2001). Among older antidepressants, imipramine and phenelzine were reported to be superior over placebo; therapeutic potential of other drugs such as mirtazapine needs to be confirmed (for review see Hoffman and Mathew 2008).

2.5 Obsessive–Compulsive Disorder

Although OCD is phenotypically different from other anxiety disorders and is primarily characterized by the presence of costly obsessions and compulsions causing marked distress, pharmacological treatment options include clomipramine and various SSRIs (see Schruers et al. 2005 for review). While non-serotonergic agents are not considered as first-line therapy and may not be effective as a stand-alone treatment, dopaminergic and norepinephrinergic drugs often contribute to augmentation strategies in 40–60% of patients with OCD who do not respond to SSRIs.

As summarized in Table 1, anxiety disorders respond to different classes of antidepressant drugs. Most of these drugs have shown superiority over placebo, but there are only few clinical studies that clearly differentiate efficacies of compounds within one class of drugs. A clear dominance of SSRIs has developed over the last 10–15 years. They are indeed effective in all forms of anxiety, but they are not necessarily more efficacious than older antidepressants. Their extensive clinical use is mainly based on their favorable tolerability. It is to be noted that all these antidepressants share similar mode(s) of pharmacodynamic action: direct or indirect influence on serotonergic and/or noradrenergic neurotransmission. The broad efficacy of SSRIs clearly demonstrates that enhanced serotonergic function – but not necessarily enhanced noradrenergic function – is crucial for the anxiolytic properties of current antidepressants. It remains to be established, if new antidepressant principles in development, like agomelatine, a melatonin MT_1 and MT_2 receptor agonist with additional 5-HT_{2C} antagonism[1] (Olié and Kasper 2007), or drugs affecting the hypothalamus–pituitary–adrenal gland axis, like antagonists of

[1]First clinical reports suggested efficacy in the treatment of GAD but this evidence needs to be further extended by additional studies, with active comparisons as well as over longer periods of time (Stein et al. 2008).

the corticotropin releasing factor (CRF) receptor 1[2] or the vasopressin V1b receptor, will be effective in anxiety disorders.

3 Preclinical Evidence

3.1 Is Anxiolytic Activity Predicted by Efficacy in Classical Antidepressant Tests?

The most used high-throughput screening models employed to detect antidepressant-like activity are the forced swim test and the closely related "dry" version, tail suspension test. These tests are based on the observations of Porsolt et al. (1977) that rats, when forced to swim in a restricted space from which they cannot escape, will eventually cease apparent attempts to escape and become immobile apart from the small movements necessary to keep their heads above the water. Several hypotheses have been suggested to explain why animals stop struggling – e.g., behavioral despair, fatigue or saving energy for survival. None of these hypotheses make it easy to relate the immobility response to the clinical symptoms of depression or anxiety disorders, and therefore such tests conducted in standard healthy laboratory animals clearly lack face and construct validity. At the time these tests were developed, knowledge of the anxiolytic effects of antidepressants was still rather limited. Therefore, the tests were labeled as being able to detect antidepressant-like properties of drugs, and this is indeed supported by most antidepressants showing efficacy in these tests. Thus, despite several false positives described (e.g., Nagatani et al. 1987; Wieland and Lucki 1990), these tests are usually said to have certain predictive validity.

The question remains, however, whether efficacy in these tests predicts antidepressant activity, anxiolytic activity, or just (sub)acute effects on release of neurotransmitters such as noradrenaline and/or serotonin. Given that clinical efficacy of antidepressants is seen typically after at least 2–3 weeks of treatment, the latter seems to be the more likely.

Similar to what is said above about the "behavioral despair" tests, other commonly used tests to detect antidepressant-like activity do not seem to provide information on potential anxiolytic activity. First, there are drugs that lack meaningful anxiolytic properties in the clinic but nevertheless are found to be fully efficacious in preclinical studies evaluating antidepressant-like activities. Second, benzodiazepine anxiolytic drugs that are effective in the treatment of anxiety disorders usually produce no appreciable effects in the antidepressant tests.

[2]First clinical studies indicated lack of clinical efficacy of CRF1 antagonists in the treatment of GAD (Coric et al. 2008; see also Steckler*** 2009). However, it may be too early to interpret this evidence because, even successful drug classes like SSRIs took several clinical trials to firmly establish their clinical usefulness.

Thus, taking all available evidence together, one comes to the conclusion that commonly used antidepressant tests are not suited for detecting anxiolytic drug activity.

3.2 Efficacy of Antidepressant Drugs in Classical "Anxiolytic" Tests

A great variety of preclinical models that allow assessment of anxiolytic drug potential are thought to exist. While they can hardly be grouped based on the potential relevance to clinical anxiety categories (however, see Sects. 3.3–3.5 for comments on animal models of PD, PTSD and social anxiety), they are easily grouped into a small number of categories based on the operating behavioral mechanisms (e.g., Griebel 1995).

3.2.1 Ethological Models: Exploratory Activity

This category includes several very popular and frequently used tests such as elevated plus maze, zero maze, open field, and light-dark box. The members of this category have a common trait in that they represent easy to administer, single-trial short tests that are based on the exploration of a novel territory that consists of non-aversive and aversive parts (e.g., dark versus light compartments in the light-dark box; closed versus open arms in the elevated plus maze, etc.). Overall, no consistent effects of antidepressant drug treatment were found in these tests. For example, in the elevated plus maze – one of the most widely used models to study effects of antidepressants on anxiety-related behavior – the majority of the studies reported no effects of antidepressant on anxiety-related behavior both after acute or chronic application (see Borsini et al. 2002 for review). In fact, some studies even reported anxiogenic-like responses to antidepressant drug administration.

3.2.2 Ethological Models: Social Behavior

This group consists of models based on social contacts and communication such as social interaction, social competition, or ultrasonic calling. Social interaction under bright light conditions is one of the most popular representatives of this category and is reported to reliably detect effects of benzodiazepine anxiolytics; antidepressants of different classes, however, produce mixed effects. One chronic study with paroxetine resulted in anxiolytic-like effects after 3 weeks of treatment (Lightowler et al. 1994), but studies with chronic administration of other antidepressants (including more members of the SSRI class) did not reveal any meaningful effects (e.g., File et al. 1999).

3.2.3 Unconditioned Stress-Induced Responses

A third subcategory consists of models in which stress-induced alteration of behavior or physiological responses are studied. While stress does seem to be a common denominator, this group is clearly very heterogeneous. Most of these stress-based procedures such as stress-induced hypothermia fail to detect anxiolytic-like potential of antidepressant drugs. However, all classes of antidepressants demonstrate efficacy against isolation-induced vocalizations in guinea pig pups (see Borsini et al. 2002 for review). In rat pups though, acute application of drugs that affect the serotonergic system appear to be effective, whereas compounds that affect the noradrenergic system exert opposite or no effects (e.g., Hodgson et al. 2008). One potential drawback of this model is that the blood brain barrier is not fully developed in pups, which makes it less suitable for screening purposes. An alternative model in adult animals is based on the vocalizations induced by mild electroshock. Such vocalizations are sensitive to acute treatment with SSRIs, clomipramine, imipramine, but not desipramine or maprotiline (see Borsini et al. 2002 for review). Similar readout (vocalizations) and sensitivity to antidepressant treatment make isolation distress calls and electroshock-induced vocalizations look similar. However, one should keep in mind that drugs may suppress vocalizations via various mechanisms including non-specific ones (e.g., sedation, analgesia) and the value of these methods in detecting novel anxiolytics is not firmly established.

3.2.4 Conditioned Fear Tests

Conditioned fear tests include models such as fear-potentiated startle but may also be extended to cover shock rod burying, active/passive avoidance, etc. Overall, no single model is found to convincingly detect antidepressant drugs with anxiolytic-like activity. However, certain members of this category do show significant promise in this regard. In the shock-conditioned freezing paradigm, SSRIs have been reported to exert lasting anxiolytic-like effects while NE reuptake inhibition is not expected to share these effects (e.g., Hashimoto et al. 2009; Inoue et al. 2006). Certain modifications of the fear conditioning protocol can make it even more attractive. For example, one may manipulate the contingency between shocks and to-be-conditioned stimuli in such a way that the latter become only partially predictive of shocks and therefore are less effective in eliciting freezing during the test. Tsetsenis et al. (2007) demonstrated that stimulation of 5-HT1A receptors in hippocampus reverses enhanced fear conditioning to such ambiguous stimuli – a hallmark of human anxiety – in 5-HT1A knockout mice. Because of the expected up-regulation of 5-HT neurotransmission, repeated administration of antidepressant drugs is expected to have an opposite effect – i.e., reduced conditioned responding to ambiguous cues.

3.2.5 Conflict Tests

In tests such as the Geller-Seifter's or Vogel conflict test, positive reinforcement contingency (food or water) is concurrent with a mild shock delivery schedule representing a punishment contingency. Overall, while classical benzodiazepine anxiolytics are very effective in these tests, antidepressant drugs are generally not active (see Borsini et al. 2002 for review). Lack of efficacy of antidepressants in these tests is quite noteworthy given the above mentioned effects of these agents on shock-induced vocalizations. Thus, it appears that while antidepressant drugs attenuate expression of behaviors directly elicited by the aversive events (i.e., vocalizations), they do not affect the behavioral significance of these events (i.e., as assessed by the response rates on the concurrent positive reinforcement schedule).

3.3 Animal Models of Panic Attacks

There are several approaches to preclinical modeling of panic attacks. The most straightforward approach is based on the views of panic attacks being simply extreme cases of anxiety. For example, a classical elevated plus maze can be modified by making it unstable and to elicit unconditioned flight/escape behaviors in rats. This new quality was associated with the gain of sensitivity to chronic, but not acute, treatment with fluoxetine (Jones et al. 2002). Alternatively, extreme anxiety in mice is readily evoked by exposure to a natural threat (a rat). Defensive and avoidance behaviors displayed by mice under conditions of the so-called mouse defense test battery are attenuated by chronic treatment with antidepressant drugs, illustrating once again the efficacy of these drugs against extreme anxiety states (see Blanchard et al. 2003 for review).

Elevated anxiety levels may also be produced by acute treatment with anxiogenic drugs such as pentylenetetrazole or electrical stimulation of brain areas implicated in the regulation of fear and escape behaviors such as dorsal periaqueductal grey. Due to its robustness and reliability, the latter becomes increasingly popular for modeling panic attacks in preclinical settings. Pharmacological studies indicate that both SSRIs such as citalopram, fluoxetine, or paroxetine, and the benzodiazepines like alprazolam reduce the flight-like escape behaviors produced by electrical stimulation of the dorsal periaqueductal grey in the rat (Hogg et al. 2006). Efficacy of SSRIs does not seem to require subchronic administration that somewhat alleviated the validity arguments for this method.

3.4 Animal Models of Social Anxiety

Reviews of the clinical signs and symptoms of social anxiety tend to suggest that this disorder can hardly be modeled in laboratory animals. Indeed, it seems difficult to imagine animal models of socially determined dysphoric ruminations, one of the

distress types found in patients with social phobia. Social nature of this disorder dictates the use of social hierarchy-derived stressors in preclinical models. Several research groups observed that (sub)chronic treatment with antidepressants enhances aggressive behavior of subdominant and subordinant rats and mice, indicative of elevated social position (Malatynska et al. 2005; Mitchell 2005).

An alternative approach relies on identification of the core operating mechanisms that underlie development and expression of social anxiety. For example, anticipatory anxiety, another distress type associated with social phobia, may have mechanisms based on classical conditioning and could therefore be amenable to preclinical modeling. The phobic symptoms associated with social phobia overlap greatly with those of other anxiety disorders (e.g., specific phobias), while patients with social phobia may have characteristics conventionally associated with other anxiety disorders (e.g., enhanced anxiety reactions to high concentrations of CO2, caffeine, or cholecystokinin; Nutt et al. 1998). Collectively, this evidence suggests that preclinical models do not necessarily have to involve social stressors.

Further, conditioned fear has long been acknowledged as an important etiological mechanism in social anxiety and clinical evidence indeed supports enhanced conditionability of aversive socially specific stimuli (Lissek et al. 2008). While the preclinical models for such social fear conditioning are still to be developed (e.g., based on the use of distress-specific ultrasound vocalizations), there are no reasons to believe that drugs that reduce conditioned fear in the conventional paradigms will not affect socially specific conditioned fear. This expectation relies at least in part on the available evidence indicating that clinically effective drugs (SSRIs, MAO inhibitors) attenuate the expression of conditioned fear responses in laboratory animals (see above). On the other hand, there are a number of various investigational drugs that effectively reduce expression of conditioned fear, but clinical data will be needed to establish predictive validity of such approach to identification of novel treatments of social anxiety.

3.5 Animal Models of Post-Traumatic Stress Disorder

PTSD develops following extreme stressful experiences and therefore animal models are based on exposures to brief sessions of intense physical or social stressors. Generally speaking, animal models that are characterized by long-lasting conditioned fear responses as well as generalized behavioral sensitization to novel stimuli following short-lasting but intense stress have a phenomenology that resembles that of PTSD in humans (see Stam 2007 for review). Animals that were subjected to brief, but intense, electrical foot- or tail-shocks or strong social stressors (predators or aggressive conspecifics) displayed gradually increasing and long-lasting hyper-responsiveness to novel stressful stimuli (e.g., exposure to the novel environment, open or lit spaces, etc.), and reduced response to appetitive stimuli (e.g., preference for sweet solution or appetitive anticipatory behaviors).

PTSD is characterized by hyperarousal, re-experiencing of the traumatic event, withdrawal or avoidance behavior, and emotional numbing; however, not every aspect of PTSD can obviously be modeled in laboratory animals and, therefore, it is rather difficult to correlate effects in the animal models with clinical efficacy. Indeed, preclinical evidence generated using stress-sensitization models suggested that benzodiazepine anxiolytics are as effective if not more effective than SSRIs in reducing behavioral hyper-reactivity induced by shock pre-exposure (van Dijken et al. 1992).

Another serious limitation of the currently available evidence is that there is not much data on efficacy of drug treatment during the early stages of post-traumatic stress (i.e., prophylactic treatment). Therefore, predictive validity of animal models can only be judged on therapeutic properties (i.e., treatment of the fully developed disorder) while preclinical data suggest that prophylactic treatment could be very effective.

3.6 Animal Models of Obsessive–Compulsive Disorder

Spontaneous behaviors resembling clinical symptoms of OCD are hardly seen in laboratory animals (see, however, Garner et al. 2004 for discussion on barbering behavior in mice) and therefore current models of OCD focus on perseverations and compulsive checking, induced either genetically or pharmacologically (e.g., quinpirole-induced checking behavior or reduction in spontaneous alternation in T-maze). There is only a limited degree of validation of these models (see Joel 2006 for review).

Other animal models where significant efforts were invested into pharmacological validation require further evaluation because of the limited experience outside the lab that originally developed the model (e.g., signal attenuation; Joel 2006) or the large number of false positives that attenuate marble burying via mechanisms varying from motor stimulation to sedation (Van Gaalen, Bespalov, Wicke, unpublished results).

3.7 "Benzodiazepine" Versus "Anxiolytic Activity" Tests

Over the last 15–20 years there were several reviews of the published evidence on the effects of antidepressant drugs in classical anxiolytic-like activity tests (e.g., Borsini et al. 2002). The overall evidence, briefly summarized above, is rather disappointing. Of course, a number of factors contributed to the lack of consistency in the study outcomes – e.g., administration routes, dose ranges tested (Soderpalm et al. 1989), species differences (Barrett and Gleeson 1991), gender effects (Rosenzweig-Lipson et al. 2007), environmental effects (Wettstein 1992), etc. However, all these discussions do not hide the main fact – classical anxiolytic

drug tests are not very sensitive to antidepressant drugs clinically effective in the treatment of anxiety disorders.

Indeed, most of these classical tests are pharmacologically validated using benzodiazepine anxiolytics and are therefore "benzodiazepine tests" rather than "anxiolytic drug tests." An elegant illustration of this difference was provided by the study by Thiebot and colleagues (1985) who suggested that at least some of the effects of benzodiazepine drugs in "anxiolytic" tests may be explained by their ability to stimulate impulsive choice. Benzodiazepines are known to reduce tolerance to delay of reward resulting in the enhanced preference for smaller but immediate rewards over delayed larger rewards. Thiebot and colleagues (1985) illustrated how these pro-impulsive properties of benzodiazepines may help understanding their effects in the conventional conflict tests. Similar arguments can be applied to tests where conflicts are less explicit but nevertheless significant (e.g., conflict between exploratory and safety drives in the plus maze and social interaction). Interestingly, antidepressants like SSRIs do not stimulate impulsive choice like benzodiazepines and after chronic administration are actually found to enhance tolerance to delay of reward (Wolff and Leander 2002). Thus, despite clinical anxiolytic potential, antidepressant drugs do not necessarily reproduce the preclinical psychopharmacology of benzodiazepines and it is not well established that effects of benzodiazepines in classical "anxiolytic" tests reflect their therapeutic effects.

3.8 Need for New "Anxiolytic" Tests Sensitive to Antidepressant Drug Treatment

There are a number of novel therapeutic candidates for treatment of anxiety and depression and a greater number of emerging targets for developing such therapies. As the discussion above suggests, one can hardly count on the classical anxiolytic tests when it comes to testing drugs with non-benzodiazepine-like mechanism of action. What are the features that the new tests should have?

First, clinical experience clearly indicates that a preclinical model should be sensitive to treatment with serotonergic antidepressants. Further, clinical efficacy has a rather delayed onset and therefore preclinical models that require subchronic drug administration are potentially of higher value. While there is no animal model that unequivocally meets all the requirements, one should mention again anxiolytic-like effects of SSRIs in the conditioned freezing paradigm as well as in the four-plate and novelty-suppressed feeding tests (see also Borsini et al. 2002 for review). Independent confirmations of these results in a larger number of studies could make these tests a valuable tool in novel anxiolytic drug development.

Second, evidence on clinical efficacy of antidepressants is obtained in patients who have been diagnosed with anxiety disorders. In contrast, most preclinical research is conducted using normal laboratory animals. The following example illustrates the significance of this discrepancy. Mice overproducing CRF exhibit

various alterations including increased anxiety-related behaviors and hypothalamus-pituitary-adrenocortical activity (Stenzel-Poore et al. 1994). Chronic treatment with citalopram decreased anxiety-related behavior in CRF overproducing animals in conditioned fear test, whereas it had opposite effects in controls (Fig. 1, left panel). Importantly, there was no such genotype dependence when effects of citalopram were studied in relation to 5-HT1A receptor autoreceptor function. 5-HT1A receptors serve both somatodendritic autoreceptor and post-synaptic heteroreceptor function. In mice, 8-OH-DPAT-induced hypothermia has been attributed to activation of somatodendritic 5-HT1A autoreceptors (Bill et al. 1991). Chronic treatment with citalopram or other antidepressants of various classes as well as repeated electroconvulsive shock therapy attenuate 8-OH-DPAT-induced hypothermia (Bill et al. 1991; Goodwin 1989). Chronic treatment of wild type and CRF overexpressing mice with citalopram results in desensitization of 5-HT1A autoreceptors, as indicated by the attenuated hypothermic response to 8-OH-DPAT administration in both wild types and transgenics (Fig. 1, right panel). This demonstrates that chronic treatment with antidepressants can lead to similar cellular changes in neurotransmitter systems under normal and pathological conditions, but that such changes may lead to opposite effects on anxiety-related behavior.

3.8.1 Social Stress

Repeated and/or prolonged exposures to stress have long been argued to present a path towards a disease model that would allow preclinical evaluation of novel antidepressant principles with anxiolytic properties. For example, Keeney and Hogg (1999) investigated the effect of the SSRI citalopram in normal mice and mice that were exposed to repeated social defeat, coupled with the stress of continuously living in proximity to the dominant mice. Mice were repeatedly tested in the dark-light transition box. As expected, control animals initially avoided the illuminated part of the apparatus but, with repeated testing, this avoidance behavior was greatly diminished. Interestingly, no such changes were seen in stressed animals which showed even greater avoidance of the illuminated part. When treated with citalopram, stressed animals spent more time in the illuminated section, but not earlier than after 2 weeks of daily injections.

More recently, a similar stressor was applied by Berton and Nestler (2006). In this study, mice were subjected to daily bouts of social defeat, followed by continuous protected sensory contact with their aggressor for 10 days and were then screened for social behavior by measuring social approach toward an unfamiliar mouse enclosed in a wire mesh. Control mice spent most of their time interacting socially with the unfamiliar mouse, while the defeated mice displayed intense aversive responses and spent less time close to the target mouse. Chronic, but not acute, treatment with fluoxetine or imipramine improved social interaction in the defeated animals. Importantly, this effect was not seen after acute or chronic

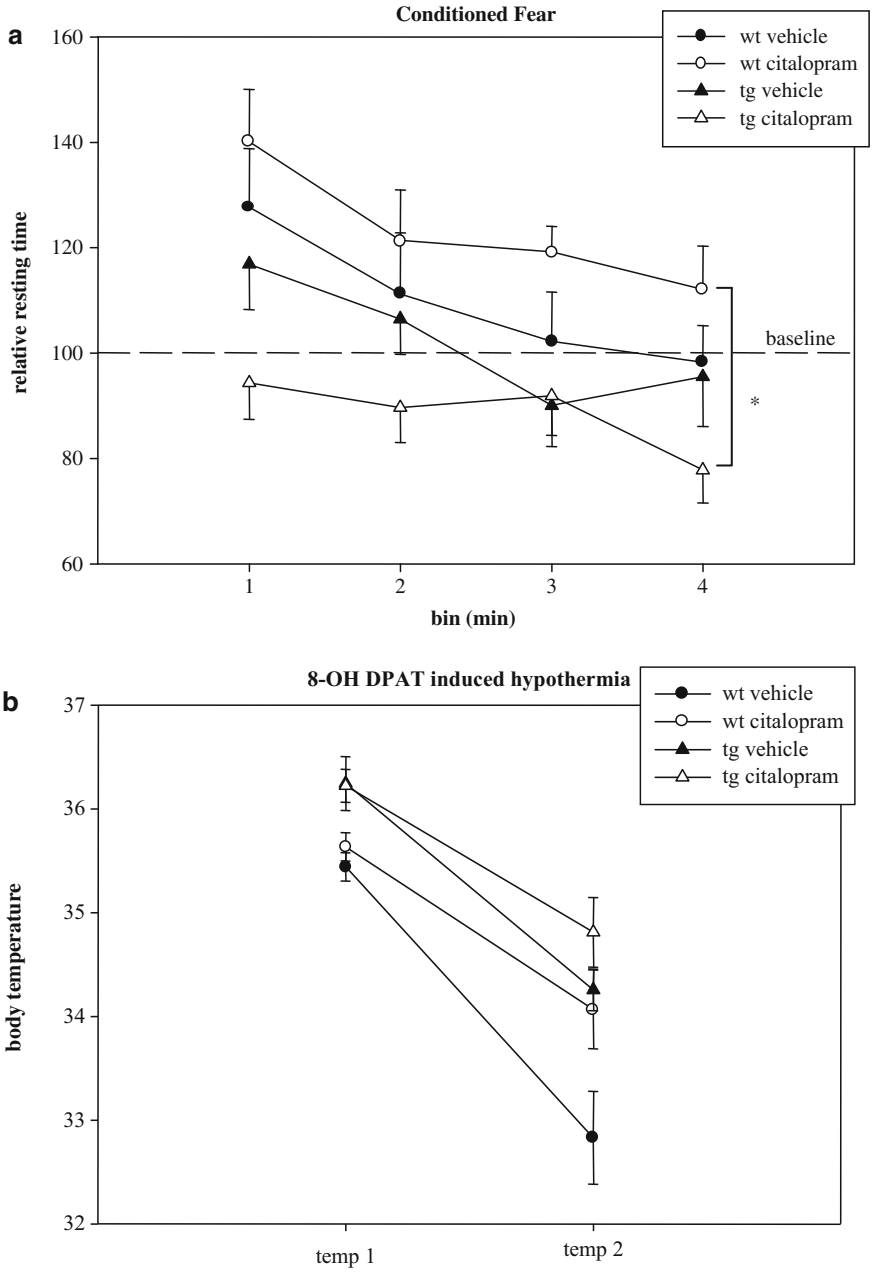

Fig. 1 Effects of chronic citalopram treatment in CRF overproducing and wild type mice on conditioned fear and body temperature. *Left panel*, change in the relative time not moving during re-exposure to the light relative to baseline (*dotted line*) 24 h after conditioning is shown. wt: wildtype; tg: transgenic. *$P<0.05$ (wt citalopram compared to tg citalopram). *Right panel*, body temperature measured under basal conditions (temp 1) and 20 min after treatment with 8-OH DPAT (temp 2). Citalopram attenuated the 8-OH DPAT induced hypothermia ($P<0.05$). From: Van Gaalen (2001)

treatment with chlordiazepoxide, suggesting that this disease model may be selectively sensitive to anxiolytic effects of antidepressant drugs.

Social stress mechanisms are also key to suggested value of the mouse defense test battery mentioned above. In this test, anxiogenic effects of fluoxetine and imipramine have been reported after acute treatment, while anxiolytic effects were shown after chronic drug application (e.g., Griebel et al. 1995).

3.8.2 Non-Social Stress

In most preclinical studies, stress is associated with exposures to aversive, painful stimulation such as electrical footshocks. In contrast, in humans, exposure to such noxious stimulation is often difficult to reveal and document. Meanwhile, both in animals and humans, stress stimuli have specific characteristics imposed by intermittent reinforcement contingencies. In other words, stressful experiences may be associated with positive or negative reinforcers delivered in a manner, which is not controlled by the subjects. Previous research suggested that compared with the ratio schedules of reinforcement, interval schedule (fixed or variable) might have aversive properties and are less preferred by the subjects (e.g., Nevin et al. 2001). Accordingly, such stressful intermittent schedules of reinforcement generate concurrent, excessive behaviors such as polydipsia (Cook et al. 1983; Falk 1971) and may even lead to adverse physiological consequences including cardiomyopathies typical of stress-related disorders (Rupp et al. 1997).

Antidepressant drugs such as SSRIs attenuate expression of schedule-induced polydipsia and these effects are characterized by remarkable delay of onset (Fig. 2; see also Hogg and Dalvi 2004). In addition to its usual significance (i.e., parallels to clinical effect), delayed onset of action in these studies helps to argue for specific mechanism of action because, when given at high-enough doses, most CNS active drugs attenuate polydipsia (via non-specific sedation or motor inhibition). Therefore, development of data analysis techniques is necessary for drugs that are expected to act faster than modern antidepressants.

3.8.3 Beyond Chronic Stress Procedures

Elevated baseline anxiety levels (i.e., disease states prior to the drug treatment) are described for a number of mouse strains where trait anxiety results from either genetic deletion of a known gene (e.g., genes for 5-HT1A, 5-HT2C receptors or neuropeptide Y) or more complex and most likely randomly introduced alterations in various neurotransmitter circuits that may be more akin to the clinical situation (e.g., BALB/c mice; Belzung and Griebel 2001). It remains to be evaluated whether such animals with trait anxiety will be in classical tests or whether they still require novel tests.

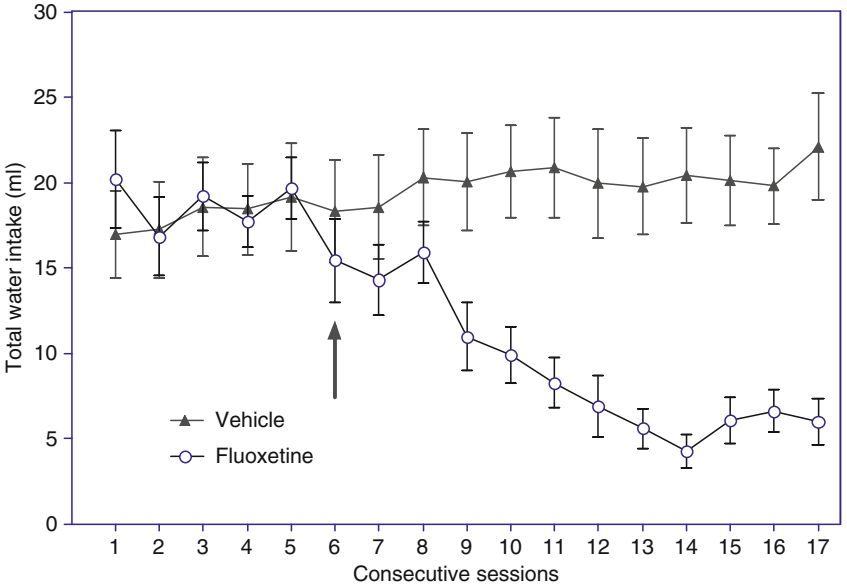

Fig. 2 Effects of repeated fluoxetine treatment on polydipsia induced by intermittent schedule of food delivery (fixed time 60 s) in rats. When water intake stabilized (ca. 3 weeks), rats received daily injection of fluoxetine (10 mg·kg^{-1}) or its vehicle prior to the 1-h long sessions (indicated by the *arrow*). Fluoxetine attenuated schedule-induced polydipsia starting after the fourth injection ($P<0.05$)

Overall, there are a number of new "anxiolytic" tests emerging that may be sensitive to antidepressant drug treatment and will therefore be very useful in the drug development process. All that has to be done is that these promising results need to be replicated by independent laboratories and evidence on predictive validity has to be extended.

As summarized in Table 2, not too many preclinical models exist where antidepressant drugs produce effects that may be interpreted as predictive of and relevant to their clinical efficacy in the treatment of anxiety disorders. Despite this generally disappointing situation, such analysis seems to suggest that treatment with SSRIs is more often associated with signs of preclinical efficacy than any other type of antidepressants. This may of course be due to the fact that SSRIs have been the most popular antidepressant medications dominating clinical use and this has affected the design and focus of preclinical studies favoring the use of SSRIs. However, this evidence may also be suggesting that, similar to the clinical situation, preclinically SSRIs are superior to other antidepressant drug classes and enhanced serotonergic function is indeed crucial for the anxiolytic properties of current antidepressants. Further, while the methodological portfolio certainly needs further work, available data on preclinical efficacy of SSRIs suggest that useful animal models are available and point at these directions for new model development.

Table 2 Summary of the preclinical efficacy of antidepressant drugs in animal models of anxiolytic activity

Animal model	TCA	MAOI	SSRI	SNRI & NRI
Ethological models				
Exploratory activity				
Social behavior			±	
Unconditioned non-social stress-induced responses				
Conditioned fear (non-social)			+	
Social stress	+		+	
Social hierarchy	+	+	+	+
Conflict tests				
PAG stimulation	+		+	
Stress sensitization		+	+	

+ positive evidence; ± mixed evidence; empty cell indicates lack of positive evidence

4 Mechanisms of Anxiolytic Action of Antidepressant Drugs

It has been repeatedly discussed that depression and anxiety disorders share a number of overlapping clinical signs and symptoms such as sleep disturbances, restlessness, irritability, difficulty concentrating, loss of control, fatigue, fear distress, etc. Many patients complaining of symptoms of anxiety in fact have depression, which is often overlooked (Fineberg and Drummond 1995). Extremely high degree of comorbidity of depression with anxiety disorders, overlapping symptoms as well as similar time-course of antidepressant drug effects in patients with depression or anxiety point at the most obvious explanation of the anxiolytic properties of antidepressant drugs: mechanisms responsible for anxiolytic effects are similar, if not identical, to those responsible for antidepressant effects of these medications.

4.1 Changes in the Neurotransmitter Systems

Acute administration of antidepressant drugs suppresses the firing rate of 5-HT neurons (Blier 2001). For both reuptake inhibitors and MAO inhibitors, these effects are explained by the density of 5-HT being greatest in the raphe nuclei. This suppression results in increased activation of 5-HT1A autoreceptors on the cell body of 5-HT neurons, which in turn exert a negative feedback action on their firing activity. This increased activation of 5-HT1A autoreceptors and resulting negative feedback occur because the firing rate of 5-HT neurons is generally proportional to 5-HT release throughout the brain. In projection areas, there is also an increase in the synaptic availability of 5-HT due to reuptake inhibition or MAO inhibition, but this enhanced level is limited by the suppression of the firing activity of 5-HT neurons. However, with prolonged treatment, the spontaneous firing of 5-HT neurons gradually returns to normal because of the desensitization of 5-HT1A autoreceptors. This desensitization is also expected to be proportional to receptor stimulation (i.e., local 5-HT levels) and therefore 5-HT1A receptors in the projection areas are affected

less than autoreceptors in somatodendritic fields. Downregulation of the latter leads to an increase in 5-HT neurotransmission and this increase is seen as the main neurochemical substrate of effects of currently used antidepressant drugs. Indirect support of this view is provided by the data indicating rapid relapse of symptoms in SSRI-sensitive depressed patients who undergo a dietary 5-HT depletion paradigm (Delgado et al. 1999). However, one should also note that there is still no convincing clinical evidence that 5-HT1A blockade accelerates onset of action of antidepressants. Thus, 5-HT1A desensitization remains a hypothesis awaiting final validation.

While 5-HT1A desensitization accounts appear to dominate the current way of thinking about antidepressant drug action, it is unclear whether this is the mechanism that is also responsible for these drugs' anxiolytic effects. Sustained administration of antidepressant drugs also enhances 5-HT transmission to NE neurons of the locus coeruleus (Szabo et al. 1999). This enhanced transmission is indicated by a marked suppression of the firing activity of these neurons resulting from an enhanced inhibitory tone exerted by 5-HT. Such inhibitory effect is, however, indirect, and is mediated by increased activation of excitatory 5-HT2A receptors on inhibitory GABAergic interneurons, the latter in turn suppressing the firing of NE neurons (Szabo and Blier 2001). Obviously, this GABAergic activation as well as attenuation of noradrenergic firing could explain at least in part the anxiolytic effects of antidepressant drugs. However, there is not much evidence directly supporting such accounts (e.g., no benzodiazepine-like anxiolytic effects of antidepressants or agents reducing the firing activity of NE neurons such as α2 agonists). It is likely that there are other neurochemical adaptations triggered by enhanced 5-HT neurotransmission that may contribute to anxiolytic activity (e.g., adaptations in β-adrenoreceptors or glutamate/NMDA receptors).

Enhanced 5-HT levels may also be responsible for clinical efficacy of drugs whose primary receptor targets are outside this neurotransmitter system. For instance, prolonged administration of selective NE reuptake inhibitors is expected to desensitize α2 receptors located on 5-HT terminals (Mongeau et al. 1994). Such indirect effects on 5-HT perhaps explain the weaker anxiolytic profile of NE reuptake inhibitors compared with SSRIs.

Overall, desensitization of auto- and heteroreceptors controlling the release of monoamines such as 5-HT is the most studied neurochemical adaptation induced by antidepressant drugs. It is unlikely to be the only possible mechanism to be exploited by future antidepressant/anxiolytic drugs. However, this knowledge is instrumental because novel target candidates can be searched by following the neuroanatomical organization of 5-HT system.

4.2 Neuroanatomical Aspects of Antidepressant Drugs' Anxiolytic Action

Serotonergic raphe cells project to a number of brain areas that are traditionally implicated in emotional processing and expression of anxiety (e.g., amygdala, hypothalamus) and the most straightforward explanation of anxiolytic properties

of antidepressant drugs would be based on inhibitory effects of elevated 5-HT on the function of these brain areas. Such account naturally leads to testing anxiolytic potency of novel drugs with alternative mechanisms of downregulating the activity of "anxiety" centers. For instance, antagonists acting at CRF1 receptors have long been hypothesized to possess anxiolytic activity (Steckler 2009). Indeed, these receptors are known to be essential in coordinating physiological response to stress and a number of studies have demonstrated the ability of CRF1 antagonists to inhibit stress-induced increases in HPA axis activity[3] (e.g., as shown by reduced corticosterone levels; Ising et al. 2007). Preclinical studies have also revealed a very appealing anxiolytic-like profile of CRF1 antagonists, which did not alter spontaneous anxiety when tested in traditional "benzodiazepine" tests (e.g., conflict tests) but were very active in tests taxing stress-facilitated anxiety (e.g., elevated plus-maze testing following the swim stress exposure; Chaki et al. 2004). Perhaps even more appealing is that CRF1 antagonists are effective in several other tests, which were claimed to be sensitive to antidepressant drug treatment (e.g., separation-induced vocalizations, mouse defensive test battery, etc.). However, as convincing as this evidence may appear, there is no clinical proof of anxiolytic activity of CRF1 antagonists and the first clinical data are rather disappointing (Coric et al. 2008) suggesting that anxiolytic effects of antidepressant drugs may have mechanisms other than simple suppression of stress-induced responses.

4.2.1 Hippocampus

One of the original members of Papez' circuit, the hippocampus, also receives relatively dense serotonergic innervation and, from a functional point of view, the hippocampus is one of the most interesting targets of anxiolytic drug action. It is well established that the hippocampus is hyperactive in anxiety disorders and there were several theories advanced to associate reduction in hippocampal activity with anxiolysis (e.g., McNaughton et al. 2007). Experimental evidence also speaks strongly in support of hippocampal involvement in anxiolytic drug action. For example, inhibition of hippocampal dentate gyrus granule cells selectively suppressed conditioned responses to ambiguous cues. In contrast, inhibition of neurons in the central nucleus of the amygdala suppressed conditioned responses to both ambiguous and non-ambiguous cues in the fear conditioning experiments (Tsetsenis et al. 2007).

For antidepressant drugs, the hippocampus is an especially important target. Over the recent 5–6 years, several studies have suggested that adult neurogenesis in the hippocampus is causally related to antidepressant drug action (see Drew and Hen 2007 for review). Several antidepressants were shown to stimulate hippocampal neurogenesis upon repeated administration. Ablation of neurogenesis via

[3]It is of note that subchronic treatment with SSRIs like fluoxetine may attenuate stress-induced behaviors but has little or no effects on stress-induced increases in ACTH and corticosterone levels (Zhang et al. 2000).

irradiation of a brain area containing the hippocampus prevented both neurogenic and behavioral (anxiolytic- and antidepressant-like) effects of antidepressants. Interestingly, non-drug inhibitors of conditioned fear (i.e., learned safety signals) enhance hippocampal neurogenesis as well (Pollak et al. 2008).

At first glance, this evidence appears to come into conflict with the data suggesting that learning (including aversive learning such as fear conditioning) enhances neurogenesis. This paradox is solved by studies demonstrating that learning increases or decreases the number of newly born cells depending on their birth date: For example, Dobrossy and colleagues (2003) divided water maze learning into two phases, an early phase during which performance improves rapidly, and a late phase during which asymptotic levels of performance are reached. The number of newly born cells increased contingently with the late phase and a large proportion of these cells survived for at least 4 weeks. In contrast, late-phase learning decreased the number of newly born cells produced during the early phase. This decline in neurogenesis was positively correlated with performance in the water maze. To understand these results, one may want to recall the difference between "perfect predictors" and ambiguous predicting stimuli mentioned above for fear conditioning studies. It is the ambiguous stimuli that are more relevant to anxiety modeling and are known to depend on hippocampal function. In case of the water maze, one could expect slower acquisition of the task in some individuals to be due to the greater ambiguity of guiding stimuli. Antidepressant drugs would be expected to reduce the impact of the ambiguous guiding stimuli, thereby enhancing hippocampal learning and neurogenesis.

Experimental support for this hypothesis would require additional studies with (sub)chronic treatment regimens of antidepressants given prior to and during the water maze training. Given that the repeated treatment with antidepressants enhances 5-HT neurotransmission and that this may inhibit hippocampal activity, it is very critical to see if anxiolytic effects can be separated from cognition-impairing properties. Such dissociation was shown for scopolamine-like muscarinic receptor antagonists and benzodiazepine anxiolytics, both of which have similar capacity to reduce hippocampal theta activity and cognitive functions. However, although benzodiazepines are anxiolytic, muscarinic receptor antagonists are not.

4.2.2 Prefrontal Cortex

Prefrontal cortex is another brain area that is critically involved in both cognitive processes and antidepressant drug action. For example, anti-OCD effects of antidepressant drugs are thought to result from increased 5-HT neurotransmission in orbitofrontal cortex (Nakao et al. 2005). OCD is often associated with hyperactivity in neuronal cortico-striato-thalamo-cortical loops and this hyperactivity can be dampened by enhanced action of 5-HT in the cortex. There is a growing body of evidence linking frontal cortical functions to other types of anxiety disorders as well. Stress-sensitization studies assessed expression of Fos protein in response to novel stressors several weeks after the repeated shock experience: in

shock-preexposed subjects, there was an elevated number of Fos-positive cells in the medial prefrontal cortex (Bruijnzeel et al. 1999). Prefrontal cortex may have an even more global function in controlling the response to stress. When a stressor is controllable (e.g., escapable), stress-induced activation of serotonergic dorsal raphe nuclei is inhibited by prefrontal cortex, and behavioral consequences of uncontrollable stress are prevented (Amat et al. 2005). It is to be noted that repeated treatment with antidepressant drugs reverses learned helplessness, the most commonly studied behavioral consequence of uncontrollable stress (Sherman et al. 1982).

The prefrontal cortex may also be involved in the mechanisms of attentional bias towards threat-related stimuli and poor inhibition of distractor processing which are characteristic of clinical anxiety (Bishop 2009). Both clinical and preclinical data indicate that prolonged stress exposure induces neuroanatomical changes in the prefrontal cortex and negatively affects performance on prefrontal cortex-dependent attentional flexibility task (Liston et al. 2006, 2009).

The above mentioned involvement of brain areas that are traditionally discussed in the context of cognitive functioning suggests contribution of learning and memory to pathophysiology of stress-related disorders as well as antidepressant drug action. Originally formulated over 40 years ago, the cognitive model of depression focuses on automatic thoughts, cognitive distortions, dysfunctional beliefs, and negative information-processing biases (Beck 2008). Traumatic experiences and the formation of dysfunctional beliefs in early life are viewed as predisposing events while congruent stressors in later life as precipitating factors. Such views can easily be transferred to describe the pathogenesis of anxiety disorders.

Is it possible that different anxiety disorders share neuroanatomical substrates? The answer is "yes and no." On the one hand, there is clearly a growing body of evidence that certain brain areas and connections are more important for some anxiety disorders than for others (e.g., cortico-striato-thalamic loops for OCD). On the other hand, antidepressant drug treatment induces neurochemical adaptations in the 5-HT system that are distributed in time and space in a manner that is not likely to depend on the type of anxiety disorder. It is possible that various anxiety disorders share some core "learning" mechanism with a specific neuroanatomical substrate and this mechanism is sensitive to antidepressant drug treatment.

4.3 Cognitive Model of Anxiety Disorders: Implications for Novel Drug Development

Even a superficial review of animal models sensitive to antidepressant drugs reveals several paradigms with strong cognitive load. For example, a number of antidepressants were shown to be effective in rats trained to press a lever to obtain food under differential reinforcement of low rates of responding schedule (DRL; Sokolowski and Seiden 1999). Under this schedule, rats need to withhold a response for a certain period of time (originally, 72 s but shorter versions are also used).

Antidepressants shift distribution of the inter-response times to the right, enhancing the total number of food reinforcers earned and reducing the overall response rate. These effects are quite characteristic of this drug class because opposite results are typically seen with psychostimulants as well as benzodiazepine anxiolytics. Specific analysis methods were developed for characterizing antidepressant drug action besides the increase in reinforcement rate. Successful performance in the DRL task requires inhibition of prepotent responses. Such inhibitory control is one of the core functions attributed to prefrontal cortex and this makes the DRL task one of the candidates for evaluating cortical cognitive aspects of antidepressant drug effects. This example of DRL indicates that strong face validity may not be necessary for tests predictive of antidepressant/anxiolytic action.

Effects of antidepressants in the DRL task are seen after the first injection. At first glance, acute efficacy speaks against the validity of this task. Indeed delayed onset of action in preclinical tests is mandated by delayed onset of clinical efficacy and is further reinforced by the current way of thinking dominated by 5-HT1A desensitization accounts. However, delayed onset of action may have other mechanisms not directly related to changes in the receptor and/or second messenger systems. For instance, any learning requires time and should the antidepressant drug action involve improved inhibitory control or any other cognitive function, it would take a certain period of time and a number of training sessions for these "cognitive" effects to be translated into improved behavioral performance in "anxiolytic" tests. If this is true, anxiolytic/antidepressant-like activity of novel drugs may not necessarily require sub(chronic) treatment and can be predicted based on the results of acute tests.

5 Summary

Antidepressant drug treatment is the clinical standard of care for all types of anxiety disorders. As discussed above, this stands in a sharp contrast to preclinical practice where most "anxiolytic" drug tests are not sensitive to antidepressants. There are at least two factors contributing to this situation.

First, traditionally "anxiolytic" drug tests have been validated by confirming their sensitivity to benzodiazepine drugs (i.e., establishing predictive validity). This approach is clearly not the most effective because it does not fully establish that classical benzodiazepine-sensitive test really reflect on therapeutic efficacy of these drugs and not their side-effects and because there may be more than one pathophysiological mechanisms via which "anxiolysis" may be produced (i.e., benzodiazepine-sensitive and benzodiazepine-insensitive).

Second, development of "anxiolytic" drug tests was often a victim to face validity requirements. For instance, laboratory rodents do avoid open spaces because for them, engagement in otherwise risky behaviors is biologically not justified. In contrast, human anxiety disorders are clearly maladaptive and reducing anxiety in diseased individuals serves a clear biological role. Another example

concerns the selection of the stress procedures for inducing anxiety states in laboratory animals. It is often assumed that only aversive stimuli that induce visible and markedly pronounced physical suffering are relevant and stressful enough. In contrast, in humans, exposure to such noxious stimulation is often difficult to reveal and document. Rather, what is common for true stress stimuli are specific characteristics imposed by intermittent reinforcement contingencies (i.e., positive or negative reinforcers are delivered in a manner, which is not controlled by the subjects). Uncertainty in a broad sense (including unstructured time) is the hallmark of human stress and may have different expressions ready for experimental modeling. These possibilities include schedule-induced behaviors that are directly based on intermittent reinforcement, conditioning to ambiguous stimuli, social stress where agonistic confrontations are possible but not predictable and not controlled by the subject, and an even larger class of behaviors that are critically dependent on the inhibition of the prepotent responses in exchange for the ambiguous possibility of later gain in reinforcement. As reviewed above, in all of these cases, antidepressant drug treatment is clearly effective.

Face validity of the model further dictates that antidepressant drug have a delayed onset of action. As argued above, the cognitive model of antidepressant drug action may be extended to cover their anxiolytic properties as well. Cognitive effects of antidepressants and any learning induced by this treatment will certainly require time for translation into improved behavioral performance and anxiolysis. One of the cognitive functions that appears to be affected by antidepressant drugs is inhibitory control. Inhibition of prepotent responding has beneficial effects in the "uncertainty" stress situations discussed above and therefore it is this cognitive function that may be critical for anxiolytic effects of antidepressants and novel anxiolytic drug development.

In conclusion, as heterogenous as they may appear, anxiety disorders share certainly more than just the label "anxiety." Their sensitivity to treatment with antidepressants such as SSRIs suggests common pathophysiological mechanisms. When these mechanisms are fully revealed and understood, truly specific anxiolytic drug tests will help identifying novel medications with improved therapeutic properties.

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Metabotropic Glutamate Receptors: Their Therapeutic Potential in Anxiety

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Abstract Psychiatric and neurological disorders are linked to changes in synaptic excitatory processes with a key role for glutamate, that is, the most abundant excitatory amino-acid. Molecular cloning of the metabotropic glutamate (mGlu) receptors has led to the identification of eight mGlu receptors, which, in contrast to ligand-gated ion channels (responsible for fast excitatory transmission), modulate and fine-tune the efficacy of synaptic transmission. mGlu receptors are G protein-coupled and constitute a new group of "drugable" targets for the treatment of various CNS disorders. The recent discovery of small molecules that selectively bind to receptors of Groups I (mGlu1 and mGlu5) and II (mGlu2 and mGlu3) allowed significant advances in our understanding of the roles of these receptors in brain function and dysfunction including anxiety. Although investigation of the role of the Group III (mGlu4, 6, 7, and 8) receptors is less advanced, the generation of genetically manipulated animals and recent advances in the identification of subtype-selective compounds have revealed some first insights into the therapeutic potential of this group of receptors.

Keywords Glutamate · Receptors · Metabotropic · Anxiety · Allosteric · Orthosteric

1 Classes of Glutamate Receptors

Glutamate is the most abundant and principle excitatory neurotransmitter in the brain. Glutamate mediates its effects via a limited number of distinct ionotropic glutamate (iGlu) receptors (i.e. NMDA [*N*-methyl-D-aspartate], AMPA [α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid], and kainate receptors) and via a number of metabotropic glutamate (mGlu) receptors. Eight mGlu receptors have been cloned to date and they are classified into three clusters based on sequence homology, preferred signal transduction pathway, and pharmacology. The Group I (located primarily post-synaptically) includes mGlu1 and mGlu5 receptors, which are coupled via Gq to phospholipase C. The Group II (located primarily pre-synaptically) receptors include mGlu2 and mGlu3 receptors, and the Group III receptors (located primarily pre-synaptically) include mGlu4, 6, 7, and 8 receptors. These receptors are coupled to Gi and inhibit stimulated cAMP formation (Schoepp 2001; Schoepp and Conn 2002).

mGlu receptors are characterized by a large extracellular ligand recognition N-terminal domain and a seven transmembrane-spanning (TM) region containing a number of conserved cysteine residues. The extracellular ligand recognition N-terminal domain has a so-called bi-lobed structure, also called the Venus fly-trap. The Venus fly trap can have an open or closed configuration in the absence or presence of an agonist, respectively. Modeling studies have indicated that the N-terminal extracellular domain of the mGlu receptor has structural similarities with bacterial periplasmic binding proteins. Like the bacterial periplasmic binding

proteins, the two globular lobes are linked by a hinge region that allows different conformational positions (open and closed). Upon binding of the agonist within the crevice that separates the two lobes, the flytrap closes and the agonist is thought to stabilize the closed conformation by making bonds with the residues of both lobes (for review see Spooren et al. (2003), Gasparini and Spooren (2007)).

2 Group I mGlu Receptor Expression in the Brain

mGlu1 and mGlu5 receptors are ubiquitously expressed on postsynaptic excitatory terminals in limbic brain regions that are involved in motivational and emotional processes. These regions include the limbic cortex (the subiculum, entorhinal, cingulate, and piriform cortical regions), the hippocampus, the amygdala, the olfactory tubercle, the periaqueductal grey, and the basal ganglia. mGlu1 and mGlu5 receptors are also expressed in thalamus and spinal cord and in periphery in the skin on nerve endings. Although the expression of mGlu1 and mGlu5 are overlapping to a high degree, the level of expression of mGlu5 is consistently higher in various regions as compared to that of mGlu1, with the exception of the cerebellum (Shigemoto and Mizuno 2000).

3 mGlu1 Receptors

3.1 Selective Tool Compounds

CPCCOEt was the first non-glutamate allosteric antagonist described for the mGlu1 receptor. This compound was useful to investigate the role of the mGlu1 subtype in vitro and the characterization of the allosteric binding site located in the TM domain. However, its limited potency and lack of bioavailability and brain penetration did not allow for a broad use in vivo. A more potent compound BAY36-7620 (Carroll et al. 2001) was subsequently described to interact at the same allosteric binding site and to inhibit the receptor's constitutive activity (Lavreysen et al. 2003). The improved in vivo properties of BAY36-7620 have allowed the detection of an early signal for analgesic and anticonvulsant effects. Further improvement was achieved with the identification of JNJ-16259685 (3,4-dihydro-2*H*-pyrano[2,3] b-quinolin-7-yl*cis*-4-methoxycyclohexyl ketone; Lavreysen et al. 2004a, b), the first highly potent, selective, and systemically active mGlu1 allosteric antagonist. This compound shows high affinity for the rat mGlu1 receptor (Ki value of 0.34 nM) and high potency to inhibit glutamate-induced increases in intracellular Ca^{2+} concentrations at the rat mGlu1 receptor (IC_{50} value 3.24 nM). JNJ-16259685 has about a 400-fold selectivity for the mGlu1 vs. the mGlu5 receptor as measured in Ca^{2+} mobilization assays. JNJ-16259685 shows good brain penetration; it

occupies mGlu1 receptors in the cerebellum, with an ED_{50} of 0.04 mg/kg s.c. Several structurally related analogs of JNJ-16259685 have been described, of which JNJ-16567083 (3-ethyl-2-methylquinolin-6-yl *cis*-4-methoxycyclohexyl ketone) has been used most frequently for in vivo studies. Recently, pharmacological properties for the mGlu1 antagonists A-841720 (9-dimethylamino-3-(*N*-hexamethyleneiminyl)-3*H*-5-thia-1,3,6-triazafluoren-4-one), FTIDC (4-[1-(2-fluoropyridine-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide), and CFMTI (2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-2,3-dihydro-1*H*-isoindol-1-one) were reported (El-Kouhen et al. 2006; Suzuki et al. 2007a, b). These compounds also have nanomolar activity and excellent selectivity for rat mGlu1 and were shown to be active in vivo. For a more comprehensive overview of the pharmacological properties of these mGlu1 receptor antagonists, we refer to a recent review.

3.2 Preclinical Efficacy

Disturbances in the neurotransmission by GABA and glutamate play a major role in the pathophysiology of anxiety, and accordingly GABA-A channel modulators and inhibitors of the NMDA receptor have shown anxiolytic-like activity in preclinical models and humans (Johnson et al. 2008; Priolo et al. 1991; Bittencourt et al. 2004; Engin and Treit 2008). The modulation of mGlu1 has been considered as an attractive target for the treatment of anxiety for several reasons. First, mGlu1 receptors are expressed in several regions involved in anxiety, for example, hippocampus, hypothalamus, periaqueductal gray, and amygdala (Simonyi et al. 2005; Lavreysen et al. 2004a; Testa et al. 1994; Shigemoto et al. 1992). Second, mGlu1 receptor antagonists are known to inhibit NMDA receptor activity (Heidinger et al. 2002; Salt and Binns 2000) and to increase GABA release (Battaglia et al. 2001; Pellegrini-Giampietro et al. 1999; Cozzi et al. 2002). Hence, it was hypothesized that mGlu1 antagonists may provide anxiolytic-like activity without evoking the side effects observed with direct GABA modulators (physical dependence, tolerance, psychomotor, and cognitive impairment) or NMDA antagonists (psychotomimetic effects and neurotoxicity).

Anxiolytic-like activities of mGlu1 receptor antagonists have been documented in a variety of animal models, for example, the lick suppression test (Steckler et al. 2005; Varty et al. 2005; Lima et al. 2008; Klodzinska et al. 2004; Tatarczynska et al. 2001), the maternal deprivation-induced ultrasonic vocalization test (Satow et al. 2008), the stress-induced hyperthermia model (Satow et al. 2008; Rorick-Kehn et al. 2005), the ethanol withdrawal-induced anxiogenic-like behavior (Kotlinska and Bochenski 2008) and the isolation-induced aggression model (Navarro et al. 2008).

Several studies have also illustrated effects of mGlu1 receptor antagonists on fear memory (Gil-Sanz et al. 2008; Nielsen et al. 1997; Gravius et al. 2005, 2006; Maciejak et al. 2008; Nadlewska et al. 2003; Kim et al. 2007; Simonyi et al. 2007).

The models used are based on aversive stimuli, and a fear response is acquired by associative learning.

However, data derived from mGlu1 receptor knockout (KO) mice have suggested that inhibition of mGlu1 receptor-mediated signaling may induce cognitive impairments in hippocampal-dependent learning and memory processes (Conquet et al. 1994; Aiba et al. 1994). Data on long-term potentiation (LTP) in mGlu1 KO mice, albeit inconsistent, have created further concerns (Silva 2003; Aiba et al. 1994; Hsia et al. 1995; Conquet et al. 1994; Bordi 1996). It is well established that mGlu1 receptors are required for NMDA- and AMPA-mediated LTP in certain synapses (although probably not in all; Anwyl 2009), which may explain their effects on cognitive types of behavior. In line with the above mentioned observations, mGlu1 receptor antagonists have been shown to impair processes involved in fear memory formation. Acquisition, consolidation and extinction of aversive or fear learning were attenuated by mGlu1 receptor antagonists (Gil-Sanz et al. 2008; Nielsen et al. 1997; Gravius et al. 2005, 2006; Maciejak et al. 2008; Nadlewska et al. 2003; Kim et al. 2007; Simonyi et al. 2007). Although these effects could be explained in the context of cognitive impairment and regarded as a side effect, the behavior assessed in these models is also associated with an expectation of potential danger that characterizes anxiety and fear. Clearly both cognitive and anxiety components are present in these models and anxiolytic-like activity of mGlu1 antagonists will affect the behavioral readout.

4 mGlu5

4.1 Selective Tool Compounds

The first subtype-selective mGlu5 receptor antagonists reported were SIB1757 and SIB1893 with IC_{50} values of 3.7 and 3.5 μM, respectively, in the inositol phosphate (IP) accumulation assay. Shortly after, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) was described, a 100-fold more potent antagonist derived from chemical variation of SIB1893 with reasonable PK, oral bioavailable, and in vivo activity. At human and rat mGlu5 receptors MPEP inhibits quisqualate-stimulated IP production with IC_{50} values of 36 and 17.9 nM, respectively, but has no significant agonist or antagonist activities at cells expressing other mGlu receptors up to 10 μM. It was shown that MPEP acts as a noncompetitive antagonist that inhibits mGlu5 receptor function and binds in the TM region, without affecting the binding of glutamate to the extracellular region. In cells overexpressing mGlu5 receptors, MPEP also inhibits constitutive activity of the receptor, suggesting that MPEP acts as an inverse agonist (for review please see Gasparini and Spooren (2007), Gasparini et al. (2008), Jaeschke et al. (2008)).

5 Efficacy in Anxiety

5.1 *Preclinically*

The anxiolytic-like properties of mGlu5 receptor antagonists are well documented and replicated in academic and industrial settings. These antagonists have activities in a wide range of conditioned (Vogel conflict, Geller Seifter, conditioned emotional response, fear potentiated startle, and four plate test) and nonconditioned (elevated plus maze, stress-induced hyperthermia, marble burying, and social exploration) anxiety tests. Aside from benzodiazepine agonists, mGlu5 receptor antagonists are the only class of compounds to have such a robust preclinical efficacy profile. The downstream pathways responsible for this anxiolytic-like effect are not fully elucidated. However, it is established that the anxiolytic-like effects are not mediated through GABAergic systems, but may relate to processes that depend on systems in the amygdala, such as neuropeptide Y, or prefrontal systems involving serotonin and noradrenaline. Importantly, mGlu5 receptors are a critical factor in fear memory formation, and also in conditioned emotional states in adverse conditions, such as (chronic) pain, that is, processes that again are highly dependent on amygdala functions (for review please see Spooren and Gasparini (2004), Gasparini et al. (2008), Jaeschke et al. (2008)).

5.2 *Clinically*

During a functional high-throughput screening at Hoffmann-La Roche's small-molecule library for mGlu5 modulators, fenobam was identified as a potent non-competitive non-MPEP-like allosteric modulator of mGlu5 receptors. Fenobam (McN-3377) was originally developed as a nonbenzodiazepine anxiolytic (by McNeil Laboratories, 1978–1982), with an unknown molecular target (Porter et al. 2005). In preclinical studies, fenobam was reported to be active in rat models of anxiety (conflict tasks including Geller-Seifter and Vogel; Patel et al. 1982), and this activity was not blocked by a benzodiazepine antagonist (Goldberg et al. 1983). In a double-blind placebo-controlled clinical trial, fenobam had an efficacy and onset of action comparable with diazepam (HAM-A/HSRC/HAM-D) (Pecknold et al. 1982). Additionally, fenobam has been reported to be active in outpatients with severe anxiety and in a single blind study (Lapierre and Oyewumi 1982). In all three studies, fenobam was reported to have a good safety profile with lack of sedation, muscle relaxation, and no interaction with ethanol, further supporting a mechanism of action that is distinct from GABA-ergic potentiation. However, fenobam has also been reported to be inactive in one phase II outpatient trial where side effects of a psychostimulant nature were reported (Friedman et al. 1980). At the time, fenobam was discontinued from further development as an anxiolytic, with indications that the molecule required further refinement (Friedman et al. 1980; Wu et al. 1995).

Addex Pharmaceuticals studied ADX10059, an MPEP derivative, in a small Phase IIa study in patients with dental anxiety. The study was a double-blind, placebo-controlled trial conducted at specialist centers. It included 50 patients with moderately severe dental anxiety that underwent a routine dental procedure. Anxiety was measured at specific time points before, during, and after the procedure by using a subjective rating scale, called the Visual Analog Scale of anxiety (VAS anxiety). Skin conductance, an objective measure of the physiological response to stress and patient ratings of study medication effectiveness, was also collected. ADX10059 did not reduce acute anticipatory anxiety in this study; however, signs of anxiolytic activity were observed in the trial. Although the differences were not statistically significant, patients in the ADX10059 group had lower increases in skin conductance at all time points from 30 min post dose, suggesting a lower level of stress. In addition, patients rated ADX10059 as more effective, with 56% rating it as "good" or "excellent" compared to 30% of those who received placebo. ADX10059 was generally well tolerated in the study and there were no serious adverse events (Addex press release). In conclusion, to date the clinical data on mGlu5 do not confirm the broad anxiolytic profile seen in animal models and in patients with fenobam. However, given the limited number of patients involved and the acute treatment design, it does not preclude that mGluR5 antagonists have potential in other forms of anxiety.

6 Group II mGlu Receptor Expression in the Brain

Group II mGlu receptors (mGlu2 and 3) are negatively coupled to adenylate cyclase activation and modulation of ion channels (Brauner-Osborne et al. 2007). Like the Group I mGlu receptors, they are widely expressed in limbic brain regions, including the limbic cortex, hippocampus, amygdala, bed nucleus of the stria terminalis, and basal ganglia. Both Group II mGlu receptors are preferentially presynaptically expressed. mGlu2 expression seems to be more restricted to neurons, while mGlu3 receptors are also expressed in glial cells (Janssens and Lesage 2001). Presynaptically, Group II mGlu receptor expression is found at the pre-terminal portion of the axon, that is, outside the active zone of glutamate release. Presynaptic activation, presumably from spillover of glutamate after synaptic release, acts as a negative feedback signal, that is, it leads to the reduction in glutamate release (Shigemoto et al. 1997). Glial mGlu3 receptors may be involved in inflammatory responses and neuroprotection. Anxiety has been suggested to be associated with enhanced glutamatergic transmission in brain areas such as prefrontal cortex and amygdala. Hence, activation of presynaptic Group II mGlu receptors, which is known to reduce glutamate release, may provide anxiolytic effects (Swanson et al. 2005). Interestingly, Group II mGlu receptors also seem an interesting target for the development of new antipsychotic drugs (Patil et al. 2007), although this is beyond the scope of this chapter.

6.1 Selective Tool Compounds

A limited number of potent, mixed mGlu2/3 receptor orthosteric agonists have been reported, such as eglumetad (LY354740), a structurally constrained analogue of glutamate that competes with endogenous glutamate for its binding site (orthosteric site). The anxiolytic-like properties of this compound were tested in both preclinical and clinical studies (see below); however, broad utilization of eglumetad was limited due to its modest oral bioavailability and brain penetration (Rorick-Kehn et al. 2007). A prodrug strategy that led to the identification of Talaglumetad (LY544344), an L-alanylamide prodrug of eglumetad, allowed to significantly improve the biodisponibility compared to eglumatad (Rorick-Kehn et al. 2006). Talaglumetad was tested in phase II in patients with generalized anxiety disorder (GAD) (see below), but further development was suspended because of toxicity (convulsions) in preclinical rat studies (Dunayevich et al. 2008).

Using mGlu2 or mGlu3 receptor KO mice, it was shown that the anxiolytic-like activity of eglumetad was absent in either type of knockout (Linden et al. 2005), suggesting that both receptor subtypes would be of relevance. In contrast, there is preclinical evidence from studies with KO mice suggesting that the mGlu2 receptor may be of more relevance for the mediation of psychosis-related behavior than the mGlu3 receptor (Spooren et al. 2000, 2002; Fell et al. 2008; Woolley et al. 2008), while neuroprotective effects of mGlu2/3 receptor agonism is abolished in mGlu3 KO mice (Corti et al. 2007). However, the true therapeutic relevance of mGlu2 vs. mGlu3 receptor activation in the clinic remains elusive due to the lack of subtype-selective, orthosteric Group II mGlu receptor agonists. In fact, the high sequence homology in the orthosteric binding sites of the mGlu2 and mGlu3 receptor made it very unlikely to discover orthosteric agonists that would be selective for one or the other subtype. An alternative strategy, aiming at selectively targeting the mGlu2 receptor, is the targeting of allosteric sites with agents acting as positive modulators (PAMs). These compounds do not activate the mGlu2 receptor at the orthosteric binding site, but bind to an allosteric site located in the TM domain of the receptor, which is different from the glutamate binding site, and facilitate the effects of glutamate at the receptor. Prototypical mGlu2 PAMs are LY487379 (Johnson et al. 2003) and BINA (Galici et al. 2006), also showing anxiolytic-like activity in animals.

7 Efficacy in Anxiety

7.1 Preclinically

There is substantial preclinical evidence supporting a role of Group II mGlu receptors in the modulation of anxiety-related behavior. Eglumetad (LY354740) has anxiolytic-like effects in a number of anxiety tests in rodents, including

fear-potentiated startle (Tizzano et al. 2002; Walker et al. 2002), the elevated plus maze (Ferris et al. 2001; Linden et al. 2004, 2005; Monn et al. 1997; Schlumberger et al. 2009), in a rat model of panic-related behavior (Shekhar and Keim 2000), the Vogel conflict test (Tatarczynska et al. 2001), fear-induced suppression of operant responding (Rorick-Kehn et al. 2006), and stress-induced hyperthermia (Rorick-Kehn et al. 2005; Spooren et al. 2002; see also Spooren et al. 2002, 2003; Swanson et al. 2005; Witkin et al. 2007, for reviews). Some of these behavioral effects seem to be regulated at the level of the amygdala (Walker et al. 2002) and hippocampus (Tatarczynska et al. 2001) and seem to depend on the interactions with GABAergic mechanisms (Ferris et al. 2001). Likewise, fear-induced suppression of operant responding was reversed by talaglumetad, and the prodrug seemed to be more efficacious than eglumetad (Rorick-Kehn et al. 2006).

Anxiolytic-like effects were also seen in rats treated with the mGlu2 PAM LY487379 in fear-potentiated startle (Johnson et al. 2003), and with BINA in the elevated plus maze and in stress-induced hyperthermia (Galici et al. 2006), suggesting that at least part of these effects are mediated via stimulation of the mGlu2 receptor. Moreover, these findings indicate that both orthosteric and allosteric modulators of the mGlu2 receptor may hold potential for the treatment of anxiety disorders in man.

Not much is known about the specific role of the mGlu3 receptor in the mediation of anxiety-related behavior. However, the finding that eglumetad lost efficacy in mGlu3 receptor KO mice (Linden et al. 2005) would suggest that this receptor also plays a role in this type of behavior.

7.2 Clinically

The anxiolytic potential of eglumetad (LY354740) was first revealed in healthy volunteers, showing activity in fear-potentiated startle (Grillon et al. 2003). Likewise, evidence for anxiolytic effects was reported in a small study with talaglumetad in healthy volunteers on CCK-4 induced panic and anxiety symptoms, although these effects reached significance only after exclusion of two subjects that did not show decreased CCK-4-induced ACTH release (Kellner et al. 2005). Similarly in a small trial involving panic disorder patients, eglumetad significantly reduced the responses to CO_2 challenge compared to placebo (Levine et al. 2002, Schoepp et al. 2003).

In contrast, eglumetad failed to reduce the number of patients having panic attacks in the final 3 weeks of the treatment or to improve the outcome on Panic Disorder Severity Scale in a small, double-blind, placebo-controlled clinical trial, which was part of a larger, multicenter phase II study (Bergink and Westernberg 2005). In this study, the number of patients analyzed were low, and paroxetine, used as positive control, also failed to demonstrate a positive result, rendering this study inconclusive.

A first human trial on GAD showed significantly greater improvement from baseline in Hamilton Anxiety (HAMA) and Clinical Global Impression Improvement

(CGI-I) scores, as well as response and remission rates in eglumetad-treated patients compared with placebo-treated patients (Michelson et al. 2005, Dunayevich et al. 2008). These positive effects were confirmed with talaglumetad, the prodrug of eglumetad, in a smaller but longer GAD trial (8 weeks instead of 5 weeks), which at the highest dose tested demonstrated significant improvement in the HAMA and CGI-I scales over placebo-treated patients. The trial was, however, discontinued in an early stage because of convulsions in preclinical safety studies (Dunayevich et al. 2008).

In all clinical studies, eglumetad and talaglumetad demonstrated a favorable side effect profile compared to the active controls used (lorazepam or paroxetine).

Taken together, the reported clinical evaluations of the mGlu2/3 agonist eglumetad or its prodrug talaglumetad clearly support the hypothesis that activation of the mGlu2/3 receptor is associated with anxiolytic efficacy and confirm the results obtained in preclinical models. However, the identification of the precise therapeutic potential of this mechanism of action will require additional clinical investigations. Given the preclinical safety issues identified with talaglumetad, the recently identified PAMs might constitute a valuable development alternative to maintain mGlu2 activating effects while avoiding the unfavorable properties (low bioavailability, preclinical safety; Johnson et al. 2003, Galici et al. 2006).

8 Group III mGlu Receptors

Similar to the other mGlu receptor groups, Group III mGlu receptors share about 60%–70% protein sequence similarity. For all four Group III receptors several splice variants have been identified, all of which result from alternative splicing at the level of the C-terminus. Activation of mGlu4, 6, 7, and 8 receptors recruit G_i proteins, leading to decreased levels of intracellular cAMP. While the availability of in vitro and in vivo tools for the Groups I and II receptors has facilitated the elucidation of their roles within the CNS, the role of the members of the Group III receptor family is less understood because of the lack of suitable subtype-specific compounds.

Group III mGlu receptors are generally expressed on presynaptic sites in the brain, where their activation can depress both glutamate and GABA release (Schoepp 2001). More studies with subtype-selective agents are awaited to further fine tune their precise role in the modulation of excitatory and inhibitory neurotransmission.

9 Receptor Expression

mGlu4 receptors are most prominently expressed in the cerebellum, but can also be found in other brain and spinal regions to some degree (Ferraguti and Shigemoto 2006). Mostly, the receptor is found at presynaptic sites, but postsynaptic

localization both in glutamatergic and GABAergic neurons has been reported (Bradley et al. 1999). Interestingly, a mGlu4 receptor variant, exclusively expressed in taste tissues and lacking about half of the N-terminus, has been suggested to be involved in "umami" taste sensation to monosodium glutamate (Chaudhari et al. 2000; Dingledine and Conn 2000).

The mGlu6 receptor has been the least studied Group III receptor in terms of its therapeutic potential for psychiatry, largely since it is mainly expressed in the retina and only low levels have been found in the brain (Nakajima et al. 1993; Shigemoto et al. 1997; Janssens and Lesage 2001). The mGlu7 receptor is highly expressed throughout the forebrain, brainstem, and spinal cord (Bradley et al. 1996; Corti et al. 1998; Kinoshita et al. 1998). The more widespread distribution of the mGlu7 receptor compared to other presynaptic mGlu receptors may indicate a more prominent role in normal regulation of synaptic glutamate and/or a role as "emergency" autoreceptor in case of high (pathophysiological) synaptic glutamate levels. Expression of the mGlu8 receptor is most pronounced in the olfactory bulb, hippocampus, cerebellum, and mammilary body (Duvoisin et al. 1995; Saugstad et al. 1997). The mGlu8 receptor has been located outside the CNS as well (Hoang and Hay 2001; Tong et al. 2002; Tong and Kirchgessner 2003).

10 Group III Selective Ligands

Unraveling the specific functions of individual type III receptors has proven difficult, because of the lack of subtype-selective compounds. As a consequence, preclinical data that support the use of specific mGlu4, 6, 7, or 8 ligands for the treatment of anxiety are still limited. Initial in vivo tests were performed using ligands that are selective over other mGlu receptors but with limited discrimination between the various Group III receptors (Group III selective ligands). The prototypic Group III agonist is L-AP4, a ω-phosphonic acid isostere of glutamate that shows low micromolar potencies at mGlu4, 6, and 8 receptors. Other Group III selective agonists include L-SOP, (R,S)-PPG, L-CCG-I, and ACPT-I (Wu et al. 1998; Wright et al. 2000; De Colle et al. 2000). While systemic administration of these compounds is hampered by their poor blood–brain-barrier penetration, intrahippocampal or intra-amygdala injections have indicated anxiolytic-like behavior by some of these compounds in a number of paradigms such as the Vogel conflict drinking test or the elevated plus maze. Group III selective antagonists include MSOP, CPPG, and MPPG (Han and Hampson 1999; Wright et al. 2000). MSOP and CPPG have also been shown to produce anxiolytic effects in Vogel's conflict drinking test in rats, although this seems to be dependent on the applied route of administration (Chojnacka-Wojcik et al. 1996; Stachowicz et al. 2006). While these initial data provided a first hint that regulation of excitability via Group III mGlu receptors may play a role in diminishing anxious behavior, it remained difficult to determine which individual subtype was responsible for these effects. The contrasting data between Group III agonists and antagonists moreover suggested

that this behavior can be influenced by different receptors expressed within different synapses.

10.1 The mGlu4 Receptor

10.1.1 Specific Tool Compounds

Although ACPT-I and (R, S)-PPG are relatively selective for mGlu4 and 8 over the other Group III receptors, there are no reports on specific mGlu4 receptor agonists. (-)-PHCCC is an mGlu4 receptor PAM (EC_{50} 6 μM when applied together with L-AP4) with limited selectivity (Maj et al. 2003; Marino et al. 2003; Beqollari and Kammermeier 2008). Novel and more potent mGlu4 receptor-specific PAMs, among which VU015504 (with a potency of about 800 and 700 nM at human and rat mGlu4 receptors, respectively), have recently been identified (Niswender et al. 2008; Engers et al. 2009).

10.1.2 Preclinical Efficacy

In vivo data using (-)-PHCCC and VU015504 indicate that the mGlu4 receptor may be a promising new target for Parkinson's disease (Marino et al. 2003; Battaglia et al. 2006; Niswender et al. 2008). Although (-)-PHCCC seems to act as an anxiolytic in the Vogel test after intra-amygdala or intrahippocampal injection in rats (Stachowicz et al. 2006), in vivo data with specific mGlu4 receptor compounds in models of anxiety are not available to date. Hence, there is so far no clear indication that mGlu4 receptors may be involved in this disease.

10.2 The mGlu6 Receptor

10.2.1 Specific Tool Compounds

(R, S)-homoAMPA is identified as the first and only selective mGlu6 receptor agonist (Brauner-Osborne et al. 1996).

10.2.2 Preclinical Efficacy

When given intrahippocampally, (R,S)-homoAMPA produces anxiolytic-like effects in the conflict drinking test (Palucha et al. 2004). Since activity is observed only at very high doses (250 nmol/rat), and given the predominant expression of the mGlu6 receptor in the retina, the role of the mGlu6 receptor in the mechanisms

responsible for anxiolytic-like effects seems, however, to be minor. Likely, the prime role of this receptor entails visual signal transduction (Masu et al. 1995).

10.3 The mGlu7 Receptor

10.3.1 Specific Tool Compounds

AMN082 is the only mGlu7 receptor-specific agonist found to date. In vitro, in mGlu7 receptor-expressing cells, it inhibits forskolin-induced cAMP accumulation with an EC_{50} of 64 nM (Mitsukawa et al. 2005). Recently, mGlu7 receptor-specific allosteric antagonists have been identified (Suzuki et al. 2007a, b). MMPIP is a relatively potent (26 nM in a Ca^{2+} mobilization assay using rat mGlu7 receptor-expressing cells, and 220 or 610 nM in a cAMP assay on the rat and human mGlu7 receptor, respectively) and selective mGlu7 receptor antagonist and represents a promising tool for elucidating mGlu7 receptor function in vivo.

10.3.2 Preclinical Efficacy

AMN082 (6 mg/kg, p.o.) increases plasma corticosterone and ACTH levels in wild-type but not in mGlu7 KO mice (Mitsukawa et al. 2005). AMN082 is also reported to be active at 5 mg/kg p.o. in stress-induced hyperthermia as well as in a social interaction test in rats[1]. Systemic administration of AMN082 (20 mg/kg p.o.) has been shown to facilitate the extinction of aversive memories in fear-potentiated startle and conditioned tasteaversion (Fendt et al. 2008).

While data with AMN082 would suggest the use of mGlu7 receptor agonists for treating anxiety and posttraumatic stress disorders, likely via a decrease in glutamatergic transmission in key brain regions, data gathered with mGlu7 KO mice suggest that inhibition of mGlu7 receptors may be of value, presumably via the control of GABA release. Data from forced swim, tail suspension, light–dark box, stress-induced hyperthermia, elevated plus maze, marble burying, and staircase test indicate that mGlu7 inactivation induces an antidepressant and anxiolytic-like phenotype (Cryan et al. 2003; Callaerts-Vegh et al. 2006). Ablation of the mGlu7 receptor also results in specific changes in key regulators of the HPA axis, in a direction opposite to that found in human depression or following chronic stress (Mitsukawa et al. 2006). To unravel the apparent discrepancies between behavioral effects seen in mGlu7 KO mice and wild-type mice treated with AMN082, it will be crucial to assess the in vivo effects of mGlu7 receptor antagonists. Evaluation

[1]Abstract from 5th International Meeting on Metabotropic Glutamate Receptors.

of mGlu7 receptor blockade for the treatment of psychiatric illnesses should, however, also include an assessment of pro-convulsant liability and cognitive dysfunction as it has been shown that mGlu7 receptor gene disruption predisposes to epilepsy (Sansig et al. 2001) and results in deficits in long-term potentiation and working memory (Callaerts-Vegh et al. 2006; Bushell et al. 2002; Holscher et al. 2004, 2005).

10.4 The mGlu8 Receptor

10.4.1 Specific Tool Compounds

Like for all Group III receptors, there is a lack of specific mGlu8 receptor ligands. To date, (S)-3,4-DCPG represents the most selective tool compound for the mGlu8 receptor. It is an orthosteric mGlu8 receptor agonist (potency of ~30 nM and ~100- to 200-fold selectivity vs. other Group III receptors; Thomas et al. 2001). No specific mGlu8 receptor antagonists or PAMs have been reported.

10.4.2 Preclinical Efficacy

(S)-3,4-DCPG's amino acid-like structure and hence poor permeability through the blood–brain-barrier makes it less suited as in vivo tool compound; thus, data generated with this compound should be interpreted with care. In rats treated with high doses of (S)-3,4-DCPG (100 mg/kg, i.p.), c-fos expression is induced in stress-related brain regions (Linden et al. 2003). (S)-3,4-DCPG induces an anxiolytic effect in the fear-potentiated startle paradigm in rats after central application (30–300 nmol; Schmid and Fendt 2006) as well as in stress-induced hyperthermia in mice after systemic application (30 and 60 mg/kg, i.p.; Rorick-Kehn et al. 2005). The compound has no effect in the Vogel conflict drinking test in rats after injection into the amygdala/hippocampal CA1 region (up to 10 nmol/rat) (Stachowicz et al. 2005). Data from genetically manipulated animals lacking the mGlu8 receptor strengthen a role of this receptor in anxiety-related disorders. mGlu8 receptor KO mice exhibit increased anxiety-related behavior in an open field (Duvoisin et al. 2005; Robbins et al. 2007) and elevated plus maze paradigm (Duvoisin et al. 2005; Linden et al. 2002). Absence of the mGlu8 receptor also diminishes exploratory behavior in a novel enclosed environment (Duvoisin et al. 2005). Collectively, these data support the hypothesis that increasing mGlu8 receptor activity either by agonists or PAMs may be a promising alternative for treating anxiety disorders. The mechanism of action is not well understood so far; evaluation of the mGlu8 receptor as a disease target using better tool compounds is therefore awaited.

11 Conclusions

In spite of the significant advances achieved in the past two decades on the understanding of brain physiology of stress and fear, the pathological dysfunctions underlying anxiety and the associated disorders remain elusive. As a consequence, the discovery and development of agents acting via mechanisms different from the serotonin transmission pathway has been largely unsuccessful.

Among the different mechanisms of action investigated, the glutamate pathway has been evaluated first using agents acting at the iGlu receptors, but their use was hampered due to their limited therapeutic index. The identification of the mGlu receptor subfamily and the discovery of selective agents acting at the different subtypes significantly enhanced the preclinical and clinical evaluation of the role of glutamate in anxiety.

The most significant advance in the field has been achieved with eglumetad and its prodrug talaglumetad, an agonist of the mGlu2/3 subtypes developed by Eli Lilly. Both agents showed a broad anxiolytic like profile in numerous rodent models for anxiety without the notorious side effects of the established anxiolytic drugs benzodiazepines. Remarkably, the preclinical profile could be confirmed clinically first in healthy subjects (antistress, antifear) and subsequently in generalized anxiety patients with either eglumetad or talaglumetad. Furthermore, the broad therapeutic index identified preclinically was also confirmed in the clinic. Unfortunately, the GAD trial with alaglumetad was stopped due to preclinical safety findings, and no further development has been reported to date. Overall, the activation of the mGlu2/3 receptor has proven to have the potential to be developed into an anxiolytic therapeutic with an improved side effect profile compared to the established therapies. In light of the multiple failures or equivocal results reported with investigative new mechanisms, the positive results with Group II agonists constitute a remarkable success in the process of identifying and validating new therapeutic approaches to anxiety.

For the Group I mGluRs, modulation of the mGluR5 subtype with a variety of selective noncompetitive antagonists showed a broad preclinical efficacy in a number of different animal models for anxiety. Additional support for the mechanism of action came indirectly from the clinical evaluation of fenobam and the anxiolytic effects seen in four out of the five clinical trials reported. As for the eglumetad and talaglumetad, the anxiolytic effects were achieved without the typical side effects of benzodiazepines, such as sedation or muscle relaxation; however, some side effects of psycho stimulant nature were reported and led to the discontinuation of the development of fenobam. The recent negative results reported for the novel agent ADX10059 (Addex Pharmaceuticals) do not allow to confirm the broad anxiolytic profile seen in animal models and in patients with fenobam. However, given the limited number of patients involved and the acute treatment design does not preclude that mGluR5 antagonists have potential in other forms of anxiety, which would require a longer duration of treatment. In contrast to the advances achieved with the mGluR5 antagonists, the mGluR1 antagonists,

which also showed anxiolytic-like effects in animals, were not developed further. The reason might rely on the perceived limited therapeutic window, in particular for cognitive function. The effect of mGlu1 antagonists on cognitive performance are documented especially for fear memory in aversively motivated tasks. In these tests, anxiolytic-like activity of the compounds may have affected the behavioral readout. Reports on working memory in positively reinforced models are inconsistent and do not allow a final conclusions. For the Group III mGlus, the development relies significantly behind the Groups I and II, presumably because of the absence of selective agents with adequate pharmaceutical properties. Therefore, the identification and the validation of potential therapeutic use of Group III mGlus was strongly impaired and relied mainly on mouse mutants and the use of non-optimal tool compounds. The recent progress made in molecular pharmacology and the identification of new agents capable to activate selectively the different subtypes will certainly contribute to further unravel the potential of Group III receptors in anxiety.

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Developing Small Molecule Nonpeptidergic Drugs for the Treatment of Anxiety Disorders: Is the Challenge Still Ahead?

Thomas Steckler

Contents

Abstract Neuropeptide systems have been considered a major opportunity for the development of novel treatment approaches for anxiety disorders based on preclinical evidence and neurochemical alterations seen in anxiety disorders. This excitement was further facilitated by the fact that drugs acting at these systems, such as CRF1 antagonists, NK1 antagonists, NK3 antagonists or CCK2 antagonists, may have unique properties not seen with drugs affecting more classical mechanisms involved in anxiety. Consequently, there have been major efforts to develop such small-molecule, nonpeptide receptor ligands. A number of these molecules have been tested in the clinic, either in trials where levels of anxiety served as a secondary measure, or in a few studies with patients suffering from anxiety disorders. But unfortunately, and despite all the efforts of the field as a whole, we still lack convincing clinical proof-of-concept for any of the neuropeptidergic approaches in patients. It must, therefore, be concluded that neuropeptide targets remain a promising approach for the development of the next generation drugs to treat anxiety disorders, but that they continue to be high-risk targets for drug development.

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1 Introduction

Drugs that target neuropeptide systems – antagonists, full or partial agonists, positive allosteric modulators, or inverse agonists – could represent an alternative approach to treat anxiety disorders. Characteristics that should make neuropeptide systems attractive as drug targets are, first, their generally more discrete neuroanatomical localization when compared to monoamines and GABA and glutamate and, second, their state-dependent effects, i.e., neuropeptides need conditions of relatively high neuronal firing to be released at quantities which activate their receptors, while classical neurotransmitters are released by low neuronal frequency bursts (Hokfelt et al. 2003). Based on the latter, we can assume that antagonists, i.e., compounds that block binding of the endogenous peptide to the receptor recognition site, are more active under conditions of increased peptide release than under basal conditions. In other words, antagonistic drugs could normalize pathological anxiety associated with increased peptide release, while leaving normal physiology in the absence of neuropeptide release relatively unaffected, thus minimizing target-related side effect burden (Steckler 2008).

When it comes to agonists, the latter advantage of antagonists may be lost as they may stimulate the neuropeptide receptor in the absence of the endogenous ligand. Thus, full or partial agonists acting at the orthosteric (i.e., endogenous ligand) binding site of the peptide receptor, will fully or partially mimic the effects of the endogenous peptide. Nevertheless, there are a spectrum of anxiety disorders, presumably with different underlying pathophysiology, and those drugs could be of relevance if there is a decrease in peptide release and consequent peptidergic hypoactivity associated with an anxiety disorder. Likewise, agonists may be of use under conditions where the peptidergic system is not directly involved in the pathophysiology of the anxiety disorder, but where it is known preclinically that activation of the specific neuropeptide receptor will lead to a reduction in anxiety and arousal, thereby providing an indirect treatment approach. However, the ability of those compounds to stimulate the receptor in the absence of the endogenous ligand imposes the inherent risk that the receptor is tonically overstimulated, as well as the potential for the development of receptor desensitization and internalization, and hence tolerance development to the drug after chronic treatment. Compared to full agonists, this risk may be somewhat mitigated with a partial agonist leading to submaximal receptor activation and hence reduced relative efficacy when compared to a full agonist, but still exists.

Numerous selective and nonselective agonists acting at neuropeptide receptors have been described. An example for a partial agonist is the drug diltiazem, an atypical L-type calcium channel blocker used since long to treat cardiac arrhythmias,

which has recently been discovered to also be a partial agonist at the ghrelin receptor (Ma et al. 2007). Ghrelin is a 28-amino acid gastrointestinal peptide with orexigenic properties. In the brain, ghrelin binds to the growth hormone secretagogue receptor (GHS1A) (Dornonville de la Cour et al. 2001; Kojima et al. 1999; Tschop et al. 2000), where one of its functions, besides regulating food intake, may be to increase anxiety-related behavior (Asakawa et al. 2001; Carlini et al. 2002, 2004; Kanehisa et al. 2006). Interestingly, some anxiolytic efficacy has been reported for diltiazem in panic disorder patients (Balon and Ramesh 1996), although this may be more attributable to the calcium channel blockade than the partial ghrelin agonism (especially as the animal literature would suggest the latter would lead to an increase, not a reduction, in anxiety).In contrast to the full and partial agonists, positive allosteric small-molecule nonpeptidergic modulators (PAMs) bind at a site different from the binding pocket of the endogenous ligand, thereby facilitating the effects of the neuropeptide, but (assuming that there is no endogenous ligand for the allosteric binding site) should have no effect on their own. Those molecules may overcome part of the problems inherent to orthosteric agonism as they again lead to phasic effects only in the presence of the endogenous neuropeptide ligand and not to tonic activation, and hence reduce the risk of overstimulation and tolerance development. Examples for such allosteric drugs acting at neuropeptide systems have been reported, e.g., again for the ghrelin receptor (Holst et al. 2005).

Yet another class of compounds is the inverse agonists. To understand this concept, it must be acknowledged that some receptors can be constitutively active. That means that the receptor signals in the absence of the endogenous ligand, i.e., there is a basal tone of receptor signaling that is further increased by phasic release and binding of the peptide. Inverse agonists will reduce the tonic signaling of the receptor in the absence of the endogenous peptide ligand. Once again using the ghrelin system as an example, it is noteworthy that the ghrelin receptor is constitutively active (Holst et al. 2003) and that inverse ghrelin agonists (albeit peptidergic) have been described (Holst et al. 2006). Such inverse agonists could be particularly useful under conditions where an anxiety disorder is caused by an increase in constitutive firing rate of a neuropeptide receptor. They will also be of benefit if there is increased peptide release associated with an anxiety disorder, but in contrast to the antagonists, may lead to unintended hypoactivity of the system.

Other means to interact with peptidergic systems could be the inhibition of peptide synthesis or breakdown. Zinc metallopeptidases, e.g., are enzymes that rapidly degrade enkephalins in the brain. Consequently, blockade of these peptidases increases extracellular concentrations of enkephalins (Nieto et al. 2005). Enkephalins in turn reduce anxiety-related behavior in animals and, indeed, anxiolytic-like effects were observed in mice following intraperitoneal administration of a zinc metallopeptidase inhibitor (Nieto et al. 2005).

Yet another strategy to modulate neuropeptide systems could be to alter the availability of the neuropeptide at its target by freeing it from its binding protein in cases where such proteins exist. For example, it has been shown that corticotropin-releasing factor (CRF) can be displaced from its binding protein by specific CRF

binding protein inhibitors (Behan et al. 1995). In this specific example, of course, one would not expect anxiolytic activity as CRF is well known to increase anxiety-related behavior (e.g., Steckler 2008).

Thus, there are several strategies that would allow one to manipulate neuropeptide systems pharmacologically and to affect anxiety states. As a caveat, however, it must also be recognized that at least some of these peptide systems are centrally and peripherally involved in major homeostatic mechanisms, such as the control of food intake and regulation of pituitary or cardiovascular function. Thus, a close monitoring or safety and tolerability profiles of drugs that target neuropeptides is warranted. Other issues that proved to be a challenge for the development of compounds that act at neuropeptide systems relates to their physicochemical properties. At least for some targets it has proved to be very difficult to synthesize compounds that show good solubility, bioavailability, brain penetrance, and metabolic stability while maintaining high efficacy and selectivity (Steckler 2008). Nevertheless, the pharmaceutical industry has succeeded in developing small molecule, nonpeptidergic compounds acting at various neuropeptide systems with acceptable drug-like characteristics and safety profile that could be of benefit for the treatment of anxiety disorders, examples of which will be discussed below.

2 Is there Clinical Proof-of-Concept with Compounds Acting at Neuropeptide Systems?

No drug acting at neuropeptidergic systems has yet been approved for the treatment of anxiety disorders. However, a number of compounds have been evaluated in clinical trials.

2.1 CRF_1 Antagonists

Possibly, the neuropeptide target that received most attention over the last years is the CRF_1 receptor. Several review articles have summarized the preclinical evidence indicating potent anxiolytic-like properties of small molecule, nonpeptidergic CRF_1 antagonists in animals (e.g., Holmes et al. 2003; Landgraf 2005; Steckler 2005, 2008; Steckler and Dautzenberg 2006; Steckler and Holsboer 1999; Zorrilla and Koob 2004) and the picture that emerges is that these compounds are particularly efficacious in animals that are in states of heightened anxiety induced by pharmacological or emotional stressors. Sites where CRF1 antagonists seem to affect anxiety-related behavior in animals are the amygdala (Bakshi et al. 2002) and the bed nucleus of the stria terminalis (Sahuque et al. 2006), key brain areas

involved in anxiety (Canteras et al. 2009). But despite intense activity in the field for many years, there are only a few published studies investigating the effects of CRF_1 antagonists in man and none has been published on the effects of CRF_1 antagonists in patients suffering from anxiety disorders. Those studies that investigated the effects of CRF1 antagonists in stress-related psychiatric disorders focused on depression, which is another disorder where CRF1 antagonists may be of benefit. Part of the reason for a focus on depression rather than anxiety may be a strategic decision of the pharmaceutical industry based on indication sequencing, because depression is generally considered to be commercially a more attractive therapeutic indication.

Studying the effects of CRF_1 antagonism with NBI-34041 in 24 healthy male volunteers, Ising et al. (2007) reported an attenuation of the psychosocial stress-induced activation of the HPA axis. However, NBI-34041 failed to affect the stress-induced increases in emotionality as measured by the State–Trait–Anxiety Inventory (STAI-X1). In contrast, some beneficial effects of CRF_1 antagonism on anxiety was suggested by data from an earlier study in depressed patients. In this open label study in 20 patients, significant reductions in both depression and anxiety scores were reported (Zobel et al. 2000). Of note, this study was primarily designed as a safety, dose-escalation study and did not include a placebo group or an active control. More recently, however, lack of efficacy was reported for the CRF_1 antagonist CP-316,311 in a 6-week randomized, placebo controlled trial in depressed patients, which used the Hamilton Depression scale as a primary endpoint but also included a secondary efficacy analysis on the Hamilton Anxiety Scale (HAM-A) (Binneman et al. 2008). Based on these three clinical studies, evidence for anxiolytic effects of CRF_1 antagonists in man is inconclusive at best, which contrasts the substantial preclinical evidence supporting a role of CRF_1 antagonists as anxiolytic drugs. However, it should also be noted that the entry criteria for anxiety (based on HAM-A) were not high, suggesting that anxiety was moderate, which may not be sufficient to detect potent anxiolytic effects

Therefore, a counter argument would be that no CRF_1 antagonist has been tested in patients with primary diagnosis of anxiety disorder and that these compounds may show efficacy in these patients. Indeed, there is evidence for abnormalities in the CRF system in certain types of anxiety disorders: a relatively consistent finding reported in several studies is an increase in cerebrospinal fluid (CSF) levels of CRF and a blunted ACTH response to CRF challenge in post-traumatic stress disorder (PTSD) (see Nemeroff et al. 2006; Steckler 2008, for reviews). A blunted response to CRF challenge has also been reported in panic disorder patients (reviewed in Gold et al. 1988; Holsboer et al. 1987; Steckler et al. 2008) and, more recently, Smoller et al. 2003, 2005) found an association between the CRF gene and behavioral inhibition in children at risk for panic disorder. Likewise, abnormalities of the CRF system have been described in obsessive-compulsive disorder (OCD) (Fossey et al. 1996). Taken together, this would suggest that drugs normalizing CRF activity, such as CRF_1 antagonists, could be of benefit for the treatment of PTSD and panic disorder, and possibly also in OCD. Until the relevant clinical trials have been performed, the jury is still out.

2.2 NK_1 Antagonists

Another target that received substantial interest in the past is the neurokinin 1 (NK_1) receptor for which the endogenous ligand is substance P. This interest was strongly triggered by a seminal paper published in 1998, where it was shown that the NK_1 antagonist aprepitant (MK-869) had anxiolytic-like properties in a guinea pig pup model measuring separation-induced distress vocalizations comparable to the anxiolytics diazepam (a benzodiazepine) and buspirone (a 5-HT_{1A} agonist) (Kramer et al. 1998). The amygdala has also been suggested to be the site of action where NK1 antagonists elicit anxiolytic-like effects in animals (Rupniak et al. 2003). Aprepitant was as efficacious as the selective serotonin reuptake inhibitor (SSRI) paroxetine in improving scores on both the Hamilton Anxiety (HAM-A) and Depression (HAM-D) scales in depressed patients with comorbid anxiety (Kramer et al. 1998). Subsequently, an improvement in HAM-A score was also reported with another NK_1 antagonist, LY-759274, in depressed patients (Kramer et al. 2004). Interestingly, in a human positron emission tomography (PET) study, aprepitant at the dose that was efficacious in depression also blocked more than 90% of the NK_1 receptors and also displaced the NK_1 ligand [F^{18}]SPA in the amygdala (Hargreaves 2002). However, in five subsequent Phase III studies, aprepitant failed to significantly improve HAM-A (and HAM-D) scores in depressed patients. In two of those studies, paroxetine was used as active comparator and showed a significant benefit on HAM-A scores in one (Keller et al. 2006), which casts some doubts on the therapeutic benefit of NK_1 antagonism in the treatment of anxiety symptoms in depressed patients and in the treatment of depression in general. These data also raise more general questions about how predictive preclinical animal models and Phase II data are of efficacy in Phase III studies (Steckler et al. 2008).

As for CRF_1 antagonists, it could be argued, however, that the real value of NK_1 antagonists for the treatment of anxiety disorders would only become apparent once such compounds have been tested in the appropriate patient population. A recent study reported that fear provocation in individuals with specific phobia was associated with a decreased uptake of a NK_1 PET ligand in the amygdala, possibly because of an increase in endogenous substance P release triggered by the fear-provoking stimuli (Michelgard et al. 2007). This hypothesis is corroborated by preclinical findings, showing that stress exposure leads to increased substance P release in the medial nucleus of the amygdala and that direct injections of a NK_1 antagonist into the medial nucleus attenuated stress-induced anxiety-related behavior in rats (Ebner et al. 2004). These findings argue in favor of a role for NK_1 antagonists in the treatment of at least some types of anxiety. In fact, the NK_1 antagonist GR205171 has been reported to improve social phobia comparable to the SSRI citalopram in patients. In this study, symptom improvement was paralleled by a reduction in regional cerebral blood flow in response to a psychosocial stressor in the rhinal cortex, parahippocampal–hippocampal regions, and also in the amygdala (Furmark et al. 2005). These are

encouraging results, but experience with NK_1 antagonists in the field of depression should be taken as a note of caution. Certainly, one would like to see confirmation of these findings in patients suffering from social phobia or other phobias in other studies (ideally Phase III trials) before efficacy of NK_1 antagonism in these disorders is taken for granted.

2.3 NK_3 Antagonists

Compared to the NK_1 receptor antagonists, far less information has been released on the potential anxiolytic properties of NK_2 and NK_3 antagonists in man, although these two NK receptor subtypes have also been suggested to play a role in the modulation of anxiety. Only one study reported failure of the NK_3 antagonist osanetant to improve panic symptoms in panic disorder patients challenged with cholecystokinin tetrapeptide (CCK-4), a peptide frequently employed to elicit panic-like symptoms in healthy volunteers and panic disorder patients (Kronenberg et al. 2005).

2.4 CCK_2 Antagonists

Based on challenge studies with CCK-4 or with the cholecystokinin receptor 2 (CCK_2) antagonist pentagastrin (a synthetic analogue of CCK-4), linkage studies, measurements of CSF levels of CCK and preclinical data obtained from animal tests, the CCK system has also been suggested to play an important role in anxiety disorders, in particular in panic disorder (see Steckler et al. 2008, for review). A number of CCK_2 antagonists have been tested in both healthy volunteers and patients suffering from panic disorder or generalized anxiety disorder, but evidence for anxiolytic activity is conflicting. Although initial studies with the CCK_2 antagonist L-366,260 suggested antipanic effects, based on studies in healthy volunteers receiving an intravenous challenge with pentagastrin (Lines et al. 1995), or in panic patients challenged with CCK-4 (Bradwejn et al. 1994), those findings were not confirmed by others (Kramer et al. 1995; Sramek et al. 1994–1995). Likewise, lack of efficacy was reported for the CCK_2 antagonist CL-988 in a number of studies in healthy volunteers following CCK-4 or lactate challenge (Bradwejn et al. 1995, Cowley et al. 1996) and in patients with panic disorder (Pande et al. 1999; Van Megen et al. 1997) or generalized anxiety disorder (Adams et al. 1995; Goddard et al. 1999). Part of the issue here may be the fact that these compounds in general show relatively low bioavailability. Unfortunately, and unlike to the situation in the NK1 field, no PET ligand exists that could be used to demonstrate central occupancy of CCK4 receptors in human to come to real conclusions. However, as it stands one has to assume that CCK_2 antagonists are not clinically effective for the treatment of anxiety disorders.

In addition to these small molecule approaches, anxiolytic-like activity has been seen following administration of some peptidergic compounds in man.

3 Atrial Natriuretic Peptide and Oxytocin

Intravenous infusions of panic disorder patients with atrial natriuretic peptide (ANP) resulted in anxiolytic effects following challenge with CCK-4 (Strohle et al. 2001; Wiedemann et al. 2001), although it is unclear how this effect was mediated as peripherally administered ANP is not brain penetrant. It has been suggested that the effects of CCK challenge could be peripherally or centrally mediated; e.g., via activation of CCK receptors expressed at vagal nerve endings or vasodilatation of cerebral arteries, both leading to secondary central effects, or in brain areas lacking a tight blood–brain barrier (Cano et al. 2003; Crawley and Corwin 1994; Rinaman et al. 1995; Sanchez-Fernandez et al. 2003). Likewise, ANP could alter measures of anxiety by either peripheral or central effects, thereby counteracting the effects of CCK-4.

Anxiolytic effects were also reported following intranasal administration of oxytocin to healthy volunteers following psychosocial stress (Heinrichs et al. 2003). Moreover, an increase in trust was seen following oxytocin administered via this route (Kosfeld et al. 2005). Intranasal administration has been suggested to result in brain penetration of a number of peptides in humans (Born et al. 2002), although direct proof in man is lacking for oxytocin. In fact, plasma oxytocin levels also increase markedly after intranasal application (Landgraf 1985), raising the possibility that the anxiolytic-like effects of oxytocin might be peripherally mediated. But in support of – primary or secondary – central effects, functional magnetic resonance imaging (fMRI) studies showed that the fear-induced activation of the amygdala (Kirsch et al. 2005) and the amygdala response to emotional faces (Domes et al. 2007) were reduced in volunteers receiving intranasal oxytocin. Clinical trials of intranasal oxytocin treatment in social phobia are underway.

A number of other small-molecule, nonpeptidergic compounds with potential for the treatment of anxiety disorders have been proposed, including vasopressin V_{1b}, neuropeptide Y Y_2, melanocortin MC_4, angiotensin AT_1, galanin GAL_3, bombesin BB_1, melanin-concentrating hormone MCH_1 and orexin OX_1 antagonists, as well as oxytoxin, δ opiate and orphanin FQ OFQ_1 agonists, but those compounds have only been tested preclinically yet. Even more neuropeptide systems have been associated with anxiety-related behavior preclinically for which at present no small molecule, nonpeptidergic compounds with anxiolytic-like properties have been reported (reviewed in Steckler 2008). A detailed discussion of these data would be beyond the scope of this chapter.

4 Is Efficacy Enough?

Summarizing the clinical trial data, one has to conclude that the evidence for neuropeptide-based therapies for anxiety disorders is still very limited, despite major efforts by the pharmaceutical industry for more than 20 years. The only

class of small molecule, nonpeptidergic compounds that showed a preliminary clinical proof-of-concept is the NK_1 antagonists, but more studies with positive outcome need to be published before firm conclusions can be drawn. Moreover, requirements are already or can be expected to increase and just showing efficacy may not be sufficient to bring a new anxiolytic to the market in the future. What will be required is the demonstration of superiority! This might be superior efficacy in particular anxiety disorders where efficacy of current medication is limited, e.g., in specific phobias or PTSD. It can be faster onset of action, for e.g., in obsessive compulsive disorder, where benzodiazepines do not work and the onset of efficacy with antidepressant drugs is delayed. It can also be an improved side effect profile, i.e., lack of cognitive impairment, sedation, ataxia, dependency, or withdrawal complications as is the case with the benzodiazepines, or lack of nausea, sexual dysfunction and weight gain, as is seen with the SSRIs (Steckler et al. 2008). However, none of these have so far been convincingly demonstrated in clinical trials with compounds targeting neuropeptide systems in patients suffering from an anxiety disorder. Moreover, given the involvement of many peptide systems in major homeostatic mechanisms, as mentioned above, there is a risk that other, mechanism-based side effect profiles will occur. For example, nephrotoxic and hepatotoxic effects were seen with the CRF_1 antagonist antalarmin in a 90-day toxicity study in rats (Horn et al. 2008). It is unclear whether these findings are structurally related to the molecule or mechanistically to CRF_1 receptor blockade, but from these findings kidneys and liver must be considered target organs for antalarmin toxicity in humans (Horn et al. 2008). Of note, elevated liver transaminases were also reported in 3 out of 20 patients treated with the CRF_1 antagonist R121919 (Zobel et al. 2000), which is structurally relatively close to antalarmin. Moreover, implantation loss of rat fetuses has been reported following treatment of pregnant rats with antalarmin during the early stages of pregnancy, which may be related to the mechanism of action, i.e., CRF1 receptor-mediated (Makrigiannakis et al. 2004). Likewise, adverse effects can be expected with some other neuropeptide targets, such as weight gain with MC_4 receptor antagonists (Kask et al. 1998; Skuladottir et al. 1999), blood pressure lowering effects with AT_1 antagonists, or proconvulsive properties with brain penetrant δ-opioid agonists (Comer et al. 1993; Yajima et al. 2000), to name just a few.

Clearly, this is not meant to devalue the potential of neuropeptidergic targets as novel therapeutic approaches to treat anxiety disorders, because the points mentioned above are not unique to the peptide field, but it points to the fact that one might be faced with new issues when developing novel drugs that act on these targets, some being more obvious than others, and that are not necessarily seen with current anxiolytic medication. The importance of this is the fact that novel anxiolytic drugs would be expected to have a clean side effect profile as possible, making a close evaluation of this profile mandatory.

5 Conclusion

Coming back to the initial question: Is the challenge still ahead when developing small molecule nonpeptidergic drugs for the treatment of anxiety disorders? From a review of the literature the answer is clearly "yes." It is evident that neuropeptide systems represent a major opportunity for the development of novel treatment approaches for anxiety disorders as drugs acting at these systems may have unique properties not seen with other targets. There is a huge literature providing evidence for an important role of neuropeptides in the mediation of anxiety-related behavior, both in animals and man. It is also evident that drugs acting at those targets might overcome some of the problems inherent in today's anxiolytic medication. However, the approach remains a high risk and, unless there is unambiguous proof-of-concept in patients, highly speculative.

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Cannabinoids and Anxiety

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Contents

Abstract The term cannabinoids encompasses compounds produced by the plant *Cannabis sativa*, such as Δ^9-tetrahydrocannabinol, and synthetic counterparts. Their actions occur mainly through activation of cannabinoid type 1 (CB1) receptors. Arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG) serve as major endogenous ligands (endocannabinoids) of CB1 receptors. Hence, the cannabinoid receptors, the endocannabinoids, and their metabolizing enzymes comprise the endocannabinoid system.

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Cannabinoids induce diverse responses on anxiety- and fear-related behaviors. Generally, low doses tend to induce anxiolytic-like effects, whereas high doses often cause the opposite. Inhibition of endocannabinoid degradation seems to circumvent these biphasic effects by enhancing CB1 receptor signaling in a temporarily and spatially restricted manner, thus reducing anxiety-like behaviors. Pharmacological blockade or genetic deletion of CB1 receptors, in turn, primarily exerts anxiogenic-like effects and impairments in extinction of aversive memories. Interestingly, pharmacological blockade of Transient Receptor Potential Vanilloid Type-1 (TRPV1) channel, which can be activated by anandamide as well, has diametrically opposite consequences. This book chapter summarizes and conceptualizes our current knowledge about the role of (endo)cannabinoids in fear and anxiety and outlines implications for an exploitation of the endocannabinoid system as a target for new anxiolytic drugs.

Keywords Cannabinoids · Endocannabinoids · Fear · Anxiety · Stress

Abbreviations

2-AG	2-Arachidonoyl glycerol (endocannabinoid; i.e., activates CB1 receptors)
AA-5HT	Arachidonoyl serotonin (synthetic inhibitor of FAAH-mediated endocannabinoid degradation and TRPV1 antagonist)
AEA	Anandamide or arachidonoyl ethanolamide (endocannabinoid and endovanilloid; i.e., activates CB1 receptors and TRPV1)
AM251	(Synthetic CB1 receptor antagonist)
AM404	(Synthetic inhibitor of endocannabinoid uptake and FAAH-mediated degradation)
CB1	Cannabinoid type 1 receptor
CB2	Cannabinoid type 2 receptor
CBD	Cannabidiol (phytocannabinoid)
CP-55940	(Synthetic CB1 receptor agonist)
Cre/loxP	(Recombinatory system used in conditional mouse mutagenesis)
FAAH	Fatty acid amide hydrolase (major degrading enzyme of AEA)
GABA	Gama-aminobutyric acid (major inhibitory transmitter of the brain)
GPR55	(G-protein-coupled cannabinoid receptor)
HU210	(Synthetic CB1 receptor agonist)
JZL184	(Synthetic inhibitor of MGL-mediated endocannabinoid degradation)
MGL	Monoacylglycerol lipase (major degrading enzyme of AEA)
PAG	Periaqueductal grey (matter brain structure orchestrating fear responses)

PPARα	(cannabinoid receptor)
SB366971	(Synthetic TRPV1 antagonist)
SR141716A	Rimonabant/acomplia^{TM} (synthetic CB1 receptor antagonist)
SR144528	(Synthetic CB2 receptor antagonist)
Δ^9-THC	Δ^9-Tetrahydrocannabinol (phytocannabinoid; major psychoactive ingredient of *Cannabis sativa*)
TRPV1	Transient receptor potential vanilloid type-1 channel
UCM707	(Synthetic inhibitor of endocannabinoid uptake)
URB597	(Synthetic inhibitor of FAAH-mediated endocannabinoid degradation)
VDM11	(Synthetic inhibitor of endocannabinoid uptake)
WIN-55212-2	(Synthetic CB1 receptor agonist)

> "Lets us hope that through better understanding of Cannabis chemistry in the brain we may also approach the chemistry of emotions."
> Mechoulam et al. (1991)

1 Introduction

The plant *Cannabis sativa* has been used for medical and recreational purposes for more than 5000 years. It has been known since these early times that smoking of Cannabis (marijuana) may induce a state of euphoria, relaxation, sense of delight, and increase in sociability. Occasionally, however, it may cause anxiety, panic, delusions or psychotic-like symptoms. The psychoactive ingredient of Cannabis could be identified and characterized only in 1964. Raphael Mechoulam and his coworker (1964) isolated it as a lipid compound, an aromatic terpenoid named Δ^9-tetrahydrocannabinol (Δ^9-THC; Fig. 1). Today we know that Cannabis extracts contain more than 70 biologically active compounds, including the nonpsychoactive cannabidiol (Fig. 1) and cannabinol.

The identification of these natural cannabinoids, or phytocannabinoids, led to the synthesis of several analogues, termed synthetic cannabinoids, which were able to mimic the effects of Δ^9-THC with higher potency and which contributed to elucidating the pharmacology of this group of substances. Among the main synthetic cannabinoids are nabilone, CP-55940, and WIN-55212-2 (Fig. 1). In addition, the refinement of animal models allowed a better characterization of their effects. For instance, with the Tetrad test a pharmacological assay was developed in which cannabinoids could be identified by their capacity to induce analgesia, hypothermia, hypolocomotion, and catalepsy. Nonetheless, it took several decades until their mechanisms of action started to be unraveled.

For a long time, Δ^9-THC and other cannabinoids were thought to exert their biological effects by changing biophysical characteristics of cell membranes. Only at the end of the 1980s a cannabinoid receptor could be identified, followed

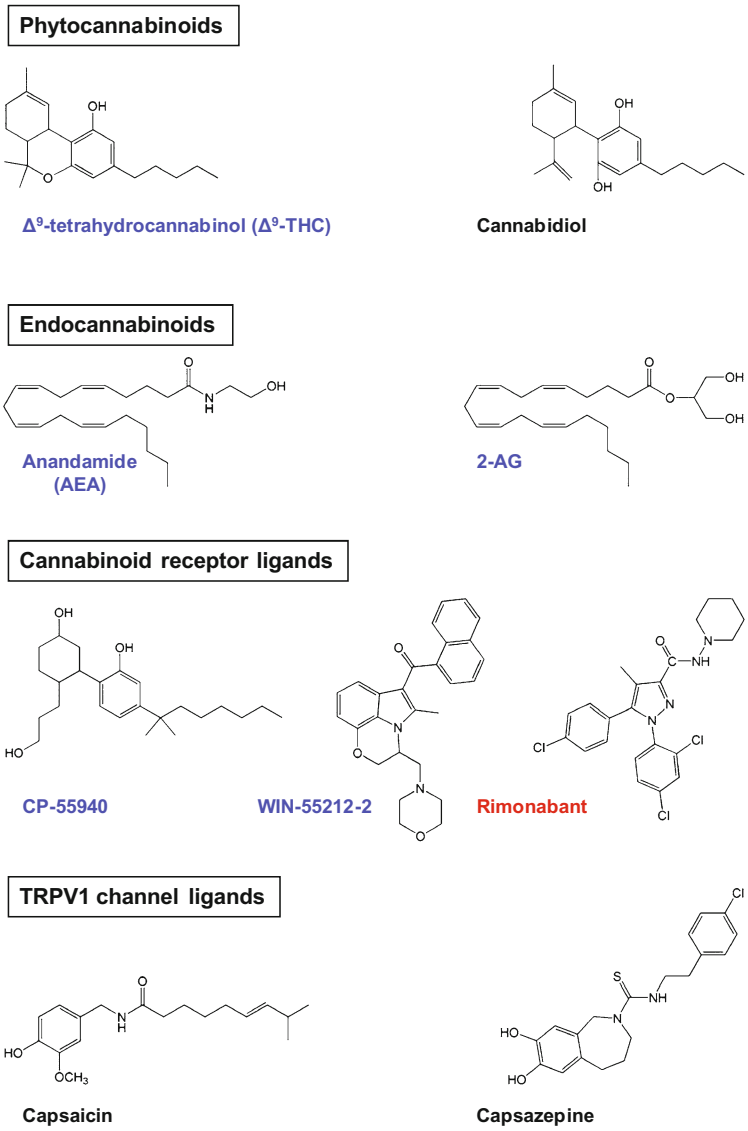

Fig. 1 Chemical structures of selected compounds that act on the endocannabinoid/endovanilloid system. Phytocannabinoids (originating from *C. sativa*), endocannabinoids and synthetic ligands activate (blue), inhibit (red) or bypass (black) cannabinoid type 1 (CB1) receptor signaling. Anandamide may activate Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels in addition to CB1, thus acting as an endovanilloid and endocannabinoid at the same time (see text for further details)

by the discovery of endogenous ligands, referred to as endocannabinoids. The endocannabinoids along with their receptors and enzymes for synthesis and catabolism constitute the endocannabinoid system. Thus, a clear distinction should be

made between the terms cannabinoids (i.e., phytocannabinoids or synthetic analogues) and endocannabinoids synthesized by vertebrates.

Today we know that the endocannabinoid system is involved in a plethora of biological functions ranging from brain development and organogenesis to control of energy expenditure, food intake, pain perception, and inflammation to regulation of cognition, stress responses, and positive as well as negative affects. This chapter will summarize current evidence concerning the effects of cannabinoids and the role of endocannabinoids in fear and anxiety, ending with conceptualizing principles of endocannabinoid action and recommendations for a pharmacological exploitation of the endocannabinoid system for the treatment of human anxiety disorders.

2 The Endocannabinoid System of the Brain

The first cannabinoid receptor was identified in the rodent brain after studies with radioactively labeled synthetic cannabinoids in 1988 (Devane et al. 1988), followed by its cloning (Matsuda et al. 1990). The biochemical characterization unveiled a 7-transmembrane, G-protein-coupled receptor. Later on, another metabotropic receptor was identified. The International Union of Basic and Clinical Pharmacology (IUPHAR) named these receptors Cannabinoid receptor type 1 (CB1) and Cannabinoid receptor type 2 (CB2), respectively (Howlett et al. 2002). Most of the psychoactive actions of Cannabis seem to be mediated via CB1 receptors. Its strong expression within the brain fits well with these neuropharmacological effects. For instance, CB1 receptors are densely expressed throughout the cerebral cortex and the hippocampal formation, possibly explaining why Cannabis smoke induces impairments in learning and memory. Furthermore, CB1 receptor density is also extremely high in the basal ganglia and cerebellum, which may account for the motor impairment induced by marijuana. Furthermore, the expression in amygdala and periaqueductal gray may account for the emotional effects and analgesia. The absence in midbrain regions responsible for the control of breathing rhythms may explain why cannabinoids, contrary to opioids, do not induce respiratory depression (Howlett et al. 2002).

The story of cannabinoids recapitulates the discovery of the mechanisms of opioid action. Morphine was isolated in the nineteenth century and opioid receptors were identified in the 1970s, followed by the characterization of endogenous ligands (endorphins) shortly thereafter. Accordingly, the race for the identification of endogenous binding partners was opened as soon as the first cannabinoid receptor had been discovered. The first endocannabinoid was identified in the porcine brain in 1992. In line with the chemistry of natural and synthetic cannabinoids, this mammalian cannabinoid proved to be also a lipid, the arachidonic acid derivate arachidonoyl ethanolamide (Fig. 1), also termed anandamide (from the Sanskrit word *ananda* for “bliss”) (Devane et al. 1992). A second endocannabinoid could be identified as 2-arachidonoyl glycerol (2-AG; Fig. 1) shortly thereafter (Mechoulam et al. 1995; Sugiura et al. 1995). Other putative

endocannabinoids are *N*-arachidonoyl dopamine, virodhamine, and noladin ether, all amide or ester analogues of arachidonic acid (Di Marzo et al. 2004; Piomelli 2003).

Endocannabinoids have several characteristics that distinguish them from classical neurotransmitters. According to the traditional view, neurotransmitters are synthesized in presynaptic neurons and stored in vesicles to be released after neural activation and subsequent calcium influx. On the contrary, endocannabinoids are synthesized from membrane lipids in postsynaptic neurons after calcium influx that follows neural activation. Anandamide synthesis primarily depends on the activity of phospholipase D, whereas 2-AG synthesis involves phospholipase C. Also diverging from the classical concept of neurotransmission, endocannabinoids immediately diffuse to the synaptic cleft, instead of being stored in vesicles (Piomelli 2003). Complementing this picture, CB1 receptors are mainly located in presynaptic terminals. Activation of CB1 receptors leads to a decrease in synaptic transmission via a complex set of intracellular signaling cascades. Thus, endocannabinoids act as retrograde messengers, which are synthesized and released on demand following depolarization of the postsynapse to reach presynaptically localized CB1 receptors, where they restrain the release of neurotransmitters (Fig. 2).

Not only the release of endocannabinoids, but also their range of action are tightly controlled in temporal and spatial terms. Endocannabinoids are internalized and hydrolyzed inside neurons. The exact uptake mechanisms responsible for these processes are still unknown. Two – mutually not exclusive – possibilities have been proposed in this context: First, endocannabinoids might cross the cell membrane along a concentration gradient in a passive process not governed by any enzyme or transport protein. Second, a protein transporter might exist, which favors the movement of endocannabinoids through the cell membrane. The later view is supported by experiments showing that the uptake process is saturable, temperature-dependent, and can be inhibited by specific compounds (Piomelli 2003). Nonetheless, an "endocannabinoid transporter" could not be identified and cloned yet.

Once inside neurons, endocannabinoids are catabolized by different enzymes. Anandamide undergoes hydrolysis by the enzyme fatty acid amide hydrolase (FAAH), an integral membrane protein identified primarily in postsynaptic sites in rodents and primates (Fig. 2). FAAH breaks down anandamide into arachidonic acid and ethanolamine. 2-AG, in contrast, is hydrolyzed mainly by presynaptically localized monoacylglycerol lipase (MGL; Piomelli 2003; Fig. 2).

In the past decade it became evident that endocannabinoid-controlled neuronal activity represents a fundamental principle of the central nervous system with large implications on brain development (Harkany et al. 2007), synaptic plasticity (Chevaleyre et al. 2006), and behavior (Moreira and Lutz 2008). This might be partially explained by the observation that CB1 receptors belong to the most abundantly expressed G-protein-coupled receptors of the brain. Nevertheless, CB1 receptors are not ubiquitously distributed throughout the entire brain, but show different levels of expression depending on the neuronal subtype. For instance, CB1 receptors are densely expressed in cholecystokinin-positive basket

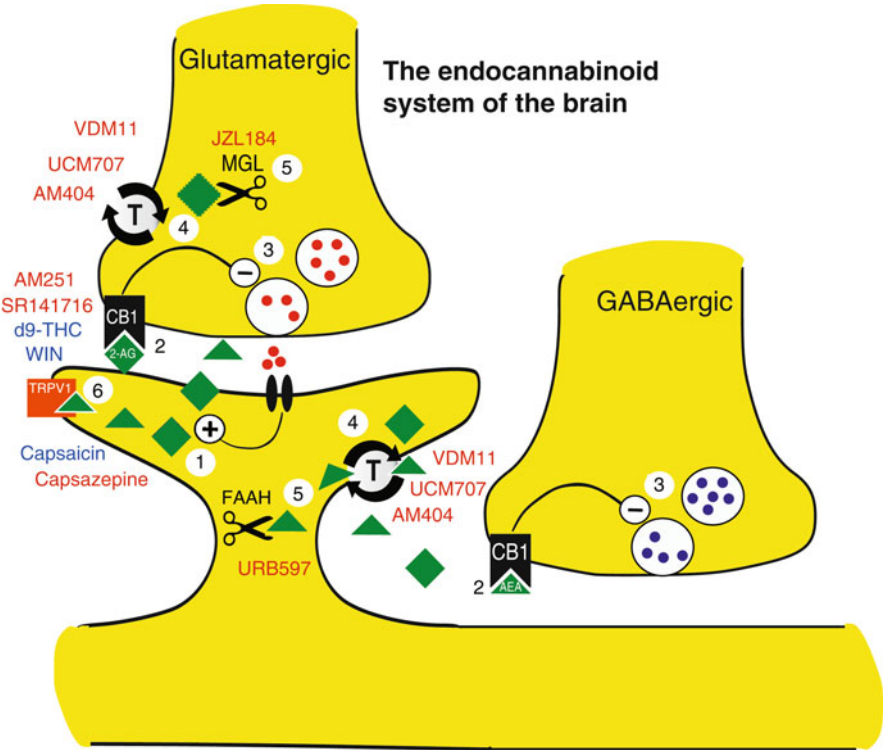

Fig. 2 The endocannabinoid system of the brain. Endocannabinoids (anandamide, AEA, and 2-AG) are synthesized on demand following depolarization of the postsynaptic membrane (1) and/or activation of postsynaptic metabotropic receptors (not shown). They diffuse into the synaptic cleft to bind to presynaptically localized cannabinoid type 1 receptors (CB1) (2). Activation of CB1 receptors leads to an inhibition of transmitter release (3). Endocannabinoid signaling is terminated by cellular uptake processes, which likely involve transporter proteins (4), followed by intracellular hydrolysis of 2-AG by presynaptic monoacylglycerol lipase (MGL) (5) and of AEA by postsynaptic fatty acid amide hydrolase (FAAH) (5). It remains to be shown that the same processes (1–5) apply to all of the different neuronal populations expressing CB1 receptors. Pharmacological compounds indicated in blue promote and those in red inhibit the respective processes (for chemical structures see Fig. 1). Noteworthy, AEA may additionally bind to cytosolic domains of postsynaptically localized Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels (6), thereby promoting activation of postsynaptic terminals

neurons that release gamma-aminobutyric acid (GABA). The density seems to be lower, though not less functionally significant, in glutamate-releasing neurons. Indeed, pharmacological stimulation of CB1 receptors may inhibit either GABA- or glutamate-mediated neural transmission (Marsicano and Lutz 2006).

In summary, the endocannabinoid system comprises the cannabinoid receptors, of which CB1 seems to be the most relevant in the brain, the endogenous ligands (endocannabinoids), such as anandamide and 2-AG, as well as the enzymes for synthesis and catabolism. Interestingly, anandamide seems to bind not only to

CB1, but also to the Transient Receptor Potential Vanilloid Type-1 (TRPV1) channel, the receptor for capsaicin, the pungent ingredient of red hot chilli pepper (Fig. 1; Ross 2003). Within the central nervous system, TRPV1 might be expressed in postsynaptic terminals (Fig. 2). Activation of TRPV1 by intracellular binding of anandamide leads to an increase in postsynaptic activity, which is in striking contrast to the consequences of extracellular binding of anandamide to presynaptic CB1 receptors, thus suggesting opposing functions of the two receptor types (Starowicz et al. 2007). Other potential cannabinoid receptors are the GPR55, a G-protein-coupled receptor, and the peroxisome-proliferator-activated receptor α (PPARα), a nuclear receptor (Brown 2007; O'Sullivan, 2007). Finally, an allosteric site has been identified at the CB1 receptor, which remains to be further investigated (Ross 2007).

3 How to Study the Role of the Endocannabinoid System

Experiments with natural or synthetic cannabinoids may provide important insights into their pharmacological characteristics as a prerequisite for their future pharmacological exploitation. However, ubiquitous activation of CB1 receptors by exogenous agonists provide little – if any – information about the role of endogenous binding partners, keeping in mind the highly abundant expression of CB1 receptors and the diversification of the endocannabinoid system in terms of the endocannabinoids (which differ in their synthesis, range/duration of action, and degradation), the neuronal subpopulations affected, and the differential role of CB1 vs. TRPV1. Consequently, the biological functions of the endocannabinoid system in general and its implication in fear and anxiety in particular can only be achieved by monitoring the activity status of the endocannabinoid system and/or by inhibiting, respectively, increasing endocannabinoid signaling.

3.1 Monitoring of Endocannabinoid Signaling

As for many other transmitter systems, indirect conclusions about a potential involvement of the endocannabinoid system in distinct biological functions can be drawn by monitoring changes in its activity status, ranging from alterations in surface expression of CB1 receptors to changes in the activity of anabolic and catabolic enzymes to changes in endocannabinoid levels within the brain. Quantification of endocannabinoid levels turned out to be technically challenging due to the physicochemical characteristics and relatively low concentrations of the endocannabinoids. Other confounding factors are that both anandamide and 2-AG may result from rapid membrane degradation, thus requiring immediate processing of biological specimens, and that 2-AG is an intracellular intermediate product of multiple metabolic processes unrelated to endocannabinoid signaling. To deal with

this problem, recent studies tried to monitor the dynamics of 2-AG (and anandamide) release by means of in vivo microdialysis, thereby focusing on the amount of endocannabinoids, which reach the extracellular space, and thus become biologically active at CB1 receptors.

3.2 Attenuation of Endocannabinoid Signaling

Rimonabant (SR 141716A; Fig. 1; Rinaldi-Carmona et al. 1994) was the first CB1 receptor antagonist synthesized. This drug has provided invaluable insights into the physiology of the endocannabinoid system as well as in the pharmacology of cannabinoids. It blocks the action of cannabinoids in animals and of marijuana in humans and had been approved for the treatment of obesity in humans in Europe. In the meantime, several other CB1 antagonists have been developed, including AM251 (Gatley et al. 1996). In vitro experiments have suggested that both rimonabant and AM251 might act as inverse agonists, rather than silent antagonists (Pertwee 2008). As for the other cannabinoid receptors, selective CB2 antagonists have also been developed, such as SR144528 (Pertwee 2008). In addition, there are also antagonists for TRPV1 receptors, such as capsazepine (Fig. 1), 6-iodonordihydrocapsaicine, iodo-resiniferatoxin, and SB366971 (Di Marzo et al. 2008).

By specific blockade of CB1 receptors (or TRPV1 channels) it is possible to measure cellular, physiological or behavioral consequences of attenuated endocannabinoid signaling. However, a certain lack of specificity of the anatagonists, in particular at higher doses, hampers the unequivocal interpretation of the findings. To circumvent these problems, mutant mice have been generated, which lack expression of CB1 (CB1-KO) or TRPV1 (TRPV1-KO). As expected, CB1-KO does not respond to cannabinoid injection (Fig. 3). Further refinements of mouse genetics led to the development of conditional CB1 receptor knockout mice, whose CB1 receptor is deleted in neuronal subpopulations, such as GABAergic or cortical glutamatergic neurons by means of the Cre/loxP system (Fig. 3). The power of this approach has been recently demonstrated in context with the dissection of the transmitter systems implicated in tetrad effects of Δ^9-THC (Monory et al. 2007; Fig. 4).

3.3 Facilitation of Endocannabinoid Signaling

Two main strategies have emerged for increasing endocannabinoid signaling at the level of their receptors: First, chemical compounds, such as AM404, VDM11 or UCM707, may interfere with the uptake of the endocannabinoids into pre- and/or postsynaptic terminals, thereby increasing the availability of endocannabinoids at CB1 receptors. However, their exact mechanisms of action still remain to be characterized. Moreover, these compounds lack specificity, as they may also interfere with endocannabinoid degradation and TRPV1 channels (Piomelli 2003).

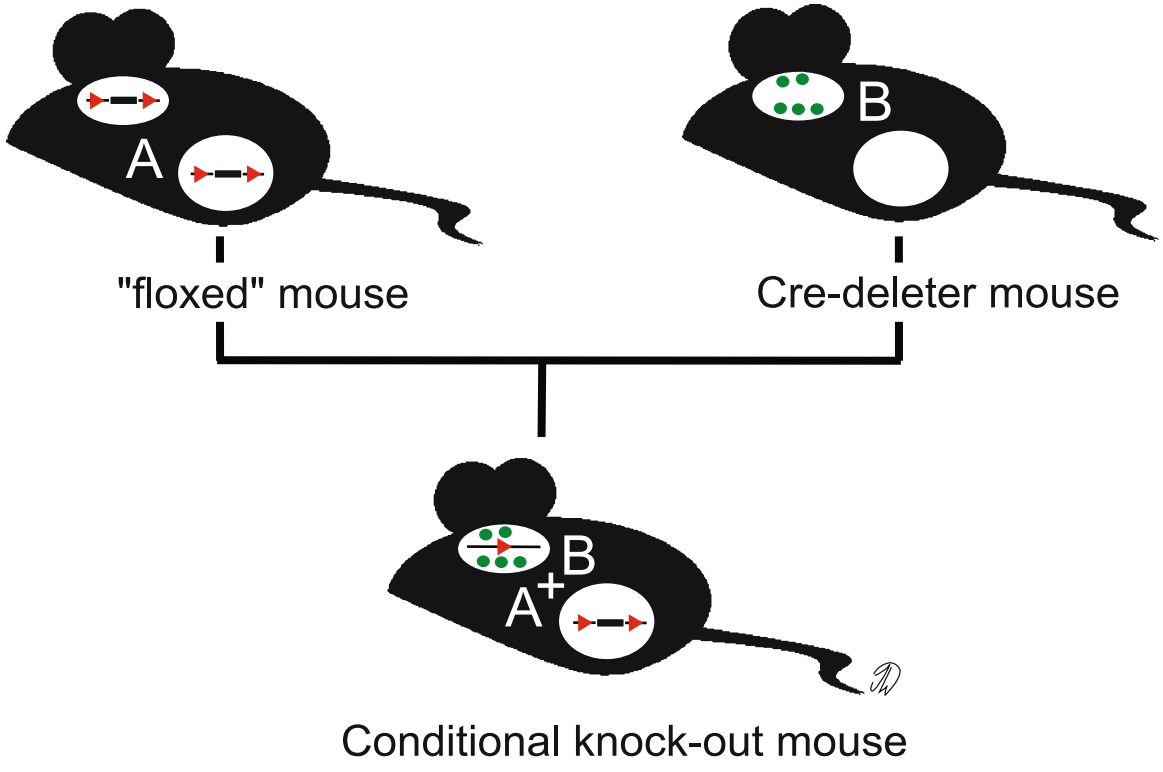

Fig. 3 Conditional mutagenesis. Schematic description of the basic principles of conditional mutagenesis based on the Cre/loxP system, illustrating that conditional mutants originate from two different mouse lines. In one line (A), the gene of interest contains two short DNA fragments (loxP sites), which are inserted into the genome by homologue recombination. These animals, in which the gene of interest is flanked by loxP (i.e., "floxed"), show normal expression of the gene product as long as they are not mated with mice of another line (B), which expresses Cre recombinase under control of a specific promoter. Expression of Cre leads to excision of DNA localized between two loxP sites. The promoter of the Cre transgene determines in which cell type and at which time point of development the recombinase is expressed and, thus, the gene of interest "knocked out" (A+B)

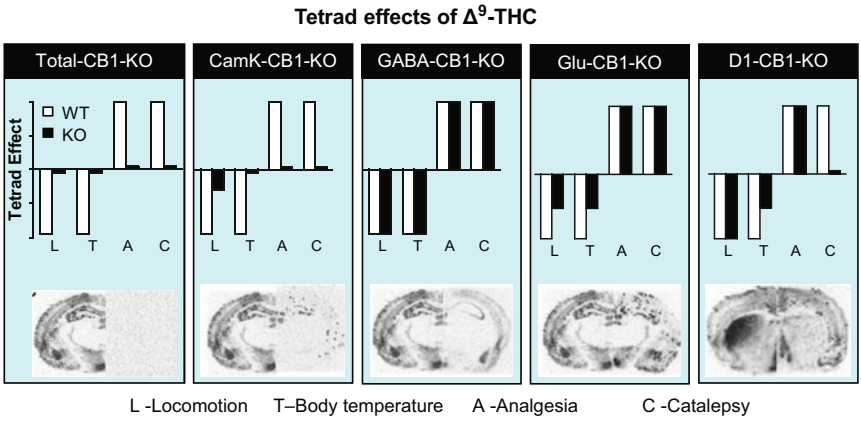

Fig. 4 Dissection of the neurochemical signature of tetrad effects of D9-THC. In pharmacological studies, the efficiency of cannabinoids is assessed by four criteria, which include reduction in locomotion, decrease in body temperature, increase in analgesia and increase in catalepsy (i.e., tetrad effects). Wild-type mice (white bars) respond to D9-THC (10 mg/kg) in the expected manner, whereas mice with complete absence of CB1 (Total CB1-KO) fail to respond at all, thus indicating the dependency of the tetrad effects on CB1. To further dissect the neurochemical signature of the tetrad effects, a variety of different conditional knockout mice that lack expression of CB1 in projection neurons of the forebrain (CamK-CB1-KO), in all GABAergic neurons of the brain (GABA-CB1-KO), in cortical glutamatergic neurons (Glu-CB1-KO) or in neurons expressing the dopamine D1 receptor (D1-CB1-KO) were treated with D9-THC. The insets depict representative in situ hybridizations of CB1 mRNA in wild-type mice (left half) and conditional mutants (right half). By means of this approach it became evident that distinct neuronal subpopulations are responsible for distinct tetrad effects (e.g., D1 receptor-expressing neurons seem to mediate the effects of D9-THC on catalepsy) (modified from Monory et al. 2007)

Second, a very promising strategy for enhancing endocannabinoid signaling is based on the inhibition of their hydrolysis. Specific inhibitors of FAAH and MGL have been developed, which are able to increase the brain levels of anandamide and 2-AG, respectively. The most prominent inhibitor of FAAH is URB597, which causes a 5-fold increase in the brain levels of anandamide (Kathuria et al. 2003), thus corroborating findings obtained with genetic deletion of FAAH (Cravatt et al. 2001). MGL, in turn, is inhibited, among others, by JZL184, which causes an 8-fold increase in 2-AG tissue content. Contrary to blockade of FAAH, MGL blockade induces tetrad effects in mice (Long et al. 2009).

4 Animal Models of Fear and Anxiety

As described in other chapters of this book in more detail, fear and anxiety responses can be assessed in an unconditioned or a conditioned manner. Animal models of unconditioned fear and anxiety are based on the conflict between two opposing innate motivations: the drive to explore a novel environment (presumably

in order to obtain food, shelter, to escape, or to find mating partners) on the one hand and avoidance of potentially dangerous places on the other hand. Behavioral paradigms most widely used in this context are the elevated plus maze (EPM), the light–dark avoidance test and the open field test, all of which measure avoidance of aversive compartments, such as of elevated open spaces (EPM), brightly lit compartments (light-dark avoidance), or the centre of an open field. Other models are the social interaction test (i.e., confrontation with a conspecific in a neutral test environment) and the Vogel conflict test (water consumption with the risk of receiving a mild electric shock; Sousa et al. 2006). Anxiolytic drugs shift the balance between approach and avoidance toward approach responses. The emotional load of the test situations can be modified, for instance, by changing the light conditions.

In the scientific literature, the terms "fear" and "anxiety" are often synonymously used, despite fundamental differences between the two emotional states. In their two-dimensional system of defense that is largely based on the conceptual work of Gray (1982), Blanchard and Blanchard (1988) and Deakin and Graeff (1991), McNaughton and Corr (2004) combined the concepts of defensive approach/avoidance and defensive distance, whereby defensive approach refers to anxiety states and defensive avoidance to fear (Fig. 5). Fear typically arises in situations with explicit (i.e., physical) confrontation with a threat, and fear responses (e.g., fight/flight, freezing, tachycardia) are often phasic and of reflexive nature. This might be explained by the fact that major relay stations and integrative centers of fear responses (e.g., periaqueductal gray, PAG) are located in the mid-/hindbrain. The

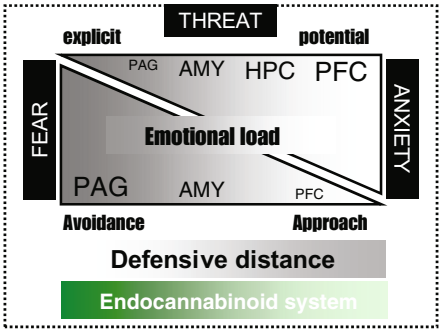

Fig. 5 The two-dimensional defense system. The two-dimensional defense system predicts that defensive distance to an explicit (fear) or a potential (anxiety) threat determines both fear (defensive avoidance) and anxiety (defensive approach) responses (modified from McNaughton and Corr 2004). A decrease in defensive distance coincides with an increase in negative emotional load. There is accumulating evidence that the endocannabinoid system becomes activated primarily in highly aversive situations with strong emotional load, thus suggesting its particular involvement in the control of fear reactions (see also Fig. 8). Fear and anxiety responses differ in the underlying neuronal circuitries (AMY – amygdala complex, HPC – hippocampus formation, PAG – periaqueductal grey, PFC – prefrontal cortex; the letter size indicates the strength of activation)

more tonic and diffuse anxiety responses, in contrast, crucially depend on the prefrontal cortex, the hippocampus and the amygdala (Fig. 5).

Animal models of conditioned fear are based on Pavlovian conditioning, whereby the animals associate an a priori neutral stimulus (i.e., cued fear conditioning) or environment (i.e., contextual fear conditioning) with a punishment (e.g., air puff or electric foot shock). On subsequent confrontation with that stimulus or environment, animals show a number of characteristic fear responses, including freezing (i.e., immobility) and potentiated startle responses. Interestingly, these fear responses are diminished following repeated exposure to the fear-eliciting stimuli in a process termed fear extinction (Myers and Davis 2007). Since conditioned fear is typically acquired in a single conditioning session, conditioning paradigms are predisposed for dissecting the role of cannabinoids and endocannabinoids in acquisition, consolidation, expression, and extinction of fear responses.

5 Role of Cannabinoids and Endocannabinoids in Fear and Anxiety – Animal Studies

Cannabinoids and drugs that interfere with the endocannabinoid system have been extensively studied in animal models of fear and anxiety. Considering that users self-administer marijuana to achieve a state of relaxation and bliss, one could infer that cannabinoids would induce anxiolytic-like effects in rodents. Yet, their effects remain controversial and a diversity of results has been reported. Actually, cannabis itself may also induce anxiety and panic attacks. Furthermore, its effects may depend on previous experiences and the context of use.

5.1 Unconditioned Fear/Anxiety

5.1.1 Natural and Synthetic Cannabinoids

Several cannabinoids have been investigated in animal models of anxiety-like behavior. Their effects tend to be complex and influenced by diverse factors. However, some common patterns start to emerge: as the main psychoactive ingredient of cannabis, Δ^9-THC has attracted considerable attention and has been investigated, among others, in the elevated plus maze and the light–dark box. Its effects tend to be biphasic – doses below 1 mg/kg generally induce anxiolytic-like effects, whereas the opposite occurs with higher doses up to 10 mg/kg. Interestingly, Δ^9-THC causes a strong activation of the hormonal stress responses at similarly high doses (Steiner and Wotjak 2008), thus indicating that it might be perceived as particularly stressful. Several synthetic cannabinoids have been investigated as well, including HU210, WIN-55212-2, and CP-55940, yielding similar results as those seen with Δ^9-THC. Though complex, their pattern of action actually

mimics the effects of cannabis in humans, which may depend on dose, environmental influences and previous stress experiences (Viveros et al. 2005).

Apart from Δ^9-THC, the only other phytocannabinoid that has been investigated in such models is cannabidiol (CBD), which also induces anxiolytic-like effects in the elevated plus maze and in the Vogel conflict test (Guimarães et al. 1990; Moreira et al. 2006). The effects of Δ^9-THC and synthetic cannabinoids are blocked by the CB1 antagonist rimonabant, though the mechanisms of action of CBD remain unknown.

Some potential explanations for the diversity of cannabinoid effects are the interference with diverse neurotransmitter systems and the action in various brain regions. Depending on the dose administered, cannabinoids could inhibit GABA or glutamate activity in the brain, thus modulating neurotransmitters with opposite functions on fear and anxiety. Alternatively, the extensive distribution of CB1 receptors in the brain suggests that cannabinoids could differentially affect negative emotions at the different brain sites of the fear and the anxiety matrix, respectively (cf. Fig. 5; Moreira and Lutz 2008).

5.1.2 Enhancing the Levels of Endocannabinoids

The experiments described above are relevant for clarifying the pharmacology of cannabinoids, though they do not provide information about the role of the endocannabinoid system. Since endocannabinoids are produced on demand followed by rapid uptake and degradation, drugs that inhibit their hydrolysis lead to an activation of CB1 signaling in a temporally and spatially restricted manner. The anandamide-transporter inhibitor AM404, for instance, induces anxiolytic-like effects in the elevated plus maze, an effect blocked by rimonabant (Bortolato et al. 2006). This drug causes an increase in the brain levels of anandamide, but not of other endocannabinoids (Bortolato et al. 2006). However, a more extensively explored strategy is the inhibition of FAAH. The prototypical compound is URB597, which induces anxiolytic-like effects, in parallel with an increase in anandamide, but not 2-AG (Kathuria et al. 2003). Contrary to CB1 agonists, no bell-shaped curves have been reported for drugs that inhibit anandamide transport or hydrolysis. However, prevention of AEA degradation not necessarily exerts anxiolytic-like effects, which seem to critically depend on the test situation (Moreira and Lutz 2008). In addition, it remains to be shown how blocking of 2-AG degradation affects fear and anxiety.

5.1.3 Inactivation of CB1 Receptors

Inhibition of CB1 signaling by pharmacological or genetic means provides the most reasonable approach for studying the involvement of the endocannabinoid system in fear and anxiety. In the majority of studies, systemic treatment with CB1 receptor antagonists induces anxiogenic-like effects (Patel and Hillard

2006), in particular if the animals are tested under highly aversive conditions, such as in brightly lit environments (Haller et al. 2004). The same holds true for CB1 knockouts, which show an increase in anxiety-like behavior only in situations with high emotional load. The mechanisms underlying these anxiogenic-like effects are still unknown. They may involve an imbalance between GABAergic and glutamatergic transmission (Moreira and Lutz 2008). Certainly, behavioral analysis of conditional mutants with cell type-specific deletion of CB1 will help to resolve this issue.

Noteworthy, a shift of anandamide actions from CB1 towards TRPV1 receptors, which exert opposite effects on neurotransmission (Di Marzo et al. 2008), may lead to an opposite behavioral phenotype, in particular since attenuation of TRPV1 signaling has anxiolytic-like consequences (Marsch et al. 2007; Terzian et al. 2009).

5.1.4 Intracerebral Injections

The diversity of the responses obtained after systemic cannabinoid injections might be due to the differential role of CB1 receptors in specific brain regions. Therefore, intracerebral injections may unveil the function of this receptor and help to clarify the effects of cannabinoids (Moreira et al. 2009). For instance, anandamide induces anxiolytic-like effects when injected into the dorsolateral PAG, an effect which is blocked by the CB1 antagonist AM251 and mimicked by the selective CB1 agonist arachidonoyl chloroethylamide (Moreira et al. 2007). Anxiolytic-like effects were also observed after injection of Δ^9-THC into the ventral hippocampus or the prefrontal cortex (Rubino et al. 2008). Therefore, these structures could be part of the brain circuitry responsible for the anxiolytic-like effects of cannabinoids (Fig. 5).

5.2 Conditioned Fear

In classical or Pavlovian conditioning paradigms, animals learn to associate a priori neutral stimuli or situations with a punishment. Subsequent encounter of these stimuli leads to pronounced fear responses, which are largely reflexive by nature (e.g., potentiated startle or freezing responses). In the absence of the predicted punishment, however, the fear responses decline both during the exposure and between exposures (i.e., within and between-session extinction). Fear extinction can result from habituation-like processes within the fear matrix as well as from relearning processes, during which the animals form a new inhibitory memory (stimulus–no-punishment association) that suppresses the original stimulus–punishment memory trace (Fig. 6). CB1 receptor signaling turned out to play an important role, not in acquisition of fear memories, but in fear extinction, since both

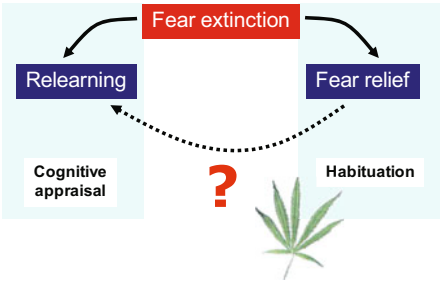

Fig. 6 Principles of fear extinction. In classical conditioning paradigms, animals and humans learn to associate a priori neutral stimuli or situations with a punishment. Re-exposure to those stimuli/situations triggers fear responses, which vanish in the absence of additional punishments. This process is termed fear extinction. It may result from relearning processes ("this stimulus/situation does not predict a punishment anymore"), which inhibit expression of the original memory trace ("this stimulus/situation predicts a punishment"). In addition, fear response may wane in a nonassociative manner via habituation-like processes (fear relief). Evidence exists that endocannabinoids are primarily involved in fear relief, which, however, might be permissive for relearning processes

genetic and pharmacological inactivation of CB1 were accompanied by sustained, nondecaying fear responses following fear conditioning (Marsicano et al. 2002). Interestingly, endocannabinoids appear to be specifically involved in extinction of *aversive* memories, since extinction of *appetitive* memories was intact in CB1-deficient mice (Hölter et al. 2005). Endocannabinoid-controlled fear extinction seems to critically depend on the amygdala, where the endocannabinoid system becomes activated during recall of aversive memories (Marsicano et al. 2002). Furthermore, systemic treatment with inhibitors of endocannabinoid uptake and/or degradation facilitated extinction of conditioned fear in a CB1-dependent manner (Chhatwal et al. 2005). Recent evidence suggests that endocannabinoids primarily affect habituation-like processes, which are thought to underlie acute fear relief rather than relearning processes per se (Kamprath et al. 2006; Fig. 6). In this context, it became evident that the averseness of a stimulus or test situation has to exceed a certain threshold, before the endocannabinoid system becomes activated and exerts its fear alleviating effects, most likely by influencing cortical glutamatergic neurons (Kamprath et al. 2009). Consequently, the endocannabinoid system seems to serve as a protective system which prevents the occurrence of exaggerated fear responses, e.g., by controlling glutamatergic transmission within the fear matrix (Fig. 7).

It is of note that extinction training resembles repeated exposures to homotypic stressors, which are associated with a priming of the endocannabinoid system (in particular of 2-AG signaling) in cortical brain structures that actively mediates long-term habituation of the stress responses during subsequent encounters (Patel and Hillard 2008). It is tempting to assume that similar processes account, at least in part, for endocannabinoid-controlled fear extinction.

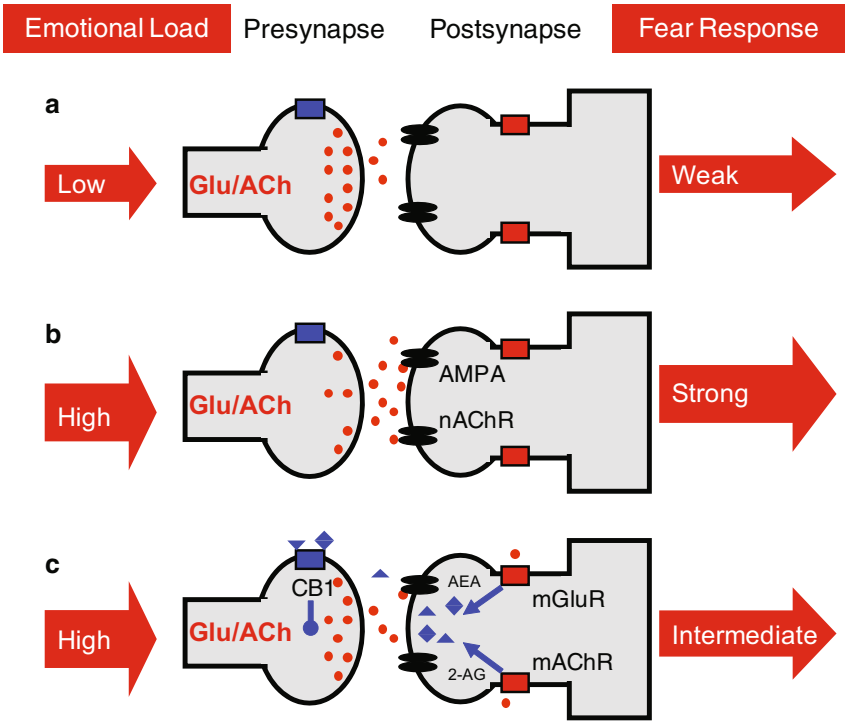

Fig. 7 A cellular model of endocannabinoid-controlled fear relief. (**a**) In situations with low negative emotional load, animals show negligible levels of fear. (**b**) In situations with high negative emotional load, in contrast, the fear matrix becomes strongly activated (see Fig. 5). (**c**) As a consequence of the intense activation of interneuronal communication, neurotransmitters [e.g., glutamate (Glu) or acetylcholine (Ach)] may reach extrasynaptically localized metabotropic receptors by synaptic spill-over, thus triggering endocannabinoid [i.e., anandamide (AEA) and 2-AG] synthesis and release. The endocannabinoids, in turn, downregulate transmitter release via presynaptically localized CB1 receptors, thereby mediating fear relief

Similar to unconditioned fear, the role of TRPV1 in fear conditioning appears to be opposite to that of CB1, since TRPV1 knockout mice show reduced levels of conditioned fear (Marsch et al. 2007).

6 Role of (Endo)Cannabinoids in Fear and Anxiety – Situation in Humans

Information concerning the role of endocannabinoids in the modulation of anxiety in humans is still quite limited. Cannabis consumption may induce a diversity of effects, ranging from relaxation and a “high” to anxiety and panic attacks (Hall and

Solowij 1998), implying a complex role of CB1 signaling on the modulation of emotional states. Pure Δ^9-THC injection may not entirely mimic the effects of Cannabis, which has been reported to induce "bliss," though also psychotic states and anxiety (Ranganathan and D'Souza 2006). In addition, CB1 receptors rapidly desensitize following their activation by Δ^9-THC, which renders it likely that part of the phenotype caused by Δ^9-THC or Cannabis intoxication relates to attenuated rather than promoted CB1 signaling.

Interestingly, clinical trials investigating the therapeutic potential of the CB1 receptor antagonist rimonabant for the treatment of obesity and cardiovascular disorders, such as the Rimonabant in Obesity (RIO) or the Strategy to Reduce Arteriosclerosis Development Involving Administration of Rimonabant—The Intravascular Ultrasound Study (STRADIVARIUS) studies, revealed the importance of endocannabinoids in the modulation of emotionality in humans, since a significant percentage of patients taking rimonabant reported feelings of anxiety and depression and an increase in suicidal thoughts (Christensen et al. 2007; Nissen et al. 2008). Rimonabant eventually reached the market in Europe as Acomplia™, only to be withdrawn shortly thereafter due to major concerns about psychiatric side-effects. Because of the same reasons, the Federal Drug Administration failed to approve the prescription of rimonabant in North America. In any case, the rimonabant saga provided evidence that endocannabinoids may tonically modulate mood and anxiety not only in lab animals, but also in humans, thus illustrating the power of translational research.

7 The Endocannabinoid System in Fear and Anxiety – Theoretical and Practical Considerations

The majority of preclinical and clinical studies report a distinctive role of the endocannabinoid system in dampening negative affects associated with fear, anxiety, and stress. However, even in normal healthy subjects, the endocannabinoid system shows a limited range of action with a lower and upper threshold (Fig. 8). The lower threshold depends on the averseness of a test situation, since the negative emotional load of a test situation has to exceed a certain threshold in order to activate endocannabinoid synthesis and release. Because of the close relationship between decrease in defensive distance, lack of controllability, and increase in emotional load (McNaughton and Corr 2004; cf. Fig. 5), this applies in particular to highly aversive situations, which cannot be avoided by the animals. It is conceivable that the spill-over of transmitters out of the synaptic cleft to extra-synaptic metabotropic receptors triggers the activation of endocannabinoid signaling, which in turn downregulates transmitter release from presynaptic butons in the vicinity of the synapse (Fig. 7).

Fear responses may depend on the defensive distance not only in quantitative, but also in qualitative terms. Rats and mice, for instance, switch from arousal and

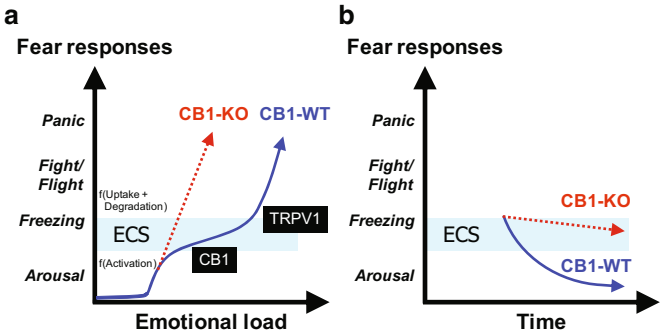

Fig. 8 The critical range hypothesis of endocannabinoid action. (**a**) The negative emotional load (cf. Fig. 5) has to exceed a certain threshold before the endocannabinoid system (ECS) becomes activated (cf. Fig. 7). After binding to CB1 receptors, the endocannabinoids delay the further increase in fear responses despite steadily increasing emotional load. However, the capacity of the endocannabinoid system is limited by efficient uptake and degradation processes. In addition, intracellularly accumulated anandamide may bind to postsynaptic TRPV1 channels. Together, these processes unleash fear responses from regulatory constraints of CB1 signaling. Consequently, in organisms with intact CB1 receptors (e.g., wild-type mice, CB1-WT), the critical range of endocannabinoid action serves as a high- and low-pass filter, which ensures moderate fear responses over a broad range of aversive conditions, without precluding exaggerated fear responses to life-threatening stimuli. This situation may become maladaptive in case of organisms with impaired CB1 signaling (e.g., CB1-deficient mice, CB1-KO), which may show inadequately strong fear responses already to situations of moderate averseness. (**b**) The same model explains dynamic changes of fear responses to stimuli/situations of a given emotional load, whereby intact CB1 signaling (e.g., in CB1-WT) mediates fear relief, whereas blocked CB1 signaling (e.g., in CB1-KO) leads to sustained fear responses

exploratory behavior (risk assessment) to behavioral immobility (freezing) to fight/flight in response to an approaching threat (e.g., a cat; Blanchard and Blanchard 1988). The adaptive value of fear reduction may become maladaptive, if the animals continue to show freezing or arousal in response to the approaching predator, when fight or flight responses would be a more adequate strategy for escaping from the dangerous situation. The endocannabinoid system seems to permit such switches in behavioral strategies despite its fear-alleviating effects, because its range of action appears to be limited by an upper threshold. This threshold is defined by highly efficient uptake and degradation processes, which omit the endogenous binding partners of the CB1 receptors. The resulting increase in anandamide in postsynaptic terminals may lead to an activation of TRPV1 receptor channels, which show a 10-times lower affinity for anandamide than CB1 (Pertwee 2008; Ross 2003) and which promote fear responses (Marsch et al. 2007; Terzian et al. 2009). We, therefore, propose that the endocannabinoid system acts as a kind of high- and low-pass filter which ensures moderate fear responses in situations with intermediate to high emotional load without precluding exaggerated fear responses in life-threatening situations (Fig. 8a). At the same time, the endocannabinoid system mediates fear relief via habituation-like processes to enduring

threatening stimuli (Fig. 8b), thus enabling the animals to return to housekeeping functions (e.g., feeding or nursing) and/or to extinguish aversive memories (Fig. 6).

In translational terms, the theoretical considerations arising from animal studies suggest that imbalances in the endocannabinoid system may contribute to human psychiatric disorders, which are associated with exaggerated fear responses, such as phobias and panic disorder, or the perseverance of aversive memories, such as posttraumatic stress disorder. Accordingly, broadening of the range of endocannabinoid action by pharmacological means might represent a novel therapeutic strategy for the treatment of these disorders. The use of cannabinoids to increase CB1 receptor signaling appears to be less promising, taking into consideration the variety of side effects (Fig. 4) and the possibility of rapid receptor desensitization. Much more promising would be to inhibit endocannabinoid uptake and degradation. In fact, inhibition of 2-AG and/or anandamide hydrolysis would enhance CB1 signaling in a temporally and spatially restricted manner. A particularly innovative approach would be to combine blockade of anandamide hydrolysis with antagonism of TRPV1, thereby promoting fear-alleviating/anxiolytic effects caused by activation of CB1 and preventing fear-promoting/anxiogenic effects mediated by TRPV1 at the same time. The success of this strategy was recently shown for arachidonoyl serotonin (AA-5HT), which exerted prominent anxiolytic effects (Micale et al. 2009).

Despite the tremendous progress in our knowledge about the physiology and pathology of the endocannabinoid system and potential consequences for health and disease in the recent years, this system still keeps a plethora of secrets waiting to be uncovered. Its pharmacological exploitation, which has started with the first use of Cannabis extracts more than 5000 years ago, has not yet come to an end, but, hopefully, will enter a new avenue away from fundamentalist concerns for the benefit of the patients.

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Pharmacological Treatment of Generalized Anxiety Disorder

David S. Baldwin, Khalil I. Ajel, and Matthew Garner

Contents

Abstract Generalized anxiety disorder (GAD) is common in community and clinical settings. The individual and societal burden associated with GAD is substantial, but many of those who could benefit from treatment are not recognized or treated. Recent evidence-based guidelines for the pharmacological management of patients with GAD have recommended initial treatment with either a selective serotonin reuptake inhibitor (SSRI) or a serotonin-norepinephrine reuptake inhibitor (SNRI), on the basis of their proven efficacy and reasonable tolerability in randomized placebo-controlled trials.

However, there is much room for improvement in both the efficacy and the tolerability of treatment. Response rates to first-line treatment can be disappointing and it is hard to predict reliably which patients will respond well and which will

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have only a limited treatment response. Many patients worry about becoming dependent on medication, a substantial proportion experience troublesome adverse effects, and these problems limit the effectiveness of pharmacological treatments in clinical practice.

The relative lack of longitudinal studies of clinical outcomes in GAD, and the small number of placebo-controlled relapse prevention studies lead to uncertainty about the optimal duration of treatment after a satisfactory initial response. There have been few investigations of the further management of patients who have not responded to first-line treatment and there is a pressing need for further augmentation studies in patients who have not responded to an SSRI or SNRI, or to other initial pharmacological approaches.

Future treatment guidelines for GAD will be influenced by emerging data for established and novel pharmacological approaches, and possibly through the more accurate identification of certain patient subgroups who are likely to respond preferentially to particular interventions.

Keywords GAD · Treatment · SSRI · SNRI · Pregabalin

1 Clinical Features, Epidemiology and Burden of GAD

GAD is characterized by excessive and inappropriate worrying that is persistent (lasting some months in ICD-10, and 6 months or longer in DSM-IV) and not restricted to particular circumstances. Patients have physical anxiety symptoms (such as tachycardia and tremor) and key psychological symptoms, including restlessness, fatigue, difficulty concentrating, irritability, and disturbed sleep (Tyrer and Baldwin 2006).

The disorder is common in both community and clinical settings. A recent review of epidemiological studies in Europe suggests a 12-month prevalence of 1.2–1.9%, and a lifetime prevalence of 4.3–5.9%: "comorbidity" with major depression is seen in three out of five cases, and a similar proportion of individuals have other anxiety disorders (Wittchen and Jacobi 2005). The functional impairment associated with GAD is similar in severity to that with major depression (Kessler et al. 1999; Wittchen et al. 2000). Patients with comorbid major depression and GAD have a more severe and prolonged course of illness, and greater impairment of social and occupational function (Judd et al. 1998; Tyrer et al. 2004). The disorder is more common in women than in men, with a mean age of onset that is somewhat later than with other anxiety disorders: it is probably the most common anxiety disorder among the population aged 55–85 years (Beekman et al. 1998).

GAD is one of the most common mental disorders in primary care settings, and is associated with increased use of health services. However, only a minority of patients present with anxiety symptoms, and GAD often goes unrecognized as doctors

tend to overlook anxiety unless it is a presenting complaint. Patients with comorbid depression are more likely to be recognized as having a mental health problem, though not necessarily as having GAD (Weiller et al. 1998; Wittchen et al. 2002).

2 Response Rates to Initial Treatment in GAD

The findings of randomized controlled trials show that approximately 40–60% of patients will "respond" to placebo and 60–75% to the SSRIs escitalopram, paroxetine, or sertraline, when using global measures of improvement, usually the Clinical Global Impression of Improvement Scale (CGI-I) (Guy 1976). Similar findings are seen in randomized controlled trials with the SNRIs duloxetine or venlafaxine, and with the novel anxiolytic drug pregabalin (Baldwin and Ajel 2007) (Table 1). There can be a striking reduction in symptom severity on the primary outcome measure, traditionally the Hamilton Rating Scale for Anxiety, HAMA (Hamilton 1959): for example, a decline from baseline in mean HAMA score of over 15 points with the optimal 10 mg/day dosage in a recent randomized controlled trial with escitalopram (Baldwin et al. 2006a). However, many patients remain troubled by significant symptoms at study end point, despite seemingly making a good overall "response" to treatment, according to the CGI-I score.

A systematic review of the findings of randomized controlled trials has established that benzodiazepines are an efficacious and rapid treatment for many patients with GAD, having similar overall efficacy to the psychological treatment cognitive therapy (Gould et al. 1997). However, benzodiazepines have limited efficacy in relieving comorbid depressive symptoms, and unwanted effects include sedation, disturbance of memory, and psychomotor function. These problems limit the overall effectiveness of benzodiazepines, as many patients discontinue treatment before the occurrence of optimal anxiolytic efficacy (Martin et al. 2007). Other potential problems include the development of tolerance, abuse or dependence, and distressing withdrawal symptoms on stopping the drug (Tyrer et al. 1983; Rickels et al. 1988). Because of these difficulties, general guidance is to use benzodiazepines only for short-term treatment (up to 4 weeks) (Baldwin et al. 2005), or in patients who have not responded to at least two previous treatments, and who remain troubled by severe, distressing, and disabling anxiety symptoms (Baldwin and Polkinghorn 2005; Nutt, 2005).

Unlike the situation in major depressive disorder, there is as yet no general consensus on what constitutes symptom remission in GAD (Ballenger et al. 1999). A post hoc analysis of randomized controlled trials with escitalopram (Bandelow et al. 2006) indicates that a HAMA score of 9 or less corresponds to the category of "borderline ill" on the CGI Severity scale (Guy 1976). Using this cut-off score, 56% of patients treated with the optimal dosage of escitalopram had remitted at the end of double-blind treatment (and 47.9% when using a lower HAMA cut-off score, of 7 or less) in the escitalopram study (Baldwin et al. 2006a). The post hoc analysis of data from the extensive clinical trial program for paroxetine using this more

Table 1 Generalized anxiety disorder: response rates in double-blind placebo-controlled studies of acute treatment with SSRIs, SNRIs or pregabalin

Study	Treatment	Dose (mg/day)	Length (Weeks)	Active response (%)	Placebo response (%)
SSRI treatment					
Pollack et al. (2001)	Paroxetine	20–50	8	62	56
Rickels et al. (2003)	Paroxetine	20, 40	8	61.7*, 68***	45.6
Rynn et al. (2001) (children)	Sertraline	≤50	9	90***	10
Allgulander et al. (2004)	Sertraline	50–150	12	63***	37
Brawman-Mintzer et al. (2006)	Sertraline	50–200	10	59.2*	48.2
Davidson et al. (2004)	Escitalopram	10–20	8	58**	38
Goodman et al. (2005)	Escitalopram	10–20	8	52***	37
Pooled analysis, three studies					
Baldwin et al. (2006a)	Escitalopram	5, 10, 20	12	70.9, 78.4**, 74.2*	63
	Paroxetine	20		66.2	
SNRI treatment					
Koponen et al. (2007)	Duloxetine	60, 120	9	63***, 65***	34
Rynn et al. (2008)	Duloxetine	60–120	10	40^{a,*}	32^{a}
Hartford et al. (2007)	Duloxetine	60–120	10	56**	42
	Venlafaxine	75–225	10	60***	
Davidson et al. (1999)	Venlafaxine	75, 150	8	62**, 49	39
	Buspirone	30		55*	
Gelenberg et al. (2000)	Venlafaxine	75–225	28	67***	33
Rickels et al. (2000)	Venlafaxine	75, 150, 225	8	NS, NS*	NR
Allgulander et al. (2001)	Venlafaxine	37.5, 75, 225	24	63**, 73***, 81^{***,b}	47^{a}
Lenox-Smith et al. (2003)	Venlafaxine	75–225	24	65**	46
Rynn et al. (2007)	Venlafaxine	Flexible dose in children 6–17 years	8	69*	48
Pregabalin treatment					
Pande et al. (2003)	Pregabalin	150, 600	4	NS, 47*	28
Pande et al. (2000)	Pregabalin	150, 600	4	No significant difference in efficacy for any treatment versus placebo	
	Lorazepam	6			

Feltner et al. (2003)	Pregabalin	150, 600	4	47.8, 49.2	42.4
	Lorazepam	6		56.3	
Rickels et al. (2005)	Pregabalin	300	4	61***	31
	Alprazolam	450		44	
		600		51**	
		1.5		45*	
Montgomery et al. (2006)	Pregabalin	400, 600	6	56*,59*	42
	Venlafaxine	75		61**	
Pohl et al. (2005)	Pregabalin	200, 400, 450	6	56**, 55**, 59**	34
Momtgomery et al. (2008) (elderly patients)	Pregabalin	150–600 (mean maximal 270)	8	58.4	48.4
Kasper et al. (2009)	Pregabalin	300–600	8	59	46
	Venlafaxine	75–225		44	

*$p<0.05$; **$p<0.01$; ***$p<0.001$, advantage for active treatment over placebo

[a]response defined as $\geq$50% reduction in HAMA score; [b]estimates from published figure; NR, not reported; NS, not significantly different from placebo

stringent criterion found that only 36% of patients undergoing double-blind treatment with paroxetine had remitted at study end point (Rickels et al. 2006).

Only few randomized controlled trials permit assessment of the relative efficacy of different treatments when compared to placebo. However, a recent analysis of findings from randomized controlled trials found an overall mean effect size of 0.39, with some differences between medication class: pregabalin, 0.50; the antihistamine hydroxyzine, 0.45; SNRI, 0.42; benzodiazepines, 0.38; SSRI, 0.36; and the azapirone anxiolytic buspirone, 0.17 (Hidalgo et al. 2007). The overall effect size in this analysis is somewhat higher than that from a previous meta-analysis (0.33) (Mitte et al. 2005), which possibly reflects differences in publication selection criteria. Estimations such as these represent post hoc analyses of pooled data, derived from randomized controlled trials that differ in design and which were not powered for the demonstration of particular levels of effect size, so care must be taken when comparing relative effect sizes. Nevertheless, there is much scope for further improvement in developing pharmacological treatments with greater efficacy than is seen with currently available medications.

3 Prediction of Response to Pharmacological Treatment in GAD

Unfortunately it is not possible to predict accurately which patients will respond well and which will have only a limited response to treatment. A greater likelihood of response to venlafaxine or the SSRI fluoxetine is associated with a shorter duration of symptoms (Perugi et al. 2002; Simon et al. 2006) and the presence of comorbid dysthymia (to venlafaxine) (Perugi et al. 2002). Other predictors of response include psychiatric comorbidity (Rodriguez et al. 2006), a history of depression or panic disorder (to venlafaxine) (Pollack et al. 2003), and the severity of psychosocial impairment (Rodriguez et al. 2006). A lower likelihood of response to escitalopram treatment is seen with lower baseline symptom severity (Stein et al. 2006), and a history of benzodiazepine use is associated with lower response to treatment with venlafaxine (Pollack et al. 2003). The presence of comorbid depressive symptoms probably does not reduce the overall response to treatment in patients with primary GAD, for the reason that although comorbid depression may delay the response to venlafaxine (Pollack et al. 2003), it does not substantially reduce overall response rates with escitalopram (Stein et al. 2005) or pregabalin (Stein et al. 2008), or affect the degree of reduction in anxiety symptoms with fluoxetine treatment (Olatunji et al. 2008).

Similar difficulties are seen in deciding how long initial treatment in GAD should continue, before it is reasonable to conclude that the chance of responding is too low to justify continuing with the current approach. An early study showed that a greater reduction in HAMA score after 1 week of diazepam treatment predicted a higher likelihood of response at 6 weeks (Downing and Rickels 1985). A limited reduction in symptom severity (i.e., a reduction in total HAMA

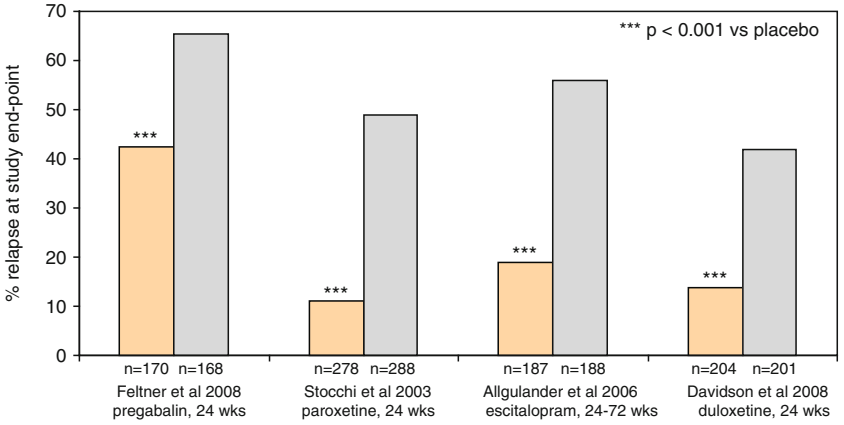

Fig. 1 Published placebo-controlled relapse prevention studies in generalized anxiety disorder

score by 25% or less) at 2 weeks of treatment predicts nonresponse to buspirone or lorazepam at 6 weeks (Laakmann et al. 1998); and the degree of response after one or 2 weeks was found to be strongly predictive of response to benzodiazepines or azapirones (or placebo) at 8 weeks (Rynn et al. 2006). Recent analyses show that the onset of efficacy (defined as a reduction in HAMA score of 20% or more) after 2 weeks of treatment is strongly predictive of response at study end point for duloxetine (Pollack et al. 2008) and escitalopram (Baldwin et al. 2009); and suggest that the likelihood of eventual response is low, if an onset of efficacy is not seen after 4 weeks of treatment (Fig. 1).

4 Optimal Duration of Treatment in GAD

Traditionally, GAD is regarded as a chronic disorder that waxes and wanes in severity over many years. In a prospective, naturalistic, longitudinal study, the probability of recovering from the index "episode" was only 58% at the end of 12 years, and over 40% of those who had recovered experienced subsequent recurrence of symptoms (Bruce et al. 2005). However, recent findings from the Zurich Study suggest there is rather more longitudinal fluidity in the diagnosis, than was previously thought (Angst et al. 2009). Continuation of antidepressant treatment beyond initial response substantially reduces the risk of early relapse and later recurrence of depressive symptoms (Geddes et al. 2003), but the value of long-term treatment in GAD is less established, due to the limited number of relapse prevention studies. Recent guidelines recommend at least 6 months of continuation treatment after initial response (Baldwin et al. 2005, Canadian Psychiatric Association 2006), but emerging data suggest that longer periods of continuation treatment may be advisable.

The value of continuation treatment in mood and anxiety disorders is usually ascertained through double-blind placebo-controlled relapse prevention studies, in

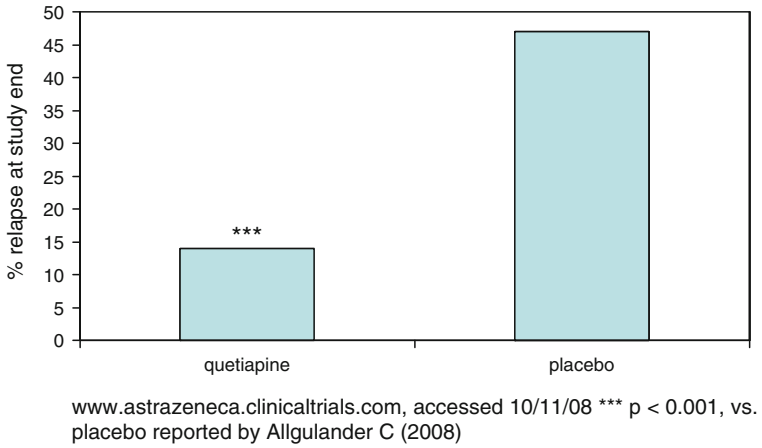

Fig. 2 Placebo-controlled relapse prevention study of quetiapine in GAD

which patients who have responded well to initial open acute treatment are randomized to either continue with active treatment, or switched to placebo. Of the five relapse prevention studies in GAD which have been published, four demonstrate the value of continuing pharmacological treatment, with escitalopram, paroxetine, pregabalin, or duloxetine (Allgulander et al. 2006; Stocchi et al. 2003; Feltner et al. 2008; Davidson et al. 2008). A formal relapse prevention study with venlafaxine did not reveal efficacy, although significantly fewer patients relapsed during venlafaxine treatment in a separate prolonged randomized double-blind placebo-controlled trial (Montgomery et al. 2002). An as yet unpublished relapse prevention study with the second generation antipsychotic drug quetiapine suggests it has some efficacy in relapse prevention (Allgulander, 2008) (Fig. 2).

The duration of double-blind treatment in the placebo-controlled relapse prevention study with escitalopram (Allgulander et al. 2006) could last for up to 18 months, so it is reasonable to recommend that treatment should continue for up to 18 months, after an initial response. The United States Federal Drug Administration has recently recommended that the double-blind relapse prevention phase in GAD studies should be preceded by 6 months of unblinded treatment (Food and Drug Administration 2006). Post hoc analyses of previously published randomized controlled trials will be able to establish whether patients with differing durations of response prior to randomization also differ in their relapse rates, and these analyses could strengthen the evidence regarding the duration of long-term treatment in GAD.

5 Management after Nonresponse to Initial Treatment in GAD

There is little consensus on the optimal next stage in patient management after a poor response to first-line treatment. Potential interventions include an increase in dosage, a switch to another evidence-based pharmacological treatment, augmentation

with an additional psychotropic drug, and the combination of medication with psychological treatment.

There is no published dosage escalation study in GAD in which patients either continue with the initial low dose or are switched to a subsequent higher dose. The findings of fixed-dose randomized placebo-controlled studies do not provide much evidence to suggest that higher doses may be preferable. By illustration, the relative efficacy of paroxetine when compared to placebo is similar for daily dosages of 20 or 40 mg (Rickels et al. 2003), and the optimal daily dosage of escitalopram is probably 10 mg rather than 20 mg (Baldwin et al. 2006a). Furthermore, fixed-dose studies with venlafaxine have produced inconsistent findings, with evidence both for (Rickels et al. 2000) and against (Allgulander et al. 2001) a dose-response relationship. However, a recent post hoc analysis of pooled data from randomized controlled trials with pregabalin suggests that higher doses (200–450 mg/day) have greater efficacy than lower doses (150 mg/day), when both are compared to placebo (Bech 2007).

Most guidelines recommend an SSRI for first-line pharmacological treatment of GAD, on the balance of efficacy and tolerability, so common second-line drug treatments include an SNRI, buspirone, the tricyclic antidepressant imipramine, pregabalin, or a benzodiazepine. The azapirone anxiolytic drug buspirone is efficacious in GAD, more so when patients have not previously been treated with a benzodiazepine: as such, it is advisable to consider use of buspirone before prescribing a benzodiazepine anxiolytic (Chessick et al. 2006).

Despite reservations about potential adverse effects (such as weight gain and metabolic syndrome) some doctors recommend an antipsychotic drug after non-response to SSRI or SNRI treatment, perhaps fearing the development of tolerance or dependence with use of benzodiazepines. The conventional neuroleptic drug trifluoperazine has proven efficacy (Mendels et al. 1986) and more recently the second-generation antipsychotic drug quetiapine has also been found efficacious, in placebo- and comparator-controlled studies (Bandelow et al. 2007a; Meredith et al. 2008). Most probably, the adverse event profile and potential long-term risks of antipsychotics will result in their usually being reserved for patients who have not responded to earlier SSRI treatment, perhaps followed by SNRI treatment. Both risperidone and olanzapine can enhance the efficacy of SSRI treatment (Brawman-Mintzer et al. 2005; Pollack et al. 2006), but currently the evidence for augmentation with quetiapine is only limited (Katzman et al. 2008; Simon et al. 2008). Potential alternative augmentation approaches include the use of pregabalin, which can enhance the effectiveness of SSRI or SNRI antidepressants (Miceli et al. 2009), the novel antidepressant drug agomelatine, which has recently been found efficacious (Stein et al. 2008), and the novel anticonvulsant drug zonisamide (Kinrys et al. 2007). Combining pharmacological and psychological approaches is often advocated in the overall management of patients with anxiety disorders, although in GAD it is uncertain whether combination treatment is superior to psychological or drug treatment given alone (Bandelow et al. 2007a, b).

6 Tolerability of Current Treatments for GAD

The tolerability profile of prescribed medication is an important consideration, particularly when recommending long-term treatment, as is the case in GAD. Adverse effects of SSRIs and SNRIs such as increased nervousness, headache and nausea, and the drowsiness associated with benzodiazepines and pregabalin, usually resolve after a few weeks of treatment, but other side effects become more important factors in the overall acceptability of treatment for patients over subsequent months. The adverse event profile of different SSRIs and SNRI is generally rather similar, although significantly fewer patients drop out due to adverse events in short-term and medium-term randomized controlled trials with escitalopram, than with paroxetine or venlafaxine (Baldwin et al. 2007a). Common concerns during longer-term treatment with SSRIs or SNRIs include the development of sexual dysfunction, weight gain, persistent disturbed sleep, and the potential for experiencing discontinuation symptoms on stopping treatment.

Treatment-emergent sexual dysfunction is probably the most common complication of SSRI treatment in depressed patients (Baldwin 2004), although some aspects of sexual function usually improve, as depressive symptoms resolve (Baldwin et al. 2006b; Baldwin et al. 2008). It is uncertain whether the same applies in the treatment of patients with GAD, in whom the complaint of loss of sexual desire is less common. Weight gain may be less troublesome with SSRIs than with many other psychotropic drugs, but the potential for gaining weight can cause concern in many patients, and there is reasonable evidence that some SSRIs can cause increases in weight of 6–10 kg after 6–12 months of treatment (Ferguson 2001). Finally, SSRIs and related drugs can have only limited benefit, or even deleterious effects, on sleep disturbance, despite beneficial effects on other depressive and anxiety symptoms (Carney et al. 2007; Cervena et al. 2005).

Discontinuation symptoms on stopping treatment are common with many classes of psychotropic drug, including SSRIs and SNRIs, as well as with benzodiazepines (Rickels et al. 1988; Baldwin et al. 2007b). Symptoms are typically mild and only transient, but many patients report severe and distressing symptoms, despite gradual discontinuation through tapering the prescribed dose of medication. Compounds differ in their propensity to cause discontinuation symptoms, but it is hard to predict which patients will be most affected. Recent research suggests that influences of diagnosis, longer duration of treatment, higher dosage, and the abrupt withdrawal of treatment are less established than previously thought (Baldwin et al. 2007b). Slow stepped withdrawal ("tapering") is often advised, in the desire to minimize the appearance of distressing discontinuation symptoms, but the value of this is not established fully and there is a need for withdrawal studies that adopt a randomized double-blind staggered design, in which both patients and doctors are unsure of whether treatment ends slowly or swiftly, or when dosage reduction occurs.

7 Conclusions

There are many psychotropic drugs and psychotherapies available for the treatment of patients with GAD, but despite this, overall clinical outcomes for many patients are often poor. The "ideal" treatment for GAD does not yet exist, as existing treatments have insufficient overall efficacy in short-term and long-term treatment and can have troublesome adverse effects when prescribed for long periods. The particular choice of treatment should be determined by the clinical features of the patient (such as the presence of comorbid depression and a history of a good response to previous treatment), patients' preferences for one approach over another and the availability of services. Doctors should counsel patients that they will not respond immediately, that sometimes symptoms can worsen in the early stage of treatment, and that long-term treatment is often needed to maintain an initial response. However, there is clearly much room for improvement, in the development of more efficacious and more acceptable pharmacological approaches in the management of this common, distressing, typically disabling, and often persistent anxiety disorder.

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Pharmacologic Treatment of Panic Disorder

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Contents

Abstract Comprehensive management of panic disorder involves a wide array treatments and interventions to reduce symptoms and increase functionality. This chapter provides an overview of the pharmacologic treatment of panic disorder including aspects of assessment, treatment selection and the biologic mechanisms of the illness.

Keywords Anxiety · Benzodiazepine · Panic · Pharmacology · SNRI · SSRI · TCA · Treatment

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1 Introduction

The anxiety disorders are among the most common psychiatric syndromes affecting up to about 15% of the population (Robins et al. 1984; Kessler et al. 1994). Panic disorder (PD), one of the most severe anxiety syndromes, is characterized by recurrent unprovoked panic attacks. During these attacks, a variety of physical symptoms may occur accompanied by a sense of doom together with a strong desire to escape (Weissman et al. 1990). This need to escape may lead people to quit jobs, avoid friends and social activities, and can lead to significant withdrawal and isolation (Roy-Byrne et al. 2006). Comprehensive management of PD involves a wide array of treatments and interventions designed to reduce symptoms and increase functionality. This chapter provides an overview of the pharmacologic treatment of PD, including aspects of patient assessment, treatment selection, and a description of the biologic mechanisms of the illness. Each mechanism will be presented with a discussion of selection, efficacy, and implementation of the treatments presented.

2 Clinical Assessment Before Treatment

There are several key steps in the clinical assessment of the anxious patient that precede the formulation of a comprehensive treatment plan. In addition to diagnosis of the specific anxiety disorder affecting the patient, the clinician needs to have a high index of suspicion for co-morbid conditions such as other anxiety disorders, mood disorders, and substance use disorders (Royal Australian and New Zealand College of Psychiatrists 2003). Furthermore, an anxiety disorder due to a medical condition or substance use (e.g., thyrotoxicosis, caffeinism, alcohol withdrawal) and medical conditions that mimic clinical anxiety (e.g., cardiac arrhythmias, seizure disorders, asthma) need to be ruled out. Thus, a thorough work-up should include a full physical examination, urinalysis, urine toxicology, electrolytes, liver functions, thyroid function tests, a full blood count, and an EKG. For patients with atypical presentations, for example, panic attacks with syncope, it is important to refer them for a neurological evaluation. A central principle of pharmacological management of anxiety disorders is the need to guide treatment by focusing on key target symptoms, such as panic attacks, phobic anxiety, or generalized anxiety. The goal of pharmacotherapy then becomes suppression or eventual blockade of the target symptom. Not all features of anxiety syndromes are directly responsive to medical interventions (e.g., agoraphobic symptoms in a PD patient). Therefore, another general rule of medical treatment is to consider the inclusion of cognitive–behavioral therapy (CBT) or other psychotherapies in the overall treatment plan. Determination of the specific features of PD that characterize a given patient's experience is an essential element of assessment and treatment planning; it is also an opportunity to begin the process of building a strong therapeutic alliance with the patient (Royal Australian and New Zealand College of Psychiatrists 2003).

Careful counseling of the anxious patient concerning the short- and long-term risks and benefits of medications is the key to satisfactory compliance as these patients are extremely side-effect sensitive.

3 Treatment Selection

A variety of psychosocial and pharmacologic interventions have proven benefits in treating PD. Treatment selection is guided by a knowledge of the efficacy profile of a given agent and consideration of potential liabilities including side-effect burden, drug–drug interactions, safety in overdose, and other nonclinical factors such as cost and availability (Canadian Psychiatric Association 2006). There are no reliable clinical predictors of treatment responsivity excepting a previous history or family history of such a response. Furthermore, despite the extensive database of neurobiological studies in PD, there are, as yet, no clinically useful biological markers of treatment responsiveness. In patients with uncomplicated PD, as well as in patients with co-morbidity, the SSRIs are now considered the first-line medications (American Psychiatric 2009). Their ease of administration and favorable toxicity/side-effect profile in comparison with older-generation agents [tricyclic and monoamine oxidase inhibitors (MAOIs)] has contributed to this trend. As mentioned earlier, there is now some evidence that SSRIs might have superior antipanic efficacy compared to other standard agents. In addition, the data from some efficacy trials suggest that SSRIs may have an earlier onset of action compared to the older generation 5-HT/NE anxiolytics (Royal Australian and New Zealand College of Psychiatrists 2003). Taken together, these characteristics strongly argue in favor of the selection of an SSRI as the first-line agent in PD. Imipramine and other tricyclics should now be considered the second-line agents for panic. Benzodiazepines are third-line, anticonvulsant the fourth-line, and the classical MAOIs would be considered fifth-line antipanic agents due to their side-effect profile and safety issues. Indeed, there appear to be few contraindications to SSRI treatment for panic. One caveat is the potential for drug–drug interactions due to hepatic P450 enzyme inhibition. For example, paroxetine and fluoxetine, in regular clinical doses, substantially inhibit the P450 IID6 isoenzyme (American Psychiatric 2009). A number of medication classes, including tricyclics, some neuroleptics, β-blockers, codeine-based analgesics, and some antiarrhythmics (e.g., flecainide and encainide), are metabolized by IID6. Fluvoxamine and, to a lesser extent, fluoxetine inhibit the P450 3A3/4 isoenzyme that metabolizes a number of classes of medications including macrolide antibiotics, nonsedating antihistamines, and calcium channel blockers. There has been one case report of EKG changes in a patient receiving both terfenadine and fluoxetine. Thus, careful clinical monitoring of patients receiving an SSRI together with any of these medications is warranted. Citalopram and sertraline appear to cause lesser degrees of P450 enzyme inhibition, and thus may be useful in the management of the panic patient on multiple medications for other psychiatric or medical reasons. Another relative contraindication would be

administration of an SSRI or indeed any antidepressant-like agent to patients with additional diagnoses of bipolar disorder or a psychotic illness. This caution is of some relevance, clinically, since PD appears to be over represented in bipolar populations (Rotondo et al. 2002). Whether SSRIs are less likely to induce hypomania than tricyclics bears further study (Howland 1996). In the bipolar/panic disordered patient, it may be preferable to avoid SSRI use and attempt stabilization of both syndromes with anticonvulsants (e.g., valproate, gabapentin, and lamotrigine), which are now being increasingly used for both syndromes. Another area of concern is the potential for serotonin syndrome (confusion, diaphoresis, agitation, fever, and tachycardia) and movement disorder to occur in SSRI/SNRI treated patients, who are subsequently given the common anti-emetic agent, metoclopramide (Fisher and Davis 2002). This observation, based on a recent report of two cases, may be the result of a pharmacodynamic interaction between SSRIs and metoclopramide. Within the SSRI class there is no available comparative efficacy data that would lead one to select one agent over another. Pharmacokinetic factors are of some importance in selecting an appropriate SSRI in PD. For example, an agent with a long $t_{1/2}$ such as fluoxetine should be considered in patients in whom the tapering phase of treatment may be complicated. Also, there may be subtle differences in side-effect profile that guide treatment selection. For example, paroxetine tends to be more sedating than other SSRIs and may be preferable in patients in whom sleep disturbance is a significant symptom. The SSRIs are generally safe agents for use in special populations such as the elderly, pregnant or lactating women, and children and adolescents (Murphy et al. 2000). There is some concern about growth-delaying effects of SSRIs exposure during the prepubertal and pubertal period (Weintrob et al. 2002). PD disproportionately affects women, and not infrequently declares itself during pregnancy or the post-partum period. Thus, it is clinically useful to know that SSRIs have a low teratogenic potential and, although excreted in breast milk, appear, thus far, to have limited effects on the newborn (Misri et al. 2000). One caveat to SSRI therapy in elderly patients is the slight risk of a syndrome of inappropriate ADH secretion, suggesting the need for electrolyte monitoring in this group.

3.1 Treatment Implementation

Many people with PD do not respond fully to a first-line treatment due to a various factors including an inability to tolerate treatment (American Psychiatric 2009). In general, panic patients are extremely side-effect sensitive, especially to the stimulant properties of agents that modify 5-HT and NE function, and therefore should be commenced on half the usual starting dose used for major depression (Louie et al. 1993). Not uncommonly, co-treatment with a benzodiazepine is necessary to minimize the impact of this side-effect. Recent work endorses the benefit of early regular co-administration of the benzodiazepine, clonazepam, with SSRIs for the rapid stabilization of moderate–severe PD (Goddard et al. 2001; Pollack et al.

2003). Gradual clonazepam tapering after several weeks of co-administration appears to be well-tolerated and limits the development of physiological dependence associated with long-term benzodiazepine use. Guidelines for the duration of SSRI therapy generally depend on the time at which a full response has been obtained. This is usually defined as a remission of both full and limited symptom panic attacks. At this time, a further 6 months of maintenance therapy is considered sufficient (Rosenbaum et al. 1996) before a trial of SSRI taper is indicated. 5-HT/NE anxiolytics have the advantage of being relatively easy to taper with this population in contrast to benzodiazepine agents. However, abrupt discontinuation should be avoided because of the flu-like syndrome that has been observed to occur in this context (Coupland et al. 1996). Additional controlled clinical research data are still needed to guide the long-term management of PD. Recent work with PD patients on imipramine suggests that they may benefit from an extended maintenance period (up to 2 years; Mavissakalian and Perel 2001). Similarly, continued SSRI therapy (18 months) appeared to prevent clinical relapse. Thus, maintenance pharmacotherapy may be necessary for some patients to protect against relapse. Large, controlled maintenance studies are underway to determine important questions concerning the ideal length of and combination of treatments, and the clinical indications for extended maintenance treatment in a subgroup of patients who might be sensitive to relapse (e.g., patients with multiple previous episodes of panic). Treatment is generally monitored by frequent assessment of clinical status and treatment compliance. Drug plasma levels are generally not needed as a guide to dosing. With respect to imipramine treatment, there appears to be a linear relationship between antipanic effects and total imipramine plasma levels (Mavissakalian and Perel 1994). Treatment resistance is a relatively uncommon problem and can usually be addressed by careful diagnostic reassessment and then moving through the top four classes of agents in a step-wise manner (Rosenbaum et al. 1996). However, partial responses are fairly common and can usually be managed by optimization of the existing regimen or co-administration of another therapeutic agent, for example, a benzodiazepine, or another 5-HT/NE anxiolytic (e.g., low-dose desipramine). If response to treatment remains unsatisfactory following an adequate trial; it is appropriate to consider a change (American Psychiatric 2009) either through augmentation with another agent or the addition of another modality (e.g., cognitive behavioral therapy). An individual approach to augmentation is required as there is little work on the benefit of other augmentation strategies in panic (e.g., lithium augmentation) that have been routinely applied in depression, where treatment-resistance is a more prevalent clinical issue.

4 Pharmacologic Treatments

Selection of a specific medication for the treatment of PD will require the consideration of the specifics of a patient's presentation and which medicine best fits in terms of properties of the medication itself (e.g., half-life) and its associated features (e.g., side effects, cost). This is because the classes of medications used

[SSRIs, serotonin/norepinephrine reuptake inhibitors (SNRIs), TCAs and benzodiazepines] are comparable in terms of efficacy (Kessler et al. 1994). As such, SSRIs and SNRIs are likely the best choice of initial pharmacotherapy for many patients with PD as they do not carry the significant side effects associated with TCAs and have no liability for abuse as is found with benzodiazepines. SSRIs, SNRIs, and TCAs all provide antidepressant effects, but all have delayed primary antipanic effects. Benzodiazepines, despite their greater propensity for side effects, are still used very frequently because of their rapid onset of action. Studies (Pollack et al. 2003) have suggested benzodiazepines in combination with antidepressants to control symptoms until the antidepressant takes effect followed by tapering of the benzodiazepine. PD patients frequently are hypersensitive to medication side effects, and it is recommended to educate patients about the likely course of both primary and side effects of the medications. It is also recommended that starting doses of SSRIs, SNRIs, and TCAs be approximately half of those given to depressed patients (Louie et al. 1993). This initial dose should be maintained for several days and then gradually increased as tolerated by the patient.

5 Mechanisms of Action

The fear circuit is highly complex, and unsurprisingly, involves several neurochemical systems that modulate and mediate responses. However, from this complexity we can draw out the key role played by the amygdala in the coordination of the components (e.g., cognitive, affective, neuroendocrine, cardiovascular, respiratory, musculoskeletal) of this circuit. Within the context of the cognitive component, it is important as a modulator of fear and anxiety responses and also as a component in the conditioning and extinction of fears. Perhaps this serves as a partial explanation for the status of PD as the most treatment sensitive of the anxiety disorders with 70–80% of patients having satisfactory responses (Rosenbaum et al. 1996). The medications used to treat PD are thought to modify components in this circuit through modification of the neurochemical systems associated with the stress response including serotonin (5-hydroxytrptamine; 5-HT), norepinephrine (NE), γ-aminobutyricacid (GABA), glutamate, and peptides.

The clinical literature concentrating on this area has been mainly derived from the studies of panic patients, and whether these data have more general implications for anxiety disorders remains to be seen. There is evidence that 5-HT mechanisms may have an important role in mediating the antipanic effects of several classes of anxiolytic agents. For example, successful SSRI treatment of PD seems related to the net enhancement of 5-HT function that occurs with the administration of these agents (Blier and Abbott 2001). This outcome appears to be due to the desensitization of somato-dendritic 5-HT_{1A} autoreceptors and possibly 5-HT_{1B} terminal autoreceptors that occurs following chronic SSRI administration (Blier 2001). In conclusion, enhancement of 5-HT neurotransmission is a common mechanism of several major classes of anxiolytic and may be a key factor in controlling pathological anxiety.

NE mechanisms may be also responsible for the therapeutic effects of some of the medications reviewed. In animal studies chronic application of both tricyclics and MAOIs has a variety of inhibitory effects on NE function, including reduction in LC firing rates, NE turnover, tyrosine hydroxylase activity, and postsynaptic β receptor number and functioning. These pre- and postsynaptic events are likely to contribute to stabilization of NE system function in syndromes associated with NE dysregulation such as PTSD and PD. In addition, 5-HT/NE interactions have been implicated in the successful treatment of panic symptoms. For example, preclinical work indicates that long-term but not short-term paroxetine administration is associated with decreased LC/NE cell firing (Szabo et al. 2000). Consistent with this finding of reduced NE activity following SSRI administration in the lab is the clinical observation that chronic SSRI treatment of panic results in the attenuation of anxiogenesis from NE system stimulation with the α-2 adrenergic antagonist, yohimbine. Newer therapeutic agents that are likely to have broad anxiolytic effects such as the 5-HT/NE reuptake blocker, venlafaxine, and the NE reuptake inhibitor, reboxetine, similarly modify LC/NE activity with chronic administration (Szabo and Blier 2001).

GABA neuronal mechanisms have been implicated in the anxiolytic effects of several classes of medications. There is evidence that $GABA_A$/NE interactions may be an important therapeutic mechanism in PD as benzodiazepine treatment blocks panicogenesis induced by yohimbine. It is conceivable, then, that enhancement of GABA function is a common therapeutic pathway for effective antipanic treatments (Jefferson 2001).

There is some evidence that antipanic treatment effects could be related to effects on heart rate variability in PD. Successful treatment (with either cognitive–behavior therapy or imipramine) is associated with normalization of heart rate variability patterns, and recent preliminary data suggest that SSRI treatment with paroxetine has a similar effect (Tucker et al. 1997). Patients with PD may have decreases in vagal tone, permitting a predominance of sympathetic activation of cardiac function. Reversal of these effects by effective treatment may decrease the tendency towards spontaneous panic.

Finally, antipanic agents may modify the functioning of the CRF/HPA axis to bring about therapeutic effects. Preclinical studies have observed decreased HPA axis function on a number of measures following chronic administration of moclobemide and the tricyclic nortryptiline (Reul et al. 1994), while benzodiazepines also appear to decrease CRF function by directly inhibiting CRF release (Plotsky et al. 1995). However, one clinical study of panic patients did not support the notion that HPA axis down modulation (and by implication reduction in central CRF function) was necessary for the occurrence of long-term clinical improvement (Abelson and Curtis 1996).

6 Serotonergic Agents

Currently, six SSRIs (fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, and escitalopram) are available in the United States (American Psychiatric 2009). Although there is no evidence for differential efficacy between them, differences in

side-effect profile, drug half-life, propensity for interactions with other medications, and the availability of generic formulations may influence choice of initial medication (Fava 2006). These drugs, because of their broad therapeutic spectrum (Haas et al. 2009; Gorman and Kent 1999), have the advantage of affording treatment for other common, co-morbid conditions (usually other anxiety disorders or mood disorders), a significant clinical problem in this population. Within this category of agents, the SSRIs are now preferred because of their favorable side-effect profile, ease of administration, and safety. Many of these agents acutely inhibit reuptake of brain 5-HT and/or NE. This is especially true of tricyclic agents, the SSRIs, and the SNRI, venlafaxine. The MAOIs and RIMAs (reversible inhibitors of MAO-A) are quite effective for PD.

Some patients with PD experience increased side effects when beginning treatment with an SSRI. Because of this, starting doses are lower than that for other indications. According to the most recent APA treatment guidelines, the recommended daily starting dose for each is as follows: fluoxetine is 10 mg or less, sertraline 25 mg, paroxetine 10 mg (IR) or 12.5 mg (CR) depending on the formulation used, 10 mg of citalopram, and escitalopram at 5–10 mg (American Psychiatric 2009). It is further recommended that this lowered starting dose be maintained for approximately 3–7 days and then gradually increased based on tolerability (Stein 2005) to standard doses as presented in Table 1. Abruptly stopping SSRIs can lead to discontinuation syndrome (Shelton 2006). Tapering treatment over a week or longer can minimize this risk.

SSRIs have few serious side effects, are rarely lethal in overdose, and have few serious effects on cardiovascular function (American Psychiatric 2009). The most common side effects of this class of medications are headaches, irritability, nausea, insomnia, sexual dysfunction, weight gain, increased anxiety, drowsiness, and tremor.

Table 1 Evidence for efficacy of serotonergic agents

Agent	Normal dosage range (mg/day)	$T_{1/2}$ (h)	FDA anxiety indications	PD[a]
SSRIs				
Fluoxetine	20–80	24–72	OCD, PD	A
Paroxetine	20–60	21	OCD, PD, GAD, SAD, PTSD	A
Sertraline	50–200	24	OCD, PD, SAD, PTSD	A
Fluvoxamine	50–300	15	OCD	A
Citalopram	20–60	35		A
Escitalopram	10–20	27–32	OCD, PD, GAD, SAD, PTSD	A
SNRI				
Venlafaxine	75–225	5–11	Extended release – PD, GAD, SAD	A
Duloxetine	40–60	12	GAD	C

SSRI Selective serotonin reuptake inhibitor; *SNRI* Serotonin norepinephrine reuptake inhibitor; *GAD* Generalized anxiety disorder; *PD* Panic disorder; *SAD* Social anxiety disorder; *OCD* Obsessive compulsive disorder; *PTST* Post traumatic stress disorder; *A* Established treatment (replicated, large scale RCTs); *B* Promising treatment approach (e.g., one or more RCTs of moderate size); *C* Limited evidence (open trials; case series literature); *D* Very limited evidence (only preclinical evidence, theoretical prediction, or anecdotal reports)

[a]Level of evidence supporting use in anxiety disorders

How these side effects are experienced is highly individual, with some being transient and some lasting the duration of treatment.

6.1 Efficacy of 5-HT and NE Reuptake Blockers

Paroxetine was the first SSRI to receive a US FDA indication specifically for PD. Both short-term and long-term (up to 12 months) efficacies have been demonstrated with paroxetine (McHugh et al. 2009). Sertraline has also exhibited both short- (Londborg et al. 1998; Pohl et al. 1998; Pollack et al. 1998) and long-term efficacies for panic, was effective in preventing relapse with chronic administration (Rapaport et al. 2001), and, over a 6-month period, was associated with reduction in the medical services utilization often seen in unstable panic patients (Roy-Byrne et al. 2001). Positive multicenter trial data have also been obtained with fluvoxamine (Asnis et al. 2001), citalopram (Leinonen et al. 2000a), and fluoxetine (Michelson et al. 2001a). Thus far, within the SSRIs class, agents appear to exhibit antipanic effects of similar magnitude (Perna et al. 2001). On the issue of between-class efficacy comparisons, findings from a recent meta-analytic study of clinical trials conducted in over 2,300 panic patients suggested that SSRIs treatment effects could even be more robust than those observed with imipramine or the benzodiazepine and alprazolam (Boyer 1995), although a more recent effect size analysis failed to find significant differences in efficacy between SSRIs and older-generation 5-HT/NE agents (Otto et al. 2001). The antipanic benefit of the serotonin/norepinephrine reuptake inhibitor (SNRI), venlafaxine, has been established (Leinonen et al. 2000b; Sheikh et al. 2000; Michelson et al. 2001b). Pharmacologic data indicate that venlafaxine may exhibit dose-dependent reuptake effects, with SRI effects occurring in lower doses and additional NRI effects becoming more apparent at higher dose levels (Asnis et al. 2001).

Of clinical importance are studies that have investigated the role of genetics in the efficacy (or lack thereof) of drug treatments. A large portion of SSRI-treated PD patients do not respond sufficiently. Recent research has shown that the presence of the long allele of the serotonin transporter (5-HTT) gene is associated with favorable response (Tiwari et al. 2009). To date, the most promising strategy in clinical practice appears to include testing of the functional CYP450 gene variants to avoid over or under dosing poor or rapid metabolizers. Pharmacogenetic studies are beginning to refine and individualize drug therapy for PD (Mancama and Kerwin 2003).

6.1.1 Tricyclic Antidepressants

There are multiple controlled clinical trials documenting the short-term efficacy of these agents in panic. The most tested agent in this regard is the tricyclic, imipramine (Jefferson 1997). Imipramine's therapeutic benefit appears to be maintained over the intermediate and longer-term, as evidenced by data from a study of over

1,000 panic patients (Cross-National and Group 1992) and a multi-site US trial (Barlow et al. 2000) in which, for panic patients with mild or no agoraphobia, CBT was at least as effective as imipramine, and both were superior to placebo, over a 9-month treatment interval. Imipramine has distinct advantages in the management of panic symptoms. It has been thoroughly scrutinized by clinical trial methodologies, has predictable clinical effects, and is inexpensive. Its main disadvantages are an appreciable side-effect burden and the potential for lethality when ingested in overdose. The degree of efficacy of imipramine in panic (approximately 60%–70% of patients are responders) has not been clearly exceeded by newer agents. Other tricyclic agents, most notably clomipramine and desipramine, appear to be similarly beneficial for PD (McHugh et al. 2009).

In conclusion, there is a wealth of clinical data documenting reliable treatment effects of both tricyclics and SSRIs for PD. These treatment effects appear to be maintained over the short- and longer-term.

6.2 Other Agents with 5-HT and NE Mechanisms

Older, but no less efficacious for PD, are the nonselective, irreversible MAOIs such as phenelzine. A number of clinical trials have confirmed their efficacy, and some studies have suggested that MAOIs may even have antiphobic properties in addition to their ability to suppress panic attacks (Liebowitz et al. 1990). The newer generation RIMAs, such as moclobemide and brofaromine, are also another viable treatment option for panic patients with several large-scale trials in the world literature, indicating their panicolytic effects (van Vliet et al. 1993; Tiller et al. 1999). The newer selective NE reuptake blocker, reboxetine, in one controlled study was efficacious and well-tolerated by PD patients (Versiani et al. 2002) and may be especially useful for those with resistant symptoms (Dannon et al. 2002). The antipanic benefit of the 5-HT_2 antagonist, nefazodone, and the selective α-2 and 5-$HT_{2/3}$ antagonist, mirtazapine, has not yet been established. However, there are now several positive open-label trials with these agents, suggesting that follow-up controlled trials could be worthwhile (Berigan et al. 1998; Bystritsky et al. 1999; Carpenter et al. 1999). These agents could be of particular benefit for the panic patient with multiple co-morbidities or those with appreciable sleep disturbance as a part of their clinical presentation.

6.2.1 Benzodiazepines

Benzodiazepeines are generally well-tolerated and highly effective medications for PD. Alprazolam has the largest number of studies supporting its efficacy (Curtis et al. 1993; Tesar et al. 1991; Dager et al. 1992; Schweizer et al. 1993), but the efficacy of clonezapam (Tesar et al. 1991; Moroz and Rosenbaum 1999; Valenca et al. 2003) and other benzodiazepines (e.g., diazepam, lorazepam) (Savoldi et al. 1990; Schweizer et al. 1992; Noyes et al. 1996) is also well-supported. Alprazolam

is efficacious, but because of its short half-life, multiple daily doses are required, which creates both clinical and practical difficulties. Clonezapam has a longer half-life, which requires fewer doses and results in less severe withdrawal symptoms and better compliance (Moroz and Rosenbaum 1999). Because of the reduced complexity of clonezapam, it is frequently the preferred medicine. The risks associated with benzodiazepines include sedation, fatigue, ataxia, slurred speech, memory impairment, and weakness (Schweizer et al. 1992). These side effects should be discussed with patients prior to treatment initiation.

6.3 Other Agents

The use of anticonvulsants in the treatment of PD has limited support. Only one randomized controlled trial of gabepentin (Pande et al. 2000) has provided support for the safety and efficacy of its use in PD. Other open-label trials have suggested utility for valproic acid in PD (Woodman and Noyes 1994) and only case-report level information is available for levetiracetam, tiagabine, and vigabatrin. Further research is needed before any of these treatments can be recommended. The use of first-generation antipsychotics in PD is not recommended. There is no evidence that they are effective and potential side effects outweigh any potential benefit (Canadian Psychiatric Association 2006). There is limited support for the use of olanzapine (Hollifield et al. 2005) and risperidone (Simon et al. 2006) with severe, treatment-resistant PD, but this must be balanced with growing concern about potential side effects (e.g., metabolic syndrome) with these agents. The use of beta-andrenergic blocking agents such as propanolol and atenolol in PD is not supported by the literature (Munjack et al. 1989; Ravaris et al. 1991), but they are occasionally used to help reduce somatic sensations by some clinicians. There is limited evidence for the potential efficacy of pindolol when used as an augmentation for patients with SSRI-resistant PD (Hirschmann et al. 2000), but given the side effect profile of these agents, they are not generally recommended. Buspirone has been investigated and found ineffective as monotherapy for PD (Sheehan et al. 1993) or as an augmentation to enhance the efficacy of CBT (Cottraux et al. 1995).

7 Novel Treatment Approaches

7.1 Anxiolytic Drug Development

Research in this area, while continuing to explore the role of 5-HT receptor subtypes (e.g., 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3) in anxiolysis, has targeted neurotransmitters and modulators beyond the monoamine systems. Neuropeptides implicated in stress and anxiety, such as CCK and CRF, have been the focus of some of these efforts. However, selective CCK_B receptor antagonists, while

initially promising in preclinical models of anxiety, have been disappointing clinical anxiolytics, partly due to their poor bioavailability (Goddard et al. 1998). A recently discovered class of hypothalamic peptide hormones, the orexins (ORX), has been implicated in anxiogenesis in some animal fear models. Preclinical studies (Johnson et al. in press) suggest that ORX neurons are more active during lactate-induced panic in panic-prone rats (with GABA deficits in the dorsomedial hypothalamus), and that ORX 1 antagonists can block lactate panic in these animals. Thus, orexin antagonists appear to be a promising class of anxiolytics, with particular relevance for the treatment of PD. In addition, Neuropeptide S plays an important role in sleep–wake cycles and also seems to have anxiolytic effects as well (Reinscheid 2008), but the mechanism is not known and much more work is needed to elucidate the details of this curious neurotransmitter.

With respect to the GABA system, there has been an extensive drug development effort focusing on benzodiazepine receptor partial agonists such as bretazenil and abecarnil (Pollack et al. 1997). The development of compounds that act similarly to endogenous anxiolytic neurosteroids (e.g., allopregnenolone) is another approach to GABA neuronal modulation that could provide future clinical benefits. Outcomes of clinical trials using GABAergic agents have been equivocal. Recent research has turned to better understanding the role of GABA and found effects on neuroactive steroid concentrations possibly related to changes in corticosterone concentrations (Zwanzger et al. 2009). GABA enhancement with the gabapentin-like agent, pregabalin, which appears to be more bioavailable than its predecessor, may prove useful across anxiety disorders (Rupprecht et al. 2009). More recently, there have been exciting drug development initiatives underway with compounds that downmodulate glutamatergic function. One group of such compounds, the metabotropic glutamate receptor agonists, seems particularly promising as anxiolytic. An example of this class is the selective mGlu2/3 receptor agonist (LY345740), which suppresses fear-potentiated startle and lactate-induced panic (Selak 2001) in animals. Recent testing in clinical populations (panic patients) has revealed that it has the capacity to attenuate CO_2-induced panic symptoms with chronic administration and thus could have clinical utility (Shekhar and Keim 2000). This drug inhibits release of endogenous glutamate via a presynaptic mechanism only when excessive glutamate release occurs, and thus has little impact on basal glutamatergic function. Newer generation compounds based on this prototype, with improved bioavailability and CNS penetration, are ready for clinical testing.

7.2 New Treatment Strategies

Augmentation strategies could be useful in terms of hastening clinical response to standard medications such as the SSRIs. For example, early co-administration of the benzodiazepine clonazepam with SSRIs (Goddard et al. 2001) can facilitate rapid stabilization of acute PD and may be a useful approach for other anxiety disorders. Coadministration of the 5-HT_{1A} antagonist, pindolol, has hastened the

onset of antidepressant effects of the SSRIs in some studies of depressed patients. This strategy, although not successful in one study of social anxiety (Schmitt and Hiemke 1999), bears exploration in other anxiety disorders. There is now preliminary evidence that the β-blocker propranolol could be a useful secondary preventive strategy for civilian trauma (Stein et al. 2001). Atypical neuroleptic agents may be useful adjunctive agents for the more severe anxiety disorders, such as chronic PTSD (Pitman et al. 2002; Stein et al. 2002). Alternatively, other treatment approaches are focusing on the neural mechanisms that may switch off or extinguish fear responses. For instance, in a recent animal study, acute administration of the anti-TB agent D-cycloserine, a partial NMDA/glycine site agonist, facilitated extinction of conditioned fear (Walker et al. 2002). It is conceivable that targeted use of this agent in combination with cognitive–behavior therapy will improve extinction of a variety of phobic disorders (Otto et al. in press).

8 Conclusions

The last three decades have seen substantial progress in the diagnosis and treatment of anxiety disorders. However, the production of an ideal anxiolytic with a rapid onset of therapeutic action minus problems of physiological dependence has remained a somewhat elusive goal. While benzodiazepines and tricyclics remain useful therapeutic agents for a number of anxiety disorders, they have been supplanted as first-line treatments by the newer 5-HT/NE anxiolytics such as the SSRIs, and the SNRI venlafaxine, which offer improved safety, a more favorable side-effect profile, and a broader spectrum of therapeutic action. PD is very responsive to a range of anxiolytic agents, including SSRIs, tricyclics, benzodiazepines, and MAOIs, and some GABAergic anticonvulsants. To date, much has been learned about short-term efficacy of the anxiolytics mentioned earlier. However, given the chronic course of many anxiety disorders, future research needs to answer many questions concerning longer-term aspects of treatment. Knowledge concerning the pathophysiology of PD and treatment mechanisms has developed considerably over the last two decades. There is renewed interest in the neural circuitry that subserves adaptive fear responses and the ways in which disturbances in this circuitry could contribute to the pathophysiology of PD (Krystal et al. 2001). Not surprisingly, drug development research is focusing on novel anxiolytics that target dysfunction at different levels of the fear circuit.

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Pharmacotherapy of Social Anxiety Disorder

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Contents

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Abstract A range of medications have been shown effective for the treatment of social anxiety disorder. The largest trials to date have been with various selective serotonin reuptake inhibitors (SSRIs). Several of these agents have been registered for the treatment of social anxiety disorder with agencies such as the FDA or EMEA, meta-analyses confirm their efficacy and safety, and expert consensus guidelines have often recommended them as a first-line pharmacotherapy of choice. Despite such advances, there are many unanswered questions in the pharmacotherapy of social anxiety disorder, including the optimal pharmacotherapy of patients refractory to first-line intervention, and the optimal sequencing of pharmacotherapy and psychotherapy. Translational research has already had an impact on concepts of treatment, and may ultimately lead to novel interventions.

Keywords Social anxiety disorder · Controlled trials · Liebowitz Social Anxiety Scale · Medication · Meta-analysis

1 Introduction

In 1985, a review of social anxiety disorder characterized it as a neglected disorder (Liebowitz et al. 1985). In the past two decades, however, a range of different medications have been studied in the treatment of this condition. These include monoamine oxidase inhibitors (MAOIs), benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), serotonin, and noradrenaline reuptake inhibitors (SNRIs), anticonvulsants, and atypical antipsychotics. In this chapter we review this body of work.

2 Irreversible Inhibitors of Monoamine Oxidase

2.1 Phenelzine

The pharmacotherapy of social anxiety disorder was given impetus with the clinical observation that the irreversible MAOI, phenelzine, was effective not only for depression, but also for social anxiety disorder (Kelly 1973). The efficacy of phenelzine in the treatment of social anxiety disorder was subsequently established in a number of placebo-controlled trials and comparative studies (Gelernter et al. 1991; Liebowitz et al. 1992; Tyrer et al. 2003; Versiani et al. 1992). The finding that the MAOIs were more effective than the tricyclic imipramine and the beta-blocker

propanolol in social anxiety disorder suggested that pharmacotherapy could contribute to dissecting the underlying neurobiology of this disorder.

A 12-week controlled study compared cognitive behavioral group therapy (CBGT) to phenelzine. The rates of response were similar at week 12, with the phenelzine group showing better outcome on certain dimensional measures (Gelernter et al. 1991). Responders were then given 6 months of maintenance treatment, and followed up 6 months later. At this point, 50% of the phenelzine group was found to have relapsed compared to 17% of the CBGT group (Liebowitz et al. 1999).

From a clinical perspective there are, however, a number of important drawbacks to the use of MAOIs. MAOI use requires patients to monitor dietary tyramine intake strictly. Numerous drugs interact with MAOIs to increase monoamines to potentially dangerous levels. Nevertheless, MAOIs continue to be regarded by some as a "gold standard" in the treatment of social anxiety disorder, and with careful monitoring they remain an effective treatment option for patients who do not respond to other first-line agents (Aarre 2003).

3 Reversible Inhibitors of Monoamine Oxidase Type A

The reversible inhibitors of monoamine oxidase A (RIMA) are selective for the A isoenzyme. The pressor responsiveness to tyramine with these drugs is less than with the irreversible inhibitors, and normalizes after cessation within about 3 days for moclobemide and 8 days for brofaromine. Thus, no dietary restrictions are required at conventional doses (Bieck and Antonin 1989).

3.1 Moclobemide

A number of randomized, controlled trials of moclobemide have been undertaken, with somewhat mixed results. For example, an early study of moclobemide, phenelzine, and placebo (Versiani et al. 1992) found that both active treatments were superior to placebo, while moclobemide was better tolerated. On the other hand, findings from subsequent fixed dose studies have been less persuasive, with only some signals of efficacy at higher doses of medication (Katschnig 1997; Noyes et al. 1997).

A 12-week, placebo-controlled study of moclobemide in patients with social anxiety disorder, which also included patients with comorbid anxiety disorders, found that the moclobemide group had a greater response at study end point (Stein et al. 2002a) than the placebo group. Response was determined using the Clinical Global Impression Scale of Improvement (CGI-I). The response in the moclobemide group was maintained for a further 6-month extension phase, and was generally well tolerated.

3.2 *Brofaromine*

Lott et al. conducted a 12-week, placebo-controlled, flexible dose study of brofaromine in social anxiety disorder (Lott et al. 1997). There was a statistically significant difference in the Liebowitz Social Anxiety Scale (LSAS) scores, in favor of brofaromine, with a greater percentage of responders at end point in the brofaromine group. Brofaromine was generally well tolerated.

Similarly, a 12-week, placebo-controlled, fixed dose study of brofaromine showed a significantly better therapeutic improvement compared to placebo on mean LSAS end point total score (Fahlen et al. 1995). Unfortunately, a commercial decision was made not to continue with the development of this agent.

4 Benzodiazepines

A 10-week, placebo-controlled study of clonazepam showed a significantly superior response rate of 78.3% in the clonazepam group, compared to 20% in the placebo group. The clonazepam group had a 31% lower mean LSAS endpoint score than the placebo group. Side effects of dizziness and unsteadiness were, however, more severe and persistent in the clonazepam treated group (Davidson et al. 1993).

Alprazolam and bromazepam are other benzodiazepines that have been investigated in social anxiety disorder, with data from controlled studies showing a significantly better therapeutic response than placebo (Gelernter et al. 1991; Versiani et al. 1997).

The combined use of clonazepam and paroxetine was investigated in placebo-controlled study (Seedat and Stein 2004). At the end of the 10-week study period, 79% in the group receiving the paroxetine/clonazepam combination had achieved responder status, compared to 43% in the placebo/paroxetine combination group. There was a trend toward significance in favor of the clonazepam/paroxetine combination group.

Clinical guidelines have typically cautioned against the use of benzodiazepines as first-line agents for social anxiety disorder because of their adverse event profile, including difficulties in withdrawal. Nevertheless, given the positive data on these agents, they can potentially be considered in the management of partial responders or nonresponders.

5 Selective Serotonin Reuptake Inhibitors

A number of clinical guidelines have argued that the SSRIs should be considered a first-line medication choice in social anxiety disorder (Baldwin et al. 2005; Ballenger et al. 1998; Bandelow et al. 2008; Swinson et al. 2006) This is a

recommendation based on meta-analyses and literature reviews of randomized controlled trials of these agents. These trials indicate that certain SSRIs are effective in the short and long term treatment of social anxiety disorder, and are well tolerated (Blanco et al. 2003; Ipser et al. 2008).

5.1 *Paroxetine*

A number of short term studies have demonstrated the efficacy of paroxetine in social anxiety disorder. A 12-week, multicenter, study comparing the efficacy of paroxetine to placebo showed that patients receiving paroxetine had a significantly greater improvement on both the LSAS and the CGI-I rating scales (Liebowitz et al. 2002). A similar 12-week study, using a flexible dose design, with the controlled release (CR) formulation of paroxetine, also demonstrated a significantly greater improvement on mean LSAS and CGI-I responder scores in patients receiving paroxetine compared to placebo (Lepola et al. 2004).

The efficacy of paroxetine in relapse prevention was investigated in a multicenter 36-week study comprising of a 12-week single blind placebo-controlled acute treatment phase, followed by a 24-week randomized, double-blind maintenance treatment phase in responders. Significantly fewer patients relapsed in the paroxetine treated group (14%), compared to the placebo treated group (39%) (Stein et al. 2002c). This finding suggests that treatment should be maintained for at least 6 months, and probably longer after an initial response is seen.

An analysis of three placebo-controlled paroxetine trials studied the role of various variables in predicting response to treatment. These included demographic, physiological, clinical, and other trial relevant data such as dose and treatment duration. Results showed that only the duration of treatment was a significant predictor of response. In addition, many patients who were non-responders at week 8, became responders by week 12 (Stein et al. 2002b). This finding suggests a conservative response to determining the duration of an initial trial of medication.

A placebo-controlled trial of paroxetine in children and adolescents showed that the treatment group had a significantly greater response (77.6%) compared to the placebo group (38.3%). Although withdrawals due to adverse events were slightly higher in the paroxetine group (5.5%) compared to placebo (1.3%), paroxetine was generally well tolerated (Wagner et al. 2004).

5.2 *Fluvoxamine*

Fluvoxamine demonstrated efficacy in the treatment of social anxiety disorder in a small placebo-controlled study (Van Vliet et al. 1994). Patients receiving

fluvoxamine showed significantly better scores on the LSAS rating scale than those receiving placebo.

A larger multicenter study of similar design confirmed this finding (Stein et al. 1999). Fluvoxamine demonstrated greater efficacy with a response rate of 42.95% compared to the placebo response rate of 22.7%.

A 10-week placebo-controlled trial was conducted in Japan on patients with social anxiety disorder (Asakura et al. 2007). The primary outcome measure was the mean change in total score of the Japanese version of the Liebowitz social anxiety scale (LSAS-J). The treatment group had a significantly greater response compared to placebo at week 10, and was safe and well tolerated.

Efficacy of the CR formulation of fluvoxamine was demonstrated in a 12-week placebo-controlled study. At end point, the treatment group had a 37% reduction in the total score of the primary outcome measure – the LSAS – compared to a 28% reduction in the placebo group (Westenberg et al. 2004).

The efficacy of long-term treatment with fluvoxamine CR was supported by a 24-week, long-term, extension phase study (Stein et al. 2003). This multicenter, placebo-controlled study included patients with social anxiety disorder who had previously shown at least a minimal improvement in symptoms with fluvoxamine CR at the end of the initial 12-week treatment. The difference in scores on the primary outcome measure – the LSAS – tended toward significance in favor of the fluvoxamine group. Analysis of data from week 12 to week 24 demonstrated similar trends in score changes, but with lower magnitude. Fluvoxamine CR was found to be safe and tolerable

An 8-week study conducted by the Research Unit on Pediatric Psychopharmacology Anxiety Study Group investigated the efficacy of fluvoxamine in a placebo-controlled trial in children with social anxiety disorder (Walkup et al. 2001). Children met DSM-IV diagnostic criteria for social anxiety disorder, separation anxiety disorder, or generalized anxiety disorder and underwent psychotherapy for 3 weeks. Subjects who did not improve were randomly assigned to receive either fluvoxamine or placebo, with both groups receiving ongoing supportive psychotherapy. Analysis of the primary outcome measure, the Pediatric Anxiety Rating Scale, showed that the fluvoxamine group had a significant decrease in mean total score compared to the placebo group. The fluvoxamine group also had a significantly greater response on the CGI-I, and fluvoxamine was generally well tolerated.

5.3 Sertraline

Liebowitz et al. conducted a 12-week, placebo-controlled study to investigate the efficacy of sertraline in social anxiety disorder (Liebowitz et al. 2003). At end point, the treatment group showed a significantly greater improvement in symptoms and responder status, compared to the placebo group. Sertraline was also generally well tolerated.

The efficacy of sertraline in relapse prevention was investigated in patients with social anxiety disorder who had initially responded to 20 weeks of treatment with sertraline (Walker et al. 2000). Response to treatment at the end of 20 weeks was determined by the CGI-I. Patients were then randomly assigned to continue with sertraline or switch to placebo for a further 24 weeks. Intent to treat end point analysis showed a relapse rate of 4% in the sertraline continuation group compared to 36% in the placebo switch group.

Compton et al. conducted an 8-week open label trial of sertraline on children with social anxiety disorder (Compton et al. 2001). Although it was a small sample size, only 36% of participants showed a response on the CG-I.

A recent study using cognitive behavior therapy (CBT), sertraline, and a combination of both, was conducted in children and adolescents. Results showed that 80.7% of participants in the combination group were rated as "much" or "very much" improved on the CGI-I scale, compared to 59.7% in the CBT only group, and 54.9% in the sertraline only group (Walkup et al. 2008)

5.4 Fluoxetine

The first placebo-controlled study on the efficacy of fluoxetine in the treatment of social anxiety disorder was published in 2002 (Kobak et al. 2002). Patients with social anxiety disorder received a fixed dose of fluoxetine or placebo for 8 weeks. Both groups had a significant improvement in symptoms from baseline to endpoint, but there was no significant separation between the two groups at end point.

Davidson et al. conducted a 14-week controlled study to compare the efficacy of fluoxetine, comprehensive CBT, placebo, and combinations thereof (Davidson et al. 2004). Patients were randomized into groups that received fluoxetine only, comprehensive cognitive behavioral therapy only, placebo only, a combination of fluoxetine and comprehensive cognitive behavioral therapy, or a combination of placebo and comprehensive cognitive behavioral therapy. The comprehensive cognitive behavioral therapy was conducted in groups of five to six patients, weekly, over the 14 weeks. Efficacy was measured by a change in score of the Brief Social Phobia Scale (BSPS) and the CGI-I responder status from baseline to endpoint. Results of both measures showed that all active treatments were significantly better than with placebo alone. However, the study showed no difference in response between the active treatment groups.

5.5 Escitalopram

A 12-week placebo-controlled study was conducted by Kasper et al. to investigate the efficacy of escitalopram in the treatment of social anxiety disorder (Kasper et al. 2005). It was a large parallel group study involving 41 centers in eight countries.

The treatment group had significantly greater improvement in therapeutic response compared to the placebo group.

A placebo-controlled, fixed-dose study with paroxetine as an active reference showed escitalopram to be significantly more effective than placebo in both the short and long-term treatment of social anxiety disorder (Lader et al. 2004).

The efficacy of escitalopram was also investigated in a 12-week open label trial in children and adolescents with social anxiety disorder (Isolan et al. 2007). The results showed that 65% of patients had responded based on the CGI-I at week 12, and the drug was generally well tolerated.

6 Serotonin and Noradrenalin Reuptake Inhibitors

6.1 Venlafaxine

Liebowitz et al. investigated the efficacy of venlafaxine in social anxiety disorder in a 12-week, placebo-controlled, flexible dose study (Liebowitz et al. 2005b). The primary outcome measure – the LSAS – showed that the venlafaxine group had significantly lower scores from week 6 through to week 12.

Remission rates, defined as a CGI-I score of 1, were significantly greater in the venlafaxine group at week 12 compared to the placebo group. Although the discontinuation rate of 17% was significantly higher among patients receiving venlafaxine than those receiving placebo, this rate was comparable to that seen in trials with the SSRIs.

Allgulander et al. compared venlafaxine extended release formulation (ER), paroxetine and placebo, in a 12-week, flexible-dose study (Allgulander et al. 2004). The primary measure of efficacy was the change in mean total score on the LSAS. Both the venlafaxine and paroxetine groups were more efficacious than placebo. There were no significant differences between the active treatment groups. Response rates at week 12, determined by the CGI-I, were 69% in the venlafaxine group, 66% in the paroxetine group, and 36% in the placebo group. Both active treatments were generally well tolerated.

Liebowitz et al. conducted a similar study on a larger population with social anxiety disorder, (Liebowitz et al. 2005a), in a 12-week, placebo-controlled study with flexible doses of venlafaxine ER, paroxetine, and placebo. Using the LSAS as the primary outcome measure, the comparison from baseline to endpoint showed a significant reduction in mean total scores in the venlafaxine and paroxetine groups compared to the placebo group. Significant reductions in scores were seen in the venlafaxine group from week 1 to week 12, and in the paroxetine group from week 3 to week 12. There were no significant differences between the venlafaxine and paroxetine groups, and venlafaxine was generally well tolerated.

A 6-month placebo-controlled trial investigated the efficacy and differences of low and high fixed doses of venlafaxine ER (Stein et al. 2005). A significant improvement was shown in the venlafaxine treatment groups compared to the placebo group, regardless of dose, as determined by the change in mean total LSAS score from baseline to endpoint. Response rates at end point, determined by the CGI-I, were 33% in the placebo group and 58% in the venlafaxine combined and individual groups, with no significant difference between dosages. Remission rates at endpoint, as defined by an LSAS score of $\leq$30 were 16% in the placebo group and 31% in the venlafaxine combined and individual groups with no significant difference between dosages.

7 Other Antidepressants

7.1 Nefazodone

The efficacy of nefazodone was investigated in patients with social anxiety disorder in a 14-week placebo-controlled trial (Van Ameringen et al. 2007). Of the patients receiving nefazodone, 31.4% showed improvement on the primary outcome measures – the LSAS and CG-I – compared to 23.5% of patients receiving placebo. There was no significant difference between the groups.

7.2 Mirtazapine

Muehlbacher et al. conducted a 10-week placebo-controlled study on 66 adult female patients with social anxiety disorder (Muehlbacher et al. 2005). The treatment group had a significantly greater change in mean total LSAS score at endpoint compared to the placebo group. Further studies on mirtazapine are, however, required with more representative groups. Mirtazapine was generally well tolerated but did have more significant side effects such as dry mouth, sedation, drowsiness, and weight gain than the placebo group.

8 Beta Blockers

The cardioselective beta adrenergic blocker, atenolol, did not show significant efficacy over placebo in a controlled study in social anxiety disorder, whereas phenelzine was significantly superior to both placebo and atenolol (Liebowitz et al. 1990, 1992).

There is also surprising little evidence to support the use of beta adrenergic blockers on an as-needed basis in people affected by performance type social anxiety such as public speaking (Hartley et al. 1983; James et al. 1983). Medication taken an hour prior to a performance may, however, be helpful, especially when there are prominent cardiovascular symptoms.

9 Anticonvulsants

9.1 Gabapentin

Gabapentin is a gamma-aminobutyric acid (GABA) analog (Fink et al. 2002). A 14-week, placebo-controlled, flexible dose study of gabapentin was conducted in patients with social anxiety disorder (Pande et al. 1999). The treatment group had significantly greater improvement in end point LSAS score than the placebo group. Patients over 35 years showed significantly better treatment effect to gabapentin compared to younger patients. Gabapentin was generally well tolerated with dizziness and dry mouth being notably more common than in the placebo group.

9.2 Pregabalin

Pregabalin is a novel GABA analog. Binding of pregabalin to voltage gated calcium channels reduce calcium influx into nerve terminals which possibly mediates its analgesic, anxiolytic, and anticonvulsant properties (Fink et al. 2002).

Pande et al. conducted a 10-week, placebo-controlled trial of pregabalin in social anxiety disorder. The high-dose treatment group (600 mg per day) had significantly greater decrease in total LSAS score compared to placebo at end point (Pande et al. 2004). Pregabalin was generally well tolerated with reported side effects including sexual dysfunction, somnolence, and dizziness.

9.3 Levetiracetam

The novel anticonvulsant levetiracetam demonstrated efficacy over placebo in a small pilot study. Based on CGI-I scores, after 7 weeks of treatment, 22% of the participants receiving levetiracetam were responders compared to 14% in the placebo group (Zhang et al. 2005).

10 Atypical Antipsychotics

10.1 Olanzapine

Olanzapine showed significant efficacy over placebo on the BSPS and the Social Phobia Inventory (SPIN) in an 8-week controlled study. There was, however, no difference between the groups in the end point CGI-I responder status (Barnett et al. 2002).

10.2 Quetiapine

Quetiapine did not demonstrate significant efficacy over placebo in an 8-week controlled study (Vaishnavi et al. 2007).

11 Other Agents

11.1 Buspirone

Although participants demonstrated promising improvement of symptoms in open label treatment (Schneier et al. 1993), statistical efficacy was not demonstrated in two placebo-controlled studies (Clark and Agras 1991; Van Vliet et al. 1997). Buspirone may, however, have a role in augmenting SSRIs in treatment resistant patients (Van Ameringen et al. 1996).

11.2 D-Cycloserine

On the basis of animal research indicating that partial NMDA agonists facilitated fear extinction, a clinical trial of combined exposure therapy and D-cycloserine administration (Davis et al. 2006; Hofmann et al. 2006) was undertaken. D-cycloserine augmentation was significantly more effective than placebo.

In a second placebo-controlled trial, patients receiving combined D-cycloserine and exposure therapy had greater improvement on certain outcome measures than patients receiving exposure therapy and placebo. Areas of improvement were symptom severity, dysfunctional cognition, and life impairment (Guastella et al. 2008). Although this strategy is yet to be recommended in clinical practice, these are important proof-of-principal trials.

12 Treatment Resistance and Augmentation

There are relatively few placebo-controlled studies on patients with treatment resistant social anxiety disorder.

A 12-week monotherapy open label trial of escitalopram was conducted on patients who had failed a previous adequate trial with paroxetine (Pallanti and Quercioli 2006). An adequate trial with paroxetine was defined as patients having received 60 mg per day for more than 12 weeks. At the end of the 12-week trial, 48.3% of patients receiving escitalopram achieved responder status on the CGI-I, and a reduction in LSAS score of greater than 35%, from baseline to endpoint. Thus, in patients who fail to respond to one SSRI, switching to another may be useful.

Open label augmentation trials of SSRIs with buspirone and risperidone in treatment resistant patients have also shown good safety, tolerability, and response, but have not always been replicated in controlled designs (Simon et al. 2006; Van Ameringen et al. 1996).

The only randomized, placebo-controlled, augmentation trial in treatment resistant social anxiety disorder to date investigated the addition of pindolol to paroxetine treatment (Stein et al. 2001). Results showed that pindolol was no more effective than placebo in augmenting the effects of the SSRI.

13 Discussion

Meta-analyses have shown that various medications are notably better than placebo in treating social anxiety disorder. Although effect sizes differ, no significant difference in efficacy has been demonstrated between these agents (Blanco et al. 2003). A recent meta-analysis showed that 55.2% of trial participants responded to medication as determined by the CGI-I, and also confirmed the benefit of longer-term medication use in treatment responders (Ipser et al. 2008). Large relapse prevention trials conducted on escitalopram, paroxetine, and sertraline found that relapse rates were 4–14% with continued pharmacotherapy, and 36–39% with placebo (Montgomery et al. 2005; Stein et al. 2002c; Stein and Stein 2008; Walker et al. 2000).

Clinical guidelines have argued that SSRIs are the first-line medication choice in social anxiety disorder, with RIMAs, SNRIs, MAOIs, and benzodiazepines all reasonable second-line agents (Baldwin et al. 2005; Ballenger et al., 1998; Bandelow et al. 2008; Swinson et al. 2006). Expert consensus has suggested that effective treatment be continued for at least 12 months or longer if the patient has a comorbid condition, a history of relapse, or an early onset of the disorder (Ballenger et al., 1998).

The few studies using a fixed dose design suggest that the dose response curve in social anxiety disorder is relatively flat (Liebowitz et al. 2002; Stein et al. 2005). It is possible that some patients may do better on a dose which is higher than the

"average" therapeutic dose, provided that it is well tolerated (Stein and Stein 2008). However, it has also been observed that more that 25% of patients who did not respond to an 8-week trial of treatment, went on to respond by week 12. Thus, before raising dosage, it may be useful to simply extend the duration of treatment (Stein et al. 2002b).

Controlled studies have generally shown CBT to be more effective than placebo in relieving symptoms of social anxiety disorder (Davidson et al. 2004; Gelernter et al. 1991; Walkup et al. 2008). Results from studies that compared medication to CBT have been equivocal, with few studies showing marked differences in short term outcomes between the treatments (Rodebaugh et al. 2004). CBT may be associated with improved long term outcomes after treatment discontinuation (Gelernter et al. 1991).

SSRIs and CBT have been compared, with neither showing consistent significant efficacy over the other (Davidson et al. 2004; Segool and Carlson 2008). To date, there has been little evidence to support the use of combined CBT and medication (Foa et al. 2002; Prasko et al. 2006) other than D-cycloserine.

Although much has been learned about the pharmacotherapy of social anxiety disorder, many areas require further research. These include studies of special populations (e.g., children and adolescents, patients with comorbid substance use), augmentation trials in treatment resistant patients, studies on the optimal sequencing of medication and psychotherapy, and longer term effectiveness studies in real world settings. Much research on the pharmacotherapy of social anxiety disorder has been driven by serendipitous clinical findings. However, there is a growing literature on the neurobiology of social anxiety disorder, and on the specific effects of treatment on underlying neurocircuitry (Furmark et al. 2002; Warwick et al. 2006). Increasing data are also available on how particular genetic mechanisms may impact not only on neurocircuitry, but also on treatment outcome in social anxiety disorder (Furmark et al. 2004; Stein et al. 2006). Knowledge of the basic neurobiology of anxiety, and increased translation of such findings in clinical settings, will hopefully lead to ongoing therapeutic advances.

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Pharmacotherapy of Post-Traumatic Stress Disorder

Lakshmi N. Ravindran and Murray B. Stein

Contents

Abstract Post-traumatic stress disorder (PTSD) is a prevalent psychiatric disorder that may result in significant social and occupational debilitation unless symptoms are recognized and treated appropriately. Considerable research effort has been devoted over the last 20 years to developing effective pharmacological treatments for this illness. At this time, the bulk of the agents investigated include antidepressants, anticonvulsants, atypical antipsychotics, benzodiazepines, and antiadrenergic agents. Herein, we review the existing evidence base for these different classes of psychotropics in PTSD. Emphasis is placed on discussion of

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evidence stemming from randomized placebo-controlled clinical trials wherever possible. A brief description of novel agents that have shown initial promise for PTSD treatment is also provided.

Keywords Pharmacotherapy · Post-traumatic stress disorder · PTSD · Treatment · Biological treatment · Medication

List of Abbreviations

BZD	Benzodiazepine
CAPS	Clinician Administered PTSD Scale
DCS	D-Cycloserine
DSM-III	Diagnostic and Statistical Manual, Third Edition
FDA	United States Food and Drug Administration
GABA	Gamma Aminobutyric Acid
GC	Glucocorticoid
HAM-A	Hamilton Rating Scale for Anxiety
HAM-D	Hamilton Rating Scale for Depression
MAOI	Monoamine oxidase inhibitor (irreversible)
PTSD	Post-traumatic stress disorder
RIMA	Reversible inhibitor of monoamine oxidase type A
RCT	Randomized clinical trial
SSRI	Selective serotonin reuptake inhibitor
SNRI	Serotonin norepinephrine reuptake inhibitor
TCA	Tricyclic antidepressant
VA	Veterans administration

1 Introduction

Descriptions of the adverse psychological consequences in soldiers following battle exposure can be found as far back as ancient Egypt and Greece. Not limited to military populations, accounts of similar symptoms in civilians can be found throughout history in reaction to natural disasters and other traumatic events. However, official recognition by the medical community of the collection of symptoms now known as post-traumatic stress disorder (PTSD) only occurred in 1980 with inclusion of the diagnosis in DSM-III. The current understanding of PTSD consists of direct or indirect exposure to a traumatic event resulting in an intense aversive reaction that is subsequently accompanied by social and

occupational dysfunction, and by a number of symptoms that can be characterized as belonging to one of three symptoms clusters: re-experiencing symptoms, avoidance/numbing behavior, or hyperarousal symptoms. Lifetime exposure to a traumatic event is not uncommon but only a relatively small proportion of those exposed will end up developing PTSD. In the US general population, lifetime prevalence of PTSD has been estimated at 6.8% (Kessler et al. 2005), but rates may be significantly higher in particular populations such as military veterans (Milliken et al. 2007), individuals with a prior history of trauma, or those with comorbid psychiatric disorders (Copeland et al. 2007). Rates may also be particularly elevated in inhabitants of areas where war and trauma exposure are common (de Jong et al. 2001).

PTSD is clearly not a rare or unimportant illness. Yet in spite of the research attention focused on finding effective treatments for it, the quest is not complete. For many years the mainstay of treatment was rest and use of sedative hypnotics – likely for treatment of hyperarousal symptoms, followed by psychotherapeutic intervention (Sargant and Slater 1940) – where even then it was recognized that early intervention was crucial. With the advent of antidepressant medications, and other more selective and better tolerated psychotropics, pharmacological approaches to PTSD treatment have been increasingly common. In this chapter, we review the evidence from randomized clinical trials (RCTs) of available psychotropic agents investigated for PTSD. Psychotherapeutic interventions in PTSD also have a considerable literature base, but discussion of these is beyond the scope of this chapter. Discussion of pharmacotherapy strategies when combined with psychotherapy are discussed elsewhere in this volume.

2 Antidepressants

2.1 Selective Serotonin Reuptake Inhibitors (SSRIs)

Over the last 20 years, SSRIs have become the treatment of choice for both depressive and anxiety disorders, in large part due to the combination of their efficacy, tolerability, and safety profiles. This holds true for PTSD treatment as well where SSRIs are the first-line agents of choice in multiple treatment guidelines (Foa et al. 2000; Ursano et al. 2004; Bandelow et al. 2008). These drugs, which as a class work to enhance serotonergic neurotransmission by preventing reuptake at the presynaptic 5-HT transporter pump (Stahl 1998), are all commonly used in the clinical treatment of PTSD, although only two, sertraline and paroxetine, have official FDA indications for this purpose.

Fluoxetine was the first of the SSRIs to be investigated for efficacy in PTSD. A sample of subjects ($N = 64$) with either combat-related or civilian-related PTSD was randomized to receive treatment with fluoxetine (up to 60 mg daily) or placebo for 5 weeks (Van Der Kolk et al. 1994). Significant reduction in overall PTSD

symptoms, as measured by the Clinician Administered PTSD Scale (CAPS) was noted in this double-blind trial, with particular improvements in symptoms of numbing/avoidance and hyperarousal. The authors also made particular note of the fact that the degree of symptomatic improvement was perceptibly greater in the subpopulation with civilian-related PTSD in this sample relative to the subpopulation with combat-related PTSD (40% vs. 15%).

Similar positive RCTs of acute treatment (<12 weeks) with fluoxetine for PTSD were subsequently published (Connor et al. 1999, Martenyi et al. 2002a) and support for the utility of fluoxetine as a relapse prevention agent has also been found. Martenyi et al. (2002b) rerandomized responders of an earlier 12-week placebo controlled trial of fluoxetine to either double-blind continuation treatment or placebo for a further 24 weeks. Continuation treatment with active medication was associated with a significantly longer time to relapse as well as additional improvement in not only PTSD but also in symptoms of comorbid disorders. Conversely, not all PTSD studies of fluoxetine report positive results. In a small sample ($N = 12$) of male veterans, Hertzberg et al. (2000) failed to detect benefit for fluoxetine following the 12-week trial, which they theorized could be related to factors including nature (civilian-related vs. combat-related), severity, or chronicity of trauma. A more recent three-arm study (Martenyi et al. 2007) compared treatment response after 12 weeks to two fixed doses of fluoxetine (20 and 40 mg) to placebo in a large sample ($N = 411$) of predominantly female subjects ($N = 294$) with PTSD. Treatment outcomes were not noticeably different between the three treatments. The authors speculated that the large proportion of females may have been a factor in these results or that, more likely, the doses of fluoxetine investigated in this study were lower than the mean doses used in similar positive trials.

The first SSRI to receive regulatory approval in the US was sertraline following the publication of two multicenter trials (Brady et al. 2000; Davidson et al. 2001a). Both trials were RCTs comparing placebo to flexibly dosed sertraline (50–200 mg daily) over a 12-week period in subjects with moderate to severe PTSD. In the first trial (Brady et al. 2000), active medication resulted in global improvement of post-traumatic symptoms with significant benefits noted for avoidance/numbing symptoms and hyperarousal. This study was also able to demonstrate that benefits of sertraline treatment were not limited to clinical symptoms, but also extended to improvements in quality of life and both social and occupational functioning. Finally, observing that the mean duration of illness in the study population was over 10 years, the authors felt that the clinical improvement with sertraline detected as early as 4 weeks into treatment was particularly noteworthy given the chronicity of illness in the studied population. Similar rates of response were observed in the subsequent study by Davidson et al. (2001a) which also confirmed that the majority of the clinical benefit derived from sertraline occurred in the first 4 weeks of the trial. As these studies used primarily civilian subjects, Zohar et al. (2002) conducted a small 10-week pilot RCT ($N = 42$) of sertraline in military veterans suffering from PTSD. In contrast to the findings of the earlier studies, improvement in post-traumatic symptoms with sertraline was associated with a numeric but not statistical advantage over placebo, with the range of improvement detected

proportionately less than that noted in the prior civilian studies (25–33% vs. 45–50%). The authors wondered if the relatively greater mean baseline severity in this military population might have played a role in the results. Friedman et al. (2007) attempted to explore this issue further in a larger 12-week RCT of subjects seen in a VA medical setting. In this population, of which 71% had combat-related PTSD, no statistical differences in outcome were detected between active drug and placebo, nor could any potential moderators of outcome, such as gender or substance abuse, be definitively identified.

In spite of these mixed results from acute phase trials, studies of continuation treatment are more supportive of sertraline use. Using a sample of patients ($N = 252$) who had previously participated in one of two acute phase RCTs of sertraline for PTSD, Londborg et al. (2001) followed these individuals in an open-label fashion during a 24-week continuation phase trial. One of the more noteworthy findings of the trial was the observation that 54% of nonresponders during the original 12-week trials subsequently converted to responders, underlining the importance of patience with extended pharmacotherapy treatment to obtain maximum clinical response. The other finding of note in this study was that 92% of the original acute phase responders maintained their recovery during this extension period. Subsequent analyses of investigational measures also showed that 20–25% of the overall improvement in PTSD symptoms during the combined 36 weeks of treatment occurred during the latter 24 weeks as did 31% of the improvement in quality of life (Rapaport et al. 2002). A subsample ($N = 96$) of the responders from this continuation-phase study was subsequently rerandomized to double-blind maintenance treatment with either sertraline or placebo for a further 28 weeks. In this instance, both relapse (sertraline 5.3% vs. placebo 26.1%) and discontinuation (sertraline 15.8% vs. placebo 45.7%) rates were significantly higher in the placebo group (Davidson et al. 2001b). These studies serve to underscore the importance of extended antidepressant treatment to elicit maximal improvement of symptoms, maintain recovery, and prevent relapse. At present, guidelines for duration of pharmacotherapy treatment recommend continuing medications for 6–12 months in cases of acute PTSD or 12–24 months for chronic PTSD; under certain circumstances, such as persistent residual symptoms or poor psychosocial functioning, an even longer treatment period may be of benefit (Foa et al. 1999; Stein et al. 2003).

Evidence from acute phase RCTs also supports the utility of paroxetine, fixed dose (20 or 40 mg) or flexibly dosed (20–50 mg), for PTSD (Marshall et al. 2001; Tucker et al. 2001). As with sertraline, the beneficial effects of paroxetine were noticeable at week 4 (Tucker et al. 2001) in one trial, and both trials showed evidence of significant improvement in measures of social and occupational function at endpoint. With the majority of previously published trials using primarily Caucasian populations, a more recent RCT by Marshall et al. (2007) was able to demonstrate that the positive effects of paroxetine (up to 60 mg) extended to a sample with a high proportion of ethnic minorities (75%). Further, benefits of the medication were seen not only for core PTSD symptoms but also for dissociative symptoms and interpersonal problems, psychopathology that often accompanies this illness.

The efficacy of other SSRIs has also been investigated, albeit to a lesser extent, for treatment of PTSD. A single double-blind trial of citalopram (20–50 mg) exists in which it is compared to sertraline (50–200 mg) and placebo. While sertraline appeared to demonstrate an advantage over the other arms on avoidance/numbing symptoms (as measured by the CAPS) following the 10 weeks of treatment, no other statistical differences in efficacy could be detected between the three groups. No other placebo-controlled RCTs have been published investigating citalopram, escitalopram, or fluvoxamine. Nevertheless, evidence from a number of open trials supports the use of these three agents in PTSD subpopulations that include military veterans, civilians, and even children and adolescents (Davidson et al. 1998; English et al. 2006; Escalona et al. 2002; Marmar et al. 1996; Robert et al. 2006; Seedat et al. 2002).

The SSRIs currently represent the mainstay of pharmacological intervention in PTSD, primarily because of their extensive evidence base relative to other pharmacological agents which is reflected in the widespread clinical use of these drugs. However, a recent report by the Institute of Medicine, published in 2007, found the evidence base for the use of pharmacotherapy, including the SSRIs was insufficient to confirm their efficacy in PTSD – a conclusion based in part on the modest effect sizes (≈0.5) seen in SSRI pharmacotherapy trials. Recommendations by the committee that larger independent trials with these drugs, in a greater variety of clinical settings, and over a greater duration are certainly valid and would no doubt enrich the literature. Nor is there any doubt that the degree of SSRI efficacy is highly variable between individuals and may be dependent on other factors which include chronicity, severity of illness, and comorbidity. Nevertheless, the prevalent use of SSRIs is unlikely to change in the near future, particularly given their relative safety and tolerability. While certain antidepressants within the SSRIs have been investigated more than others, clinicians are inclined to treat their efficacy as equal across the class. As such, choice of a particular agent tends to be based on side-effect profile and prior response. Prospective trials that investigate the use of systematic augmentation, combination, and switching strategies which include both psychotherapeutic and psychopharmacologic options specifically targeting residual symptoms will be a crucial area of future research.

2.2 Serotonin Norephinephrine Reuptake Inhibitors (SNRIs)

There are four SNRIs currently available on the market – venlafaxine XR, duloxetine, milnacipran, and desvenlafaxine. Although trials of duloxetine for PTSD are currently in progress, to date the only published PTSD RCTs involving SNRIs have focused on venlafaxine XR. This drug, whose mechanism is thought to involve differential reuptake of serotonin and norepinephrine at either end of the dosing spectrum, has demonstrated efficacy for both acute-phase and continuation treatment of PTSD, with comparable benefits to SSRIs. In their 12-week trial, Davidson et al. (2006a) compared flexibly dosed venlafaxine XR (37.5–300 mg)

to sertraline (25–200 mg) or placebo in subjects with moderate to severe PTSD ($N = 538$). Remission rates following venlafaxine treatment (mean maximum dose 224.6 mg) were significantly better than placebo (30.2% vs. 19.6%, $p < 0.05$), with particular efficacy noted for symptoms of avoidance/numbing and hyperarousal. Differentiation in outcomes between venlafaxine XR and placebo were seen as early as week 2 and overall efficacy and tolerability between venlafaxine and sertraline were similar. Davidson et al. (2006b) also subsequently published the results of a 24-week double-blind RCT comparing venlafaxine (37.5–300 mg) to placebo. In this sample ($N = 329$), venlafaxine was once again superior to placebo with respect to improvement in overall PTSD symptoms and the individual symptom clusters with the exception of hyperarousal symptoms, although there was a trend toward significance ($p = 0.06$). Changes in measures of resilience, stress vulnerability, and quality of life similarly favored venlafaxine. Although the number of trials is few, the efficacy and tolerability reports from these large RCTs support the notion that venlafaxine represents a reasonable alternative to SSRIs for pharmacologic treatment of PTSD.

2.3 Tricyclic Antidepressants (TCAs)

With the advent of newer, more tolerable antidepressants, TCAs have largely fallen out of favor as first-line pharmacological agents for anxiety disorders. Nevertheless, published RCTs investigating TCAs do exist. Desipramine, a TCA thought to work primarily by inhibiting norepinephrine reuptake into the presynaptic neuron, was investigated in an 8-week double-blind cross-over trial (Reist et al. 1989). During each of the 4-week active treatment period, hospitalized male veterans with PTSD ($N = 18$) received either desipramine (50–200 mg) or placebo with an intervening 4-day switchover. In this trial, desipramine was not associated with significant changes in PTSD symptoms. However, it should be noted that no validated observer-rated scale of PTSD was used; rather items from the Hamilton Rating Scale for Depression (HAM-D) thought to be relevant to PTSD were selected post-hoc. Similarly, the active treatment period was quite short (only 4 weeks) and it may very well have been insufficient to see a therapeutic effect.

An alternate TCA, amitriptyline, was used in an 8-week RCT of 46 veterans with combat-related PTSD (Davidson et al. 1990). Amitriptyline, a mixed norepinephrine/serotonin reuptake blocker, did demonstrate superiority over placebo on several outcome measures (including both the HAM-D and the Hamilton Rating Scale for Anxiety (HAM-A)), but not the structured interview of PTSD symptoms. Of note, the rates of response in this trial were quite low with 64% of subjects receiving amitriptyline and 72% of those receiving placebo still meeting criteria for PTSD at endpoint. Two other controlled trials investigating TCAs for PTSD have also been reported. These are discussed below in the section below entitled: "Monoamine oxidase inhibitors."

The underwhelming results from the above trials in the context of both the drug–drug interactions and the adverse event/toxicity profiles of the TCAs (which include both anticholinergic and antiadrenergic effects) have largely relegated these drugs to third or fourth line options. Nevertheless, TCAs may still have a place in PTSD treatment in special cases such as comorbid depression or intolerance to other agents.

2.4 Monoamine Oxidase Inhibitors

The mechanism of action of monoamine oxidase inhibitors involves enhancing availability of monoamines via inhibition of the enzyme monoamine oxidase responsible for their breakdown. The conventional monoamine oxidase inhibitors, referred to as MAOIs, work by irreversible inhibition of the enzyme; in contrast, a newer more selective subgroup of MAOIs work via *reversible* inhibition of monoamine oxidase type A and are thus termed RIMAs. As with TCAs, the use of conventional MAOIs is increasingly rare as a result of both their side-effect profile and the necessity of a low-tyramine diet to decrease risk of hypertensive crises. In one early report, the MAOI phenelzine was used in a double-blind cross-over trial in 13 subjects with PTSD. Outcomes following use of phenelzine (30–75 mg) during the 5-week treatment periods were not significantly different from placebo (Shestatzky et al. 1988). In contrast, two positive RCTs have been reported in which phenelzine was compared to imipramine and placebo. In the first of these 8-week double-blind studies, Frank et al. (1988) found active treatment with phenelzine (15–75 mg) and imipramine (50–300 mg) to both be effective in reducing post-traumatic symptoms in the sample of 34 veterans with PTSD. Kosten et al. (1991) similarly found both medications to be effective in their larger sample of male veterans ($N = 60$). However, with a 44% symptom improvement in the phenelzine-treated group compared to 25% improvement in the imipramine group, use of an MAOI seemed to be associated with added benefit. In spite of these mixed findings, no larger definitive placebo-controlled trials have since been conducted nor, to our knowledge, have any investigations compared more novel agents (e.g., SSRIs) to MAOIs for this purpose. Results from such trials would be helpful in more clearly delineating a role for MAOIs in current pharmacotherapy strategies for PTSD.

As noted earlier, reversible inhibitors of monoamine oxidase A (RIMAs) are a more selective subtype of MAOIs. These drugs, which include brofaromine and moclobemide, do not require the same dietary restrictions as the MAOIs and are generally more tolerable. Although RIMAs are not available in the US, they are available in other parts of the world such as Europe and Canada. Studies of brofaromine, a mixed RIMA/serotonin reuptake inhibitor, in PTSD have resulted in inconsistent findings. While Katz et al. (1994) found an advantage for brofaromine over placebo in the treatment of individuals with PTSD of greater than 1 year duration, these findings were not replicated in the subsequent multicenter trial reported by Baker et al. (1995). Moclobemide has not been investigated in any placebo controlled trials, but in an open label comparison to fluoxetine and

tianeptine all three drugs were found to be similarly effective (Onder et al. 2006). Nevertheless, these results have not been replicated in a double-blind trial nor are there any other RCTs of moclobemide for PTSD.

2.5 Other Antidepressants

Nefazodone is an older antidepressant thought to work by a dual mechanism of action: potent postsynaptic antagonism of serotonin 5-HT2A receptors as well as modest inhibition of presynaptic serotonin/norepinephrine reuptake (Davis et al. 1997). A 12-week RCT of nefazodone found it to be superior to placebo for improvement in both PTSD and depressive symptoms (Davis et al. 2004); however, the sample size ($N = 41$) was limited. McRae et al. (2004) published results of a head-to-head comparison with sertraline. In this study, nefazodone (up to 600 mg) and sertraline (up to 200 mg) were both similarly effective treatments for PTSD with comparable tolerability. Once again, however, sample size was small ($N = 37$). Amid concerns of liver toxicity, nefazodone was recently withdrawn from the market in Europe, Canada, Australia, and New Zealand. While it is still available in the US, these concerns have noticeably curtailed its use and may prevent further investigation of this drug in clinical trials.

A small 8-week placebo-controlled trial ($N = 30$) of bupropion SR, a combined norepinephrine/dopamine reuptake inhibitor, failed to distinguish active medication from placebo on PTSD outcomes (Becker et al. 2007). Similarly, an earlier RCT that investigated bupropion SR as a smoking cessation aid in veterans with chronic PTSD did not find it efficacious on the secondary measure of PTSD improvement (Hertzberg et al. 2001).

Mirtazapine is a newer antidepressant with a novel mechanism of action resulting in increased overall norepinephrine and serotonin neurotransmission. It works presynaptically to inhibit the α_2 heteroreceptors on serotonergic neurons and the α_2 adrenergic autoreceptors. It also works to selectively block serotonergic 5-HT_2 and 5-HT_3 receptors on the postsynaptic neuron, as well as having potent antagonist effects at histaminic H1 receptors which may contribute to its sedative effects. Evidence from open trials (Bahk et al. 2002; Chung et al. 2004; Kim et al. 2005) suggest that mirtazapine may be a useful agent in both short-term and long-term treatment of PTSD, with comparable efficacy to sertraline. Only a single placebo-controlled trial of mirtazapine has been reported in the literature (Davidson et al. 2003). In this 8-week pilot study ($N = 29$), mirtazapine (up to 45 mg) showed benefit over placebo on a global improvement measure of PTSD, but failed to show any significant advantage on other outcome variables (e.g., Davidson Trauma Scale). Based on the measures, effect sizes for mirtazapine ranged from moderate to strong, but no subsequent placebo-controlled trials have been performed to validate these findings.

3 Anticonvulsants

Kindling, a phenomenon by which repeated subthreshold electrical stimulation progressively acts to produce profound brain changes such that eventually spontaneous events occur with minimal or no stimulation, is one mechanism proposed to explain PTSD symptom development. For instance, the repeated recall of conditioned fear memories that may eventually result in spontaneous flashbacks or intrusive memories is a process that resembles kindling (Post et al. 1997). Based on their putative antikindling effects, the effects of several anticonvulsant agents (as either monotherapy or adjunctive treatment) for PTSD have been reported in the literature; these include divalproex, lamotrigine, tiagabine, topiramate, carbamazepine, levetiracetam, phenytoin, gabapentin, and vigabatrin. Though the case studies and open trials provide some preliminary evidence of support, results from the few controlled trials are more mixed.

The first RCT of an anticonvulsant in PTSD involved lamotrigine, which is thought to work by inhibition of glutamate transmission (Hertzberg et al. 1999). In this 12-week pilot trial ($N = 15$), lamotrigine (50–500 mg) appeared to be superior to placebo for re-experiencing and avoidance/numbing symptoms, but the small sample size and lack of subsequent replication preclude more definitive conclusions. In another trial, responders ($N = 18$) following 12 weeks of open-label tiagabine treatment (up to 16 mg) were randomized to an additional 12 weeks of double-blind continuation with either active drug or placebo (Connor et al. 2006). Although there were no differences in rates of relapse between the two groups during this period, a trend towards significance ($p = 0.08$) was observed for subjects who received ongoing tiagabine to achieve remission. Unfortunately, a subsequent, larger multicenter RCT ($N = 232$) comparing 12 weeks of double-blind tiagabine (up to 16 mg) to placebo failed to show group differences on any of the primary or secondary efficacy measures (Davidson et al. 2007). Similarly, an 8-week placebo-controlled trial of divalproex monotherapy (1000–3000 mg), specifically for treatment of PTSD hyperarousal symptoms, in military veterans ($N = 85$) failed to show benefit over placebo for any of the PTSD symptom clusters, nor for any other symptoms of depression and anxiety (Davis et al. 2008a). Topiramate is an anticonvulsant thought to promote GABA-ergic but inhibit glutamatergic transmission. In their 7-week double-blind trial ($N = 40$), Lindley et al. (2007) compared topiramate augmentation (50–200 mg) to placebo. Although no benefit was noted for this treatment regimen, it should be noted that the dropout rate for topiramate-treated subjects was noticeably higher than for the placebo-treated group (55% vs. 25%), and mainly related to adverse events. Topiramate monotherapy (25–400 mg) was also investigated in a 12-week double-blind RCT ($N = 38$) of civilians with non-combat related PTSD (Tucker et al. 2007). In this case, topiramate did show benefit over placebo for the re-experiencing cluster of symptoms, but statistical differences between groups was not seen in overall CAPS scores.

Based on the results of above studies, the use of anticonvulsants in PTSD has only very limited support. The evidence, which mainly suggests a role for either

topiramate or lamotrigine monotherapy, is based on single trials of each medication, each with an underpowered sample size. As such, treatment recommendations that involve these medications must be interpreted with caution. Nevertheless, a role for anticonvulsant agents in PTSD may exist particularly in specific subpopulations; for instance, lamotrigine in subjects with comorbid PTSD and bipolar disorder or topiramate for individuals with an underlying seizure disorder. Treatment studies that examine anticonvulsant use in these specific clinical populations would be helpful.

4 Atypical Antipsychotics

In clinical practice, atypical antipsychotics, with their dual dopaminergic and serotonergic mechanisms, are frequently used as adjunctive treatment to standard antidepressant treatment for both mood and anxiety disorders. Since up to 40% of individuals with PTSD may experience concurrent psychotic symptoms, the use of an antipsychotic certainly has a rational basis. However, the increased popularity of these medications may also stem from their relatively lower risk of unpleasant side-effects such as tardive dyskinesia and Parkinsonism commonly seen with more traditional antipsychotics. Further, the sedating qualities (thought to be due to histaminic H_1 receptor blockade) seen with a number of atypicals have also been used to target the sleep disturbances common to many psychiatric disorders including PTSD. Interestingly, in spite of their relative common usage, the evidence base for using atypicals in PTSD is still quite limited. Currently, seven atypical antipsychotics have FDA approval in the United States; these include olanzapine, risperidone, quetiapine, ziprasidone, aripiprazole, clozapine, and most recently paliperidone. Of these agents, only case-reports or open trials exist for clozapine, ziprasidone, quetiapine, and aripiprazole in PTSD. The only published RCTs of atypicals in PTSD involve either risperidone or olanzapine.

Five double-blind placebo-controlled trials of adjunctive risperidone (0.5–8 mg) in PTSD have been reported (Bartzokis et al. 2005; Hamner et al. 2003; Monnelly et al. 2003; Reich et al. 2004; Rothbaum et al. 2008). Of these, three (Bartzokis et al. 2005; Monnelly et al. 2003; Reich et al. 2004) reported improvement in PTSD symptoms within the re-experiencing or hyperarousal clusters but no improvements were seen in the avoidance/numbing cluster. In contrast, Hamner et al. (2003) found in their sample of 40 combat veterans diagnosed with chronic PTSD and associated psychotic symptoms, adjunctive risperidone improved the psychotic but not overall post-traumatic symptoms. In the most recent study (Rothbaum et al. 2008), subjects who did not remit following 8 weeks of open label sertraline were randomized to a further 8 weeks of placebo or risperidone augmentation. At the end of the study, no differences were found between groups on CAPS overall score or individual clusters. However, post-hoc analyses did reveal an advantage for risperidone in terms of improved sleep. Only a single RCT of risperidone monotherapy in PTSD has been published (Padala et al. 2006). In this 12-week study of women ($N = 20$)

with PTSD stemming from sexual assault or domestic abuse, flexibly dosed risperidone (mean dose of 2.62 mg) was well-tolerated and superior to placebo on the primary outcome measure – score on the 8-item Treatment Outcomes PTSD Scale, but not on secondary outcomes (CAPS, HAM-D, or HAM-A).

The utility of olanzapine as an adjunctive agent has been investigated in a single RCT. Stein et al. (2002) randomized male veterans ($N = 19$) with combat-related PTSD but minimally responsive to an adequate trial of SSRIs to double-blind augmentation with olanzapine (10–20 mg) or placebo for 8 weeks. Olanzapine was associated with a significantly greater reduction in overall CAPS scores, relative to placebo, as well as greater improvements in sleep and depressive symptoms, although clinician ratings of global improvement did not differ statistically between groups. Of concern, however, was the mean 13 lb weight gain following olanzapine treatment which may be a significant issue impacting compliance and general health. As with risperidone, only a single RCT of olanzapine as monotherapy in PTSD has been reported (Butterfield et al. 2001). In contrast to the results of the risperidone study, the results of this 10-week double-blind placebo-controlled pilot study ($N = 15$) did not show differences in PTSD outcomes between olanzapine (5–20 mg) and placebo. Once again treatment with olanzapine resulted in significant weight gain (mean weight gain with olanzapine: 11.5 ± 4.4 lb vs. mean weight gain with placebo: 0.9 ± 0.06 lb) highlighting the need to weigh the benefits of olanzapine use against the potential health risks. The mixed results of RCTs involving these two medications in PTSD underscore the need for larger, more definitive clinical trials investigating these agents to justify their increasingly common use in the psychopharmacological management of PTSD.

5 Benzodiazepines and Other Hypnotics

Benzodiazepines (BZDs) have long played an important role in the management of anxiety disorders. Efficacy, rapid onset of action, and tolerability are all key factors in their popularity. However, with growing concerns about possible development of tolerance with long-term use and potential for abuse, and the development of newer, more tolerable antidepressants, the use of BZDs has declined considerably. Currently, BZD use in PTSD is largely directed towards symptomatic management of hyperarousal symptoms such as sleep disturbance. However, despite the long history of BZD use for anxiety, very few RCTs exist investigating their efficacy in PTSD.

In a 12-week cross-over trial (5-week treatment periods) of 10 subjects, alprazolam monotherapy showed some benefit for nonspecific anxiety, but not for specific PTSD symptoms (Braun et al. 1990). Cates et al. (2004) attempted to validate the common practice of providing BZDs for PTSD-related sleep disturbances with their 5-week, randomized, single-blind cross-over trial (2-week treatment periods) of clonazepam (1–2 mg). In their very small sample ($N = 6$),

clonazepam showed modest numerical but no statistical superiority over placebo on any of the sleep-related measures.

Two additional studies have investigated the effects of BZD administration in the acute aftermath of trauma. Gelpin et al. (1996) prospectively compared 13 trauma survivors who received either clonazepam or alprazolam in the days following the trauma (range 2–18 days) to 13 controls matched for symptom severity. At 6-month follow-up, 69% of the BZD-treated group but only 23% of controls had developed PTSD. In their single-blind trial, Mellman et al. (2002) randomized 21 subjects recently admitted to a trauma center to treatment with temazepam (15–30 mg) or placebo for 1 week. At 6-week follow-up, 50% of the temazepam-treated group but only 27% of the placebo-treated group had developed PTSD. Although both studies are limited by small samples, they suggest that early BZD administration following trauma is not helpful in the prevention of PTSD. There is also a commonly held clinical misconception that, because of the high rates of substance abuse among those with PTSD, it is necessary to exclude BZDs from the pharmacological management of these patients despite a lack of evidence to support this belief. It is possible that this faulty idea and the negative results of the studies above have contributed to the lack of recent research investigating BZDs in PTSD. Nevertheless, a review of medications prescribed for PTSD within the VA system revealed that BZDs are among the commonest class of medications used in its management (Mohamed and Rosenheck, 2008). Since BZDs are cost-effective, useful, and commonly prescribed anxiolytics, but have only been investigated in two very small trials, there is evidently an urgent need for larger clinical trials to either validate the earlier results or to re-explore potential utility of BZDs in the prevention or treatment of PTSD.

6 Antiadrenergic Agents

Dysregulation of the noradrenergic system is one of the proposed mechanisms involved in the pathophysiology of PTSD. The presence of the hyperarousal symptoms, such as hypervigilance and sleep disturbance, as well as abnormalities in different biological measures of norepinephrine suggested to researchers that a hyperadrenergic state was at play in PTSD. Based on this hypothesis, a number of investigators proposed a role for antiadrenergic medications in its treatment. Prazosin, a centrally acting selective α_1 adrenergic antagonist, is one agent that has received considerable interest lately for its effects on PTSD-related sleep disturbance. A double-blind, 20-week, cross-over trial in combat veterans with PTSD ($N = 10$) found prazosin (up to 10 mg) significantly more effective than placebo for improving sleep quality and decreasing distressing dreams (Raskind et al. 2003). Further, on the secondary measure of changes in CAPS scores, prazosin was associated with significant improvement across all symptom clusters. Extending these findings to a larger sample of combat veterans ($N = 40$) in an 8-week parallel-group study, prazosin (up to 15 mg) was again associated with improvements in overall sleep

quality and frequency of trauma-related nightmares (Raskind et al. 2007). Similarly, positive findings were seen in a 7-week placebo-controlled cross-over trial (3-week treatment periods) of civilians with PTSD ($N = 13$) (Taylor et al. 2008). Along with improvements in sleep quality and a shift in dream content from nightmares to more "normal" subject matter, prazosin was also associated with a substantial increase in total sleep time (94 min).

Two RCTs of guanfacine, a centrally acting α_2 adrenergic agonist, in PTSD have been reported. In the first, an 8-week, double-blind trial of veterans with PTSD ($N = 63$) guanfacine failed to demonstrate superiority over placebo on measures of post-traumatic symptoms, sleep, or depression (Neylan et al. 2006). Similarly, negative results were also found in a more recent 8-week RCT (Davis et al. 2008b). Clonidine, commonly used as an antihypertensive agent because of its effects on decreasing sympathetic outflow, has a similar mechanism to guanfacine. Although a single paper reported that a combination of clonidine and imipramine was helpful in the symptomatic improvement of Cambodian refugees suffering from chronic PTSD and major depression (Kinzie and Leung, 1989), no RCTs have been published to confirm this.

Although no RCTs have reported on the use of β-blockers to treat post-traumatic symptoms once they have developed, three controlled studies have examined their utility in the secondary prevention of PTSD following trauma exposure. Based on literature that propranolol appears to impair recall of emotionally arousing material (Cahill et al. 1994), investigators argued that β-blockers administered in the peri-traumatic period might inhibit the consolidation and subsequent ability to retrieve and replay traumatic memories. However, findings from these studies are inconsistent. Pitman et al. (2002) randomized 41 patients in the emergency department who had just experienced a traumatic event and had physiological signs of arousal (thought to predict those at risk of PTSD development) to treatment with either placebo or propranolol (160 mg in four divided doses) for 10 days, with the first dose of study medication administered within 6 h of the traumatic event. While no differences were found between groups on CAPS scores at either 1- or 3-month follow-up, the authors contended that propranolol-treated subjects had reduced physiologic responsiveness to trauma-related cues. The results must also be interpreted with caution because of the elevated attrition rate in the propranolol group. Later, Vaiva et al. (2003) prospectively followed 11 emergency department trauma patients who agreed to an open trial of propranolol (40 mg TID) for 7 days followed by a taper period comparing them to a group of 8 similar patients who refused propranolol but agreed to be assessed in follow-up by a psychiatrist blinded to the subject's treatment status. At 2-month follow-up, fewer propranolol-treated subjects met criteria for PTSD (9% vs. 37.5%, $p = 0.012$).

A more recent double-blind, 3-arm trial randomized 48 physically injured patients admitted to a surgical trauma center to 14 days of propranolol (up to 40 mg TID), gabapentin (up to 400 mg TID), or placebo (Stein et al. 2007), with the first dose of medication administered within 48 h of injury. In contrast to the earlier results, neither of the study drugs was significantly better than placebo in preventing acute stress disorder at 1-month follow-up nor PTSD at 4-month follow-up.

These earlier studies have focused on using β-blockers to inhibit retrieval of fear memories. More recently, Kindt et al. (2009) used a fear-conditioning paradigm to demonstrate that β-adrenergic receptors were also involved in the reconsolidation of fear memory. But more importantly, they showed that following acquisition of a fear response, the administration of propranolol given prior to reactivating the fear memory resulted in rapid elimination of the behavioral response to the fear – in this case, measured by eyeblink startle reflex. Of note, declarative memory remained unaffected. These findings have interesting clinical implications for PTSD. For instance, the use of a β-blocker prior to undergoing prolonged exposure therapy sessions (which is based on recalling aspects of the traumatic memory) may facilitate extinction of behavioral fear responses and accelerate recovery.

7 Other Agents

Even with multiple pharmacological options, rates of response to standard treatments in PTSD are often lower than clinicians would like. When the usual augmentation and combination strategies fail, novel treatments may be of use. Preliminary results from case series and open trials have been published for multiple agents including, among others, memantine (Battista et al. 2007), baclofen (Drake et al. 2003), dehyroepiandosterone (Sageman and Brown 2006), and cyproheptadine (Clark et al. 1999), not to mention other members of the medication classes discussed above. However, two novel agents that have been investigated in a controlled fashion are cortisol and D-cycloserine (DCS).

Hypothalamic-pituitary-adrenal axis dysfunction has been implicated in PTSD. Studies of glucocorticoids (GCs) and memory have found that elevated GC levels, as seen during acute stress, may enhance consolidation of emotional, or traumatic, memories (Roozendaal et al. 2006). One model of PTSD suggests that these memories get retrieved, replayed, and reconsolidated thereby strengthening the traumatic memory – this may potentially contribute to the frequency of intrusive memories and other re-experiencing type symptoms (Pitman 1989). However, elevated GC levels have also been found to impair memory retrieval, particularly for emotionally arousing material (Buchanan et al. 2006). Aerni et al. (2004) suggested that the administration of exogenous GCs (e.g., cortisol) may be useful in impeding the recall of traumatic memories thus allowing newer nontraumatic memories to be encoded and resulting in eventual fear extinction. Three patients were randomized to 1 month of daily cortisol (10 mg) and placebo for 2 months in this randomized, double-blind, cross-over trial. While the results must be very cautiously interpreted, the authors found that cortisol treatment was associated with modest improvement of both re-experiencing and avoidance-numbing type symptoms.

Based on literature supporting a role for glutamatergic dysfunction in PTSD (Nair and Singh 2008), DCS, a partial agonist of the *N*-methyl-D-aspartate (NMDA)

receptor, has been investigated as a treatment. Results from a 12-week, double-blind, cross-over trial (4-week treatment periods) of 11 subjects suggest that DCS (50 mg) may be effective for numbing/avoidance symptoms of PTSD, although subjects also demonstrated a degree of improvement during placebo treatment (Heresco-Levy et al. 2002). Both of the agents discussed here would be served by replication with larger samples to clarify their utility for PTSD, particularly given their unique mechanisms of action.

8 Conclusions

Several classes of psychotropic medications have been investigated for efficacy in PTSD. Of the effective medications, the largest evidence base exists for antidepressants and particularly for the SSRIs. In spite of the conclusion of the Institute of Medicine that there was insufficient evidence to support their use in PTSD, most clinicians continue to use these drugs as first line agents. Their general tolerability, benefits across the multiple symptom clusters of PTSD, and efficacy for the comorbid psychiatric disorders that commonly occur with PTSD (e.g., depression) ensure that SSRIs will remain a favored treatment option for now. Although SNRIs represent a viable first line alternative to SSRIs, management strategies beyond this are less clear. Based on the available evidence, reasonable next steps might include augmentation with an atypical antipsychotic, anticonvulsant (e.g., lamotrigine), or even the antiadrenergic agent prazosin. The use of concurrent psychotherapy, such as prolonged exposure, might also be helpful. This lack of clarity regarding optimal treatment strategies in the face of partial or nonresponse to standard first-line medications highlights the need for larger, definitive trials that address these questions. A trial, similar to the Sequenced Treatment Alternatives to Depression ("STAR*D") study (Rush 2007) might be helpful in clarifying guidelines regarding these issues in PTSD.

Future research should also focus on the development of novel pharmacological agents that specifically target PTSD. The medications that are currently in use for PTSD have, in large part, been developed for other disorders before being investigated for post-traumatic symptoms. A better understanding of the underlying pathophysiology and neural circuitry affected in PTSD will be helpful in detecting unique pharmacologic targets or mechanisms which can in turn be used to develop specific compounds of use for this illness. As with other psychiatric disorders, an additional goal of future research should be identifying predictors of response to specific interventions to allow for individualized treatment; hopefully, the fields of genetics and neuroimaging will be useful for this purpose. Regardless of what type of treatment is provided, be it psychotherapeutic or pharmacologic in nature, ensuring effective delivery and compliance will be crucial in achieving the ultimate goal of treatment: remission of symptoms and optimal psychosocial function.

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Pharmacological Treatment of Obsessive-Compulsive Disorder

Helen Blair Simpson

Contents

Abstract Obsessive-compulsive disorder (OCD) is a disabling neuropsychiatric disorder. Two treatments have been proven efficacious for the symptoms of OCD: pharmacological treatment with serotonin reuptake inhibitors and cognitive-behavioral therapy (CBT) consisting of exposure and response prevention. This chapter will focus on pharmacological treatments. The only medications which have proved effective for OCD in multisite randomized controlled trials are serotonin reuptake inhibitors, which include clomipramine (a noradrenergic and serotonergic reuptake inhibitor) and the selective serotonin reuptake inhibitors. General guidelines for the use of these medication will be presented, focusing on key issues that arise in clinical practice such as what dose to use, time to response,

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management of side effects, and duration of treatment. Because many OCD patients will have either a partial response or no response to these medications, evidence-based strategies for managing both partial responders and nonresponders will be described, including the evidence supporting augmentation with other medications or with CBT, and switching to other medications. Finally, novel strategies which are based on an increased understanding of the brain mechanisms underlying OCD and which are under investigation will be reviewed.

Keywords OCD · Medications · Serotonin reuptake inhibitors · Treatment guidelines · CBT

1 Introduction

Obsessive compulsive disorder (OCD) is a neuropsychiatric condition characterized by recurrent, intrusive, and distressing thoughts, images, or impulses (obsessions) and repetitive mental or behavioral acts which the individual feels driven to perform (compulsions) to prevent or reduce distress. OCD typically starts in childhood or adolescence (Kessler et al. 2005), persists throughout a person's life (Skoog and Skoog 1999), and produces substantial impairment in social, family, and work functioning (Huppert et al. 2009). With a lifetime prevalence of up to 2–3% (Kessler et al. 2005; Robins et al. 1984), the World Health Organization has identified OCD as one of the most disabling of all medical and psychiatric conditions in the industrialized world (Murray and Lopez 1996).

Two treatments have proved efficacious for the symptoms of obsessive-compulsive disorder (OCD): pharmacotherapy with serotonin reuptake inhibitors and cognitive-behavioral therapy (CBT) consisting of exposure and response prevention. This chapter will focus on pharmacotherapy for OCD. The evidence reviewed below for which medications are efficacious for OCD comes from randomized controlled trials, open trials, and case reports; the findings from randomized controlled trials are considered the most robust because patients are randomly assigned to different treatment conditions, and evaluations are done blind to treatment group. At the same time, not all questions of importance have been or can be addressed in this type of trial design. Thus, the clinical guidelines below must rely not only on data from controlled trials, but also from open trials, case reports, and clinical experience.

The only medications proved effective as monotherapy for OCD in multisite randomized controlled trials are serotonin reuptake inhibitors. This led to speculation that OCD is due to abnormalities in the brain serotonin system (Insel et al. 1985). However, despite many attempts to demonstrate serotonin abnormalities in the brains of people with OCD, it remains unclear whether serotonin reuptake inhibitors reduce OCD symptoms by correcting serotonin dysfunction or by enhancing serotonin modulation of brain circuits whose underlying dysfunction is unrelated to serotonin. Thus, serotonin reuptake inhibitors are used in OCD, without a clear sense of their mechanism. Yet they are known to work.

General guidelines for the use of serotonin reuptake inhibitors will be presented first, focusing on key issues that arise in clinical practice.Later, because many OCD patients will have either a partial response or no response to serotonin reuptake inhibitors, evidence-based strategies for managing both partial responders and nonresponders will be described. Finally, novel medication strategies which are based on an increased understanding of the brain mechanisms underlying OCD and which are under investigation will be reviewed.

2 Serotonin Reuptake Inhibitors in the Treatment of OCD

Serotonin reuptake inhibitors include clomipramine, a tricyclic antidepressant, and the selective serotonin reuptake inhibitors (i.e., fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram, and escitalopram). The efficacy of serotonin reuptake inhibitors for OCD has been reviewed elsewhere (Koran et al. 2007). In brief, serotonin reuptake inhibitors lead to improvement in 40–60% of people with OCD, and OCD patients who receive an adequate trial will achieve 20–40% reduction in their OCD symptoms on the average (Greist et al. 1995b; Pigott and Seay 1999). Thus, serotonin reuptake inhibitors typically lead to amelioration rather than elimination of symptoms. Specific issues that arise when using serotonin reuptake inhibitors for OCD are discussed below.

2.1 Comparative Efficacy

Serotonin reuptake inhibitors are not thought to differ from each other in efficacy for OCD, with the possible exception of clomipramine as discussed below. This makes sense if their mechanism of action is due to the blockade of the serotonin transporter since they are all serotonin reuptake inhibitors. On the other hand, there are clinical reports of OCD patients who do not respond to one serotonin reuptake inhibitor but do respond to another.

The one serotonin reuptake inhibitor with some evidence for greater efficacy than the others is clomipramine, a tricyclic antidepressant that is both a serotonin reuptake inhibitor and a norephinephrine reuptake inhibitor. In meta-analyses, clomipramine has been shown repeatedly to lead to greater effects than the other serotonin reuptake inhibitors (Ackerman and Greenland 2002; Eddy et al. 2004; Jenike et al. 1990). On the other hand, the clomipramine studies were done earlier in time than the studies using selective serotonin reuptake inhibitors and thus likely included more treatment-naïve samples. In head-to-head comparisons, clomipramine has not been found to be superior to fluoxetine, fluvoxamine, or paroxetine. However, these studies were not designed to detect small but potential, clinical, meaningful differences between treatments (Freeman et al. 1994; Koran et al. 1996; Lopez-Ibor et al. 1996; Mundo et al. 2000; Pigott et al. 1990; Zohar and Judge 1996). Even if somewhat more effective, clomipramine is not considered a first-line treatment for OCD because of its potential to cause more severe side effects (as described below).

In sum, treatment guidelines (Koran et al. 2007) recommend that patients first be started on a selective serotonin reuptake inhibitor. If there is no response, they should be switched to another and eventually tried on clomipramine. With regard to the decision concerning which selective serotonin reuptake inhibitor is to be tried first, the clinician should consider prior treatment response, the safety and acceptability of particular side effects for the individual patient (see below), and the potential for drug–drug interactions.

2.2 Dose

Clinical trials that randomize patients to different doses of the same medication (i.e., fixed dose studies) provide data on the effects of dose. In OCD, fixed dose studies of fluoxetine, paroxetine, and citalopram found that higher doses of serotonin reuptake inhibitors produced a somewhat higher response rate and/or greater degree of improvement than lower doses (Hollander et al. 2003; Montgomery et al. 1993, 2001), although one fixed dose sertraline study did not find significant differences between the effects of 50 and 200 mg/day (Greist et al. 1995a). The consensus is that high doses of serotonin reuptake inhibitors are most efficacious in OCD (Greist et al. 1995b). According to Koran et al. (2007), the recommended dose is: fluoxetine 60–80 mg/day; fluvoxamine 200–300 mg/day; paroxetine 60 mg/day, sertraline 200 mg/day, citalopram 60–80 mg/day; escitalopram 20–40 mg/day; clomipramine 250 mg/day. It is advised that patients be started at a low dose for tolerability (e.g., fluoxetine 10 or 20 mg/day) and the dose be increased every week or every other week (as tolerated) to doses at least as high as listed above before deciding that a patient is resistant to any particular serotonin reuptake inhibitor.

When patients do not respond to the doses above, doses even higher have been tried in clinical practice (e.g., fluoxetine or citalopram 120 mg/day, paroxetine 100 mg/day, escitalopram 60 mg/day). To examine the efficacy of this strategy, Ninan et al. (2006) randomized patients who had not responded to 16 weeks of sertraline (titrated from 50 to 200 mg/day) to continued sertraline (200 mg/day) or to increased sertraline (250–400 mg/day). Although the high dose group had significantly greater improvement than the lower dose group, the difference in additional improvement was clinically modest, and both groups were still left with clinically significant symptoms. Thus, increasing selective serotonin reuptake inhibitors to higher than recommended doses may be effective for certain OCD patients, but this is not a highly effective strategy for all.

In sum, guidelines recommend starting a serotonin reuptake inhibitor at a low dose and titrating upwards as tolerated until the recommended doses outlined above are achieved. However, individual patients may respond better at lower or even higher doses than currently recommended. Thus, clinical judgment is required when implementing these recommendations.

2.3 Time to Response

Many of the randomized controlled trials of serotonin reuptake inhibitors for OCD did not show a significant difference between placebo and active medication before 6 weeks. Thus, an adequate trial has been defined as the maximum dose comfortably tolerated for a minimum of 6 weeks (Koran et al. 2007), or an 8–12 week trial overall, given usual titration schedules. It is important that the delayed response to treatment is explained to patients so that patients don't prematurely stop their serotonin reuptake inhibitor before it has time to show any effects.

It is not known why high doses of a serotonin reuptake inhibitor for a relatively long period of time are required in OCD. El Mansari and Blier (2006) proposed that serotonin reuptake inhibitors decrease OCD symptoms by increasing synaptic serotonin in the orbito-frontal cortex and that this increase does not occur until the presynaptic autoreceptors that regulates serotonin release are desensitized. Data from their animal studies suggest that desensitization of the autoreceptors in the orbitofrontal cortex requires 8 weeks (unlike the 3 weeks required for other areas of the brain), leading them to propose that this phenomenon explains the delayed time to response in OCD.

2.4 Side Effects

Selective serotonin reuptake inhibitors are generally well-tolerated. Potential side effects include gastrointestinal problems (e.g., nausea or diarrhea), agitation, sleep disturbances (e.g., insomnia, vivid dreams), increased tendency to sweat, and sexual side effects (e.g., decrease in libido, trouble ejaculating, delayed orgasm). In addition, weight gain or fatigue can occur. In addition, there are clinical reports of specific side effects with certain agents that are presumed due to their unique pharmacological characteristics (e.g., more sedation and constipation due to the mild anticholinergic effects of paroxetine, more anorexia and activation due to the 5HT2C antagonism of fluoxetine (Stahl 2008)). Thus, it is possible for a patient to tolerate one serotonin reuptake inhibitor, but not another.

The first step in managing side effects is to decrease the dose, assuming the therapeutic benefit can be maintained. If this is not possible, specific interventions can be considered. For example, gastrointestinal distress can be minimized by starting on a low dose and titrating upwards slowly. Sexual side effects may be helped by using a one-day "drug holiday" before engaging in sexual activity or adding a counteracting pharmacologic agent (e.g., buproprion or sildenafil). Sweating can be treated with anticholinergic agents like benztropine. Weight gain can be minimized by engaging the patient in a weight maintenance program from the start. Insomnia may respond to switching the time the medication is taken to the morning, introducing sleep hygiene measures, or adding a sedative-hypnotic agent (Koran et al. 2007).

Clomipramine is both a serotonin and norepinephrine reuptake inhibitor. It also blocks several other receptors, including muscarinic cholinergic receptors, H1 histamine receptors, alpha-1 adrenergic receptors, and sodium channels in the heart and brain. Thus, in addition to the side effects described above, clomipramine can cause anticholinergic effects (e.g., dry mouth, constipation, delayed urination), antihistamine effects (e.g., sedation, weight gain), antiadrenergic effects (e.g., orthostatic hypotension), and cardiac conduction abnormalities or seizures. As a result, a medical evaluation including an electrocardiogram is necessary before starting clomipramine. Because of clomipramine's greater potential for adverse events, guidelines (Koran et al. 2007) recommend trial of one or more selective serotonin reuptake inhibitors before trying clomipramine.

Serotonin reuptake inhibitors can cause serotonin syndrome if combined with other agents that increase serotonergic activity at central and peripheral serotonergic receptors. The symptoms of serotonin syndrome can include cognitive abnormalities (e.g., headache, agitation, mental confusion), autonomic abnormalities (e.g., shivering, sweating, fever, hypertension, tachycardia), and somatic effects (myoclonus, hyperreflexia, tremor). Thus, serotonin reuptake inhibitors are contraindicated with agents like monamine oxidase inhibitors.

Serotonin reuptake inhibitors are metabolized by the liver but differ in the degree to which they alter metabolism through the hepatic cytochrome P450 enzyme system, and thus in their potential for drug–drug interactions (Koran et al. 2007). Escitalopram, citalopram, and sertraline have the fewest known interactions with other drugs. Serotonin reuptake inhibitors also differ in their rate of metabolism and thus the length of time they remain in the body (i.e., their half-life). Fluoxetine has the longest half-life (e.g., 2–4 days for fluoxetine and 7–9 days for its active metabolite norfluoxetine); paroxetine and fluvoxamine have the shortest (<24 h). A long half-life can be advantageous if a patient misses a dose, since drug concentrations only decrease slightly; however, in the event of nonresponse or a drug–drug interaction, a longer washout period would be necessary.

In sum, there are no clear differences in efficacy between the serotonin reuptake inhibitors (with the possible exception of clomipramine), and they share many side effects due to their inhibition of serotonin reuptake. However, they have unique pharmacological properties that presumably explain observed differences in side effects in individual patients; they also differ in their potential for drug–drug interactions and their pharmacokinetics.

2.5 Suicidality

There has been a long-standing concern that antidepressants (including serotonin reuptake inhibitors) might lead to suicidal ideation and behavior (i.e., suicidality). One recent meta-analysis found an association between selective serotonin reuptake inhibitors and suicidality in pediatric patients participating in randomized, placebo-controlled trials for major depressive disorder, OCD, and other anxiety

disorders (Hammad et al. 2006). However, other studies have found no association between selective serotonin reuptake inhibitors and likelihood of suicide attempts in depressed patients (Gibbons et al. 2007; Simon et al. 2006). While the link between serotonin reuptake inhibitors and suicidality is still under study, it is well-documented that untreated depression increases the likelihood of completed suicide.

In OCD patients without depression, it has not been proven that serotonin reuptake inhibitors lead to suicidal attempts. On the other hand, many OCD patients have comorbid depression. In the National Comorbidity Survey Replication, 40% of people with lifetime OCD were found to have a lifetime diagnosis of major depressive disorder (Ruscio et al. 2008). In people seeking treatment, the rates are estimated to be even higher (Rasmussen and Eisen 1994). Thus, since untreated depression is a risk factor for completed suicide and serotonin reuptake inhibitors may increase suicidal ideation, careful monitoring of suicidal thoughts and behaviors is advised in all OCD patients when starting a serotonin reuptake inhibitor.

2.6 Duration of Treatment

Clinical trials that treat patient with medications for a certain period of time and then randomly assign responders to continue on that medication or to be switched to pill placebo are called double-blind drug discontinuation studies. These studies help determine the risk of relapse if medication is stopped.

There are four double-blind discontinuation studies of serotonin reuptake inhibitors in adults with OCD (Hollander et al. 2003; Koran et al. 2002; Pato et al. 1988; Romano et al. 2001). The studies examined different serotonin reuptake inhibitors, used different designs (e.g., length of follow-up, procedure for placebo substitution), and had different relapse definitions. Not surprisingly, the studies came to different conclusions about the risk of relapse if medication is stopped, with relapse rates (or discontinuation due to insufficient clinical response) after double-blind switch to placebo ranging from a high of 89% within 7 weeks to a low of 24% within 28 weeks. The risk of relapse remains unclear given the limited number of studies and the methodological differences between them. Based on this literature and clinical consensus, practice guidelines (Koran et al. 2007) recommend that an OCD patient who has improved during an adequate trial of a serotonin reuptake inhibitor should stay on that medication for 1–2 years. If a patient wants to discontinue the medication, it is advised that the medication be slowly tapered (e.g., 10–25% every 1–2 months).

2.7 Drug Discontinuation Syndrome

If stopped abruptly, serotonin reuptake inhibitors can lead to a drug discontinuation syndrome. This can include flu-like symptoms (e.g., dizziness, nausea/vomiting, headache, and lethargy) as well as agitation, insomnia, myoclonic jerks, and paresthesias (Tamam and Ozpoyraz 2002; Zajecka et al. 1997). Drugs with a

shorter half-life (e.g., paroxetine) are more likely to cause this than those with a longer half-life and an active metabolite (e.g., fluoxetine). To avoid withdrawal, it is recommended that clinicians taper the medication slowly; substituting a serotonin reuptake inhibitor with a longer half-life is also a strategy used in clinical practice.

2.8 The Role of Venlafaxine or Duloxetine in the Treatment of OCD

Like clomipramine, venlafaxine and duloxetine are both serotonin and norephinephrine reuptake inhibitors. Because they inhibit serotonin reuptake, both are presumed effective for the treatment of OCD. To date, the only published study of duloxetine in OCD is a case series of four patients with OCD who had either partial or no response to a serotonin reuptake inhibitor (SRI); after being switched to duloxetine up to 120 mg/day, three responded to duloxetine (Dell'osso et al. 2008). Larger and controlled studies are needed to confirm this preliminary observation.

Venlafaxine has been studied more extensively, but the findings from different studies conflict. The only randomized controlled trial of venlafaxine in OCD found no significant difference between venlafaxine and placebo (Yaryura-Tobias and Neziroglu 1996). However, the sample was small (n = 30), the maximum dose was 225 mg/day, and the trial was only 8 weeks long. Other randomized controlled trials have compared venlafaxine (or venlafaxine XR) to either paroxetine (Denys et al. 2003) or clomipramine (Albert et al. 2002). These trials were limited by the lack of a placebo group, but each was 12 weeks in length and used higher doses of venlafaxine (e.g., 225–300 mg/day). In both studies, venlafaxine was as efficacious as the serotonin reuptake inhibitor to which it was compared. Finally, in a crossover study of nonresponders (Denys et al. 2004b), when paroxetine nonresponders were switched to venlafaxine, only 3 of 16 (19%) responded, whereas 15 of 27 (56%) venlafaxine nonresponders responded when switched to paroxetine. These findings suggest that paroxetine is more efficacious than venlafaxine in a patient with limited response to the other medication.

In sum, venlafaxine is often tried in clinical practice in OCD patients who have not responded to several selective serotonin reuptake inhibitors and cannot tolerate clomipramine. However, the evidence supporting the use of venlafaxine in OCD is not as strong as the evidence for the serotonin reuptake inhibitors, and there is little data supporting the use of duloxetine.

3 Limitations of Serotonin Reuptake Inhibitors in the Treatment of OCD

Although serotonin reuptake inhibitors are the only medications proven as monotherapy for OCD, they have important limitations: only up to 65% of people will respond to an adequate trial; the typical response is a 20–40% reduction in

symptoms; and patients can relapse if the medication is discontinued. Because many OCD patients will have a clinically meaningful but modest reduction in symptoms (e.g., 25–50% reduction in symptoms) and others will have no response (e.g., <25% reduction in symptoms), clinicians who treat OCD patients will commonly be faced with both problems. Strategies for each are discussed below.

3.1 Partial Response to Serotonin Reuptake Inhibitors

There are two proven augmentation strategies for patients with partial with partial response to serotonin reuptake inhibitors: the addition of antipsychotics and the addition of CBT consisting of exposure and response prevention. Each strategy is described below.

3.1.1 Antipsychotic Augmentation

Haloperidol, risperidone, quetiapine, and olazapine have all been shown in randomized controlled trials to augment the effects of serotonin reuptake inhibitors (Bystritsky et al. 2004; Denys et al. 2004a; McDougle et al. 2000; McDougle et al. 1994). There are also negative findings from studies of quetiapine (Carey et al. 2005) and olanzapine (Shapira et al. 2004) augmentation, but these may have been due to methodological issues. A systematic review of antipsychotic augmentation (Bloch et al. 2006), which combined nine double-blind, randomized controlled clinical trials involving 278 subjects, concluded that about 1/3 of OCD patients will be helped by antipsychotic augmentation, with strong evidence supporting the efficacy of haloperidol and risperidone.

Given the delayed onset of action of serotonin reuptake inhibitors in OCD, it is recommended that antipsychotic augmentation only be implemented after an OCD patient has failed to respond to a maximal dose of a serotonin reuptake inhibitor for at least 12 weeks (Koran et al. 2007). Low antipsychotic doses appear effective (e.g., <3 mg for risperidone or the equivalent), and the time to response is about 2–4 weeks. Therefore, the antipsychotic should be terminated after 1 month of intervention in those who do not clearly benefit. This minimizes the known risks of antipsychotic exposure, which can include metabolic syndrome, tardive dyskinesia, and neuroleptic malignant syndrome (Marder et al. 2004).

Despite their proven efficacy as an augmentation strategy, questions remain about the use of antipsychotics in OCD, including whether some agents are more effective than others, their long-term efficacy (since the randomized controlled trials have studied short-term effects), and the risk of relapse upon discontinuation. One small retrospective study suggested that 13 of 15 patients had a return of OCD symptoms when their antipsychotic was discontinued (Maina et al. 2003). In addition, the efficacy of newer antipsychotics like aripiprazole remains unclear, although case reports suggest benefit in some patients (Storch et al. 2008).

3.1.2 CBT Augmentation

Two randomized controlled trials have demonstrated the benefit of adding CBT, consisting of exposure and response prevention, to people with partial response to serotonin reuptake inhibitors. Tenneij et al. (2005) compared the effects of continuing medication (paroxetine or venlafaxine) versus adding exposure and response prevention in patients who had mild OCD symptoms on average. Adding exposure and response prevention (18 45-min sessions over 6 months) was superior to medication alone, but the effects were modest. Simpson et al. (2008) recruited patients on an adequate dose for at least 12 weeks of any serotonin reuptake inhibitor who still had clinically significant OCD symptoms. Patients were randomized to CBT, consisting of either exposure and response prevention or stress management therapy (17 90-min sessions over 8 weeks). Patients who received exposure and response therapy had significantly greater decrease in symptoms, and more of them achieved mild to minimal symptoms at the end of treatment (33% vs. 4%). The addition of stress management therapy had minimal effect on OCD symptoms.

Together, these findings support the use of exposure and response prevention in augmenting the effects of serotonin reuptake inhibitors in OCD. The addition of exposure and response prevention is clearly safer than the addition of antipschotics, given the known risks of the latter (Marder et al. 2004). On the other hand, exposure and response prevention is not widely available, it is more time-consuming, and some patients refuse it. Moreover, the long-term efficacy of short-term augmentation has yet to be shown.

3.1.3 Other Augmentation Strategies

Many other medications have been tested to see if they can augment the effects of serotonin reuptake inhibitors in OCD. Several medications found promising in case reports or open trials did not show clear efficacy in small placebo-controlled trials. These include lithium, buspirone, clonazepam, L-triiodothyronine, pindolol, and desipramine (Koran et al. 2007). Other augmentation strategies that appear promising await confirmation in larger randomized controlled trials. These include the addition of clomipramine to a selective serotonin reuptake inhibitor (Szegedi et al. 1996), the addition of the anticonvulsant topiramate (Van Ameringen et al. 2006), and the addition of morphine sulfate (Koran et al. 2005a). There is increasing interest in agents that modulate the glutamate system, as described in more detail below.

3.2 Nonresponders to Serotonin Reuptake Inhibitors

If a patient does not respond to one selective serotonin reuptake inhibitor, it is recommended that the clinician switch to another and eventually to clomipramine (Koran et al. 2007). Data on the efficacy of switching is extremely limited. The

American Psychiatric Association guidelines suggest that no more than half of patients will benefit from switching, and the effectiveness of this strategy diminishes as the number of failed adequate trials increases.

There is a paucity of data supporting the use of medications other than serotonin reuptake inhibitors as monotherapy for OCD. The data for venlafaxine and duloxetine in OCD are described above. One small double-blind discontinuation study suggested that mirtazapine (an antagonist at presynaptic alpha-2 adrenergic, 5HT2, and 5HT3 receptors) might help some OCD patients (Koran et al. 2005b). An open-label study suggested that tramadol (an agonist at the u opioid receptor as well as a modulator of noradrenergic and serotonergic systems) might be effective in OCD (Shapira et al. 1997). Other small studies suggested that stimulants (and specifically D-amphetamine) might reduce OCD symptoms (Insel et al. 1983; Joffe and Swinson 1987; Joffe et al. 1991).

Another strategy for nonresponders to serotonin reuptake inhibitors is to augment with antipsychotics, since many of the studies demonstrated the efficacy of antipsychotic augmentation in OCD recruited patients with little or no response to serotonin reuptake inhibitors. Antipsychotics as monotherapy are not thought to be effective for OCD (McDougle et al. 1995; cf.Connor et al. 2005). Adding cognitive behavioral therapy to nonresponders to serotonin reuptake inhibitors has also been found to be an effective strategy in an open trial (Kampman et al. 2002). However, given the efficacy of CBT as monotherapy (Foa et al. 2005), it is not clear if any benefit accrues in maintaining a patient on a serotonin reuptake inhibitor that has led to no improvement in their OCD symptoms, unless the serotonin reuptake inhibitor has helped a comorbid condition like depression.

For the OCD patient who has failed serotonin reuptake inhibitors, CBT, and several augmentation strategies, brain modulation therapies, and even brain surgery are often considered. Current modalities include transcranial magnetic stimulation, deep brain stimulation, gamma knife surgery, and vagus nerve stimulation, with very limited evidence for each of them (Mantovani and Lisanby in press).Given the irreversibility of surgery and the potential complications from deep brain stimulation, these modalities are reserved for highly treatment-refractory patients

4 Novel Medications in OCD

Because of the limitations of serotonin reuptake inhibitors, there has been increasing interest in developing novel medications for OCD. One approach has been to focus on agents that affect other aspects of the serotonin system. For example, based on their proposal that serotonin reuptake inhibitors are therapeutic in OCD because they increase synaptic levels of serotonin (5-HT) and activate postsynaptic 5-HT2 receptors, El Mansari and Blier (2006) have proposed that an antagonist of presynaptic 5-HT1D(B) receptors (to prevent the negative feedback these autoreceptors normally exert on serotonin release) or a selective 5-HT2

agonist (to directly stimulate postsynaptic 5-HT2 receptors) might produce quicker or better effects in OCD.

Another approach has been to investigate agents which modulate the glutamate system due to the confluence of genetic and brain imaging findings implicating glutamatergic abnormalities in OCD (Pittenger et al. 2006). Several agents have shown promise in case reports or open-label studies, including topiramate (an anticonvulsant that inhibits glutamate action, Van Ameringen et al. 2006), *N*-acetylcysteine (an aminoacid derivative that reduces synaptic glutamate, Lafleur et al. 2006), memantine (a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, Aboujaoude et al. 2009; Pasquini and Biondi 2006; Poyurovsky et al. 2005), and riluzole (an antiglutamatergic agent that reduces glutamatergic neurotransmission in several ways, Coric et al. 2005). A randomized placebo-controlled trial of riluzole is underway (Pittenger et al. 2006).

Finally, several agents are being examined whose aim is not to target OCD symptoms but to improve learning from CBT. The best example to date is the use of D-cycloserine to enhance extinction learning, the presumed basis of exposure therapy. D-cycloserine is a partial agonist at the NMDA receptor. Known to facilitate fear extinction in animal models, D-cycloserine has been shown to improve outcome from exposure treatment in people with a fear of heights (Ressler et al. 2004) and in people with social phobia (Hofmann et al. 2006). There are three published studies on the effects of D-cycloserine augmentation of exposure and response prevention therapy in OCD (Kushner et al. 2007; Storch et al. 2007; Wilhelm et al. 2008). In two of these studies, D-cycloserine reduced the time to response (Kushner et al. 2007; Wilhelm et al. 2008); one study found that the people who received D-cycloserine also had a lower rate of dropout from the treatment (Kushner et al. 2007). Other medications that might also facilitate extinction learning and thereby enhance CBT outcome are being investigated (Davis et al. 2006; Ressler and Mayberg 2007).

5 Conclusion

OCD is a severe and disabling illness. The only proven medications as monotherapy for OCD are the serotonin reuptake inhibitors. Although helpful for many patients, some patients do not respond and many who respond achieve only a partial response and are left with clinically meaningful residual symptoms. Proven augmentation strategies include the addition of antipsychotics and CBT consisting of exposure and response prevention. For patients who do not respond to serotonin reuptake inhibitors, CBT, or their combination and who do not respond to antipsychotic augmentation of serotonin reuptake inhibitors, alternative strategies can be tried (as outlined above), but evidence supporting them is limited. Given the limitations of our current pharmacological approaches, there is great need for novel medication strategies for OCD. A better understanding of the brain mechanisms that underlie OCD has begun to lead to novel targets for drug development.

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