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**ORIGINAL ARTICLE**

# Differential expression of VAMP2/synaptobrevin-2 after antidepressant and electroconvulsive treatment in rat frontal cortex

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## ABSTRACT

The biological basis for the therapeutic mechanisms of depression is still unknown. We have previously performed expressed-sequence tag (EST) analysis to identify some molecular machinery responsible for antidepressant effect. Then, we developed our original cDNA microarray, on which cDNA fragments identified as antidepressant-related genes/ESTs were spotted. In this study, with this microarray followed by Western blot analysis, we have demonstrated the induction of vesicle-associated membrane protein 2 (VAMP2/synaptobrevin-2) in rat frontal cortex not only after chronic antidepressant treatment, but also after repeated electroconvulsive treatment. On the other hand, expression of SNAP-25 and syntaxin-1 was not changed by these treatments. These components make a soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor complex with VAMP2 and mediate the synaptic vesicle docking/fusion machinery. In conclusion, it is suggested that VAMP2/synaptobrevin-2 plays important roles in the antidepressant effects. Our results may contribute to a novel model for the therapeutic mechanism of depression and new molecular targets for the development of therapeutic agents.
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**Keywords: differential cloning; gene expression; depression; SSRI; microarray**

## INTRODUCTION

Depression is one of the major psychiatric diseases that is estimated to affect 12–17% of the population during the lifetime of an individual.[1] It has been demonstrated that typical antidepressants acutely inhibit the monoamine reuptake in nerve terminals resulting in significant increase in synaptic concentrations of monoamines, noradrenaline or serotonin. However, there is a latency period of several weeks before the onset of clinical effect of antidepressants. Repeated electroconvulsive treatment (ECT) is another therapy that is widely used, particularly in the treatment of drug-resistant depression. It is an efficient treatment modality, although the basis for its therapeutic mechanism is still unknown. The delay of clinical effect from the beginning of antidepressants and ECT could be the result of indirect regulation of neural signal transduction systems or changes at the molecular level by an action on gene transcription. Indeed, there are selective effects of antidepressants on specific immediate early genes and transcription factors including c-fos,[2,3] zif268,[2] NGFI-A[4,5] and the phosphorylation of CRE-binding protein.[6] It is also reported that ECT affects the expression of c-fos, junB and Narp.[7,8] These molecules activate or repress genes encoding specific proteins by binding to a regulating element of DNA. These functional proteins may be involved in critical steps in mediating treatment-induced neural plasticity.

We have recently performed expressed-sequence tag (EST) analysis to identify some common biological changes induced after chronic treatment of two different classes of antidepressants, imipramine (a tricyclic antidepressant) and sertraline (a serotonin-selective reuptake inhibitor (SSRI)). Identification of quantitative changes in gene expression that occur in the brain after chronic antidepressant treatment can yield novel molecular markers that would be useful in the diagnosis and treatment of depression. Until now, we have molecularly cloned cDNA fragments as ESTs, which were named after antidepressant-related gene-s(ADRG).[9] More recently, for high-throughput secondary screening of candidate genes, ADRG cDNAs were spotted on glass slides, and we developed our original cDNA microarray (ADRG microarray).[10,11] An important task for the future will be to ascertain which of these changes in gene expression are directly relevant to the therapeutic effects of antidepressants. In this study, we used ADRG microarray to search for some genes commonly induced after chronic antidepressant treatment and repeated ECT.

Here, we first reported that the expression of ADRG14, a vesicle-associated membrane protein 2 (VAMP2/synaptobrevin-2), is commonly increased in rat frontal cortex after chronic antidepressant treatment and repeated ECT. VAMP2 is a key component of the synaptic vesicle docking/fusion machinery that forms the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) complex.[12–15]

## RESULTS

### Identification of ADRG14 as VAMP2

In the present study, we used an ADRG microarray for high-throughput, secondary screening to identify genes commonly affected by antidepressant and ECT. Figure 1 shows the pseudo-color image of the ADRG microarray after hybridization with samples obtained from sertraline- (a) or ECT-treated (b) rat frontal cortex. As expected, we obtained low background and consistent results in duplicated experiments. After normalization of the signals for both negative and positive controls, several spots of interest on the ADRG microarray showed increased or decreased fluorescence intensities after chronic sertraline treatment or repeated ECT. Interestingly, the fluorescence intensities of the spots for ADRG14 were increased 2.3 times in the sertraline group and 2.1 times in the ECT group, when compared to controls. These data were reproducible and interassay variation was negligible, when the differences of fluorescence intensities ($\pm$2-fold) were regarded as significant.

The size of ADRG14 fragment obtained from initial EST analysis was 224 bp. Sequence and homology analysis of ADRG14 with the EMBL/GeneBank database showed perfect matches to rat VAMP2 gene (NM012663[16]).

The induction of VAMP2 after chronic antidepressant treatment with two different classes of antidepressants, imipramine or sertraline, was also confirmed by RT-PCR analysis. The reproducible band corresponding to VAMP2 (784 bp) existed on a gel. As shown in Figure 2, we have demonstrated that the treatment with either imipramine or sertraline significantly induced the expression of VAMP2 mRNA levels (134.8$\pm$11.0% or 141.6$\pm$7.8%, $P<0.05$, ANOVA followed by the Dunnett's test, respectively) after normalization by GAPDH expression. The expression of VAMP2 was also significantly increased in repeated ECT-treated rat frontal cortex (159.2$\pm$10.4%, $P<0.05$, ANOVA followed by the Dunnett's test).

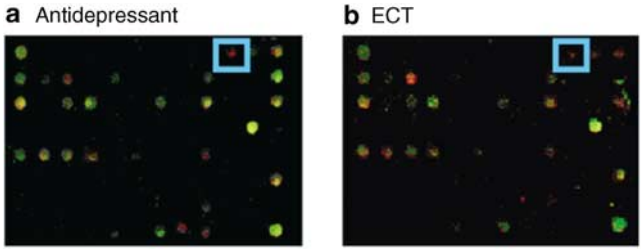


**Figure 1 Image analysis of ADRG microarray after hybridization with fluorescence probes. Ninety-six spots representing ADRG1-96 are shown here. The pseudo-color image of control group data (green) and chronic sertraline treatment group (red) were overlapped (a). As expected, we obtained low background and consistent results in duplicated experiments. In addition, the pseudo-color image of control group data (green) and repeated ECT group (red) were also overlapped (b). The spot with blue rectangle represents ADRG14 (VAMP2/synaptobrevin-2). Interestingly, the fluorescence intensities of the spots were increased 2.3 times in the sertraline group and 2.1 times in the ECT group, when compared to controls.**

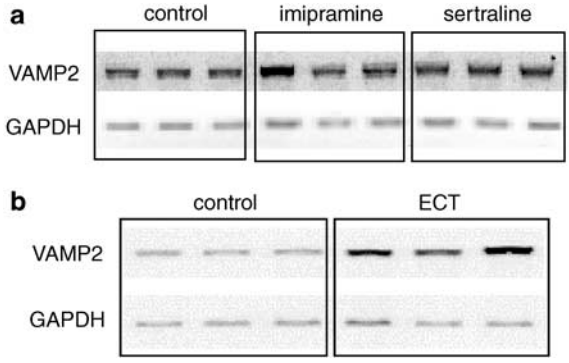


**Figure 2 Induction of VAMP2 mRNA after chronic antidepressant treatment (a) and repeated ECT (b) revealed by RT-PCR. Total RNA was extracted from rat frontal cortex treated either with vehicle (control, lanes a1–3), 10 mg/kg of imipramine (lanes a4–6) or 10 mg/kg of sertraline (lanes a7–9) for 21 days, and used for RT-PCR analysis. Total RNA samples extracted from rat frontal cortex treated either with sham operation (control, lanes a1-3) or repeated ECT (lanes a4-6) were also used for RT-PCR analysis. The reproducible single band corresponding to VAMP2 at the size of 784 bp existed on a gel. This figure represents a typical result of three independent experiments. Treatment with imipramine or sertraline increased the expression of VAMP2 in rat frontal cortex when compared to control samples (a). In addition, repeated ECT also increased the expression of VAMP2 in rat frontal cortex when compared to control samples (b).**

### Western Blot Analysis using Anti-VAMP2 Antibody

Genes are transcribed to various mRNAs and then translated into proteins that may be post-translationally modified and subsequently function as the ultimate effecting molecules in the cell. To determine whether the increase of VAMP2 mRNA levels was associated with a change of protein content, we analyzed VAMP2 immunoreactivity in rat frontal cortex with Western blot analysis. As shown in Figure 3, the existence of a single band of approximately 18 kDa was confirmed. Treatment with imipramine or sertraline induced a significant increase in VAMP2 immunoreactivity (418.0±22.3% or 387.2±17.5%, respectively) when compared to control samples (Table 1). Interestingly, VAMP2 immunoreactivity was also significantly increased after repeated ECT (175.6±10.1%, Figure 3, Table 1). These observations indicate that changes of VAMP2 gene expression may contribute to the therapeutic efficacy of chronic antidepressant treatment or repeated ECT. On the other hand, a single administration of antidepressant or acute ECT did not affect VAMP2 immunoreactivity when compared to control samples (Figure 3).

We then investigated the expression of two other proteins of regulated secretory pathways, syntaxin-1 and SNAP-25, because they make a SNARE complex with VAMP2. Concomitantly, immunoreactivity for syntaxin and SNAP-25 was unaffected by chronic antidepressant treatment or repeated ECT (Figures 4 and 5). In addition, a single administration of antidepressant or acute ECT did not affect syntaxin and synaptosome-associated protein of 25 kDa (SNAP-25) immunoreactivity when compared to control samples (Figures 4 and 5).

**Table 1 Induction of VAMP2 immunoreactivity after chronic antidepressant treatment with two different classes of antidepressants, imipramine or sertraline, and repeated ETC**

| *Treatment* | *VAMP2 expression* |
|---|---|
| Control | 100.0±7.1 |
| Imipramine | 418.0±22.3* |
| Sertraline | 387.2±17.5* |
| Control | 100.0±4.1 |
| Repeated ECT | 175.6±10.1* |

Data are expressed as % of the control data (mean±SEM) of three independent experiments.
*$P<0.05$, ANOVA followed by the Dunnett's test.

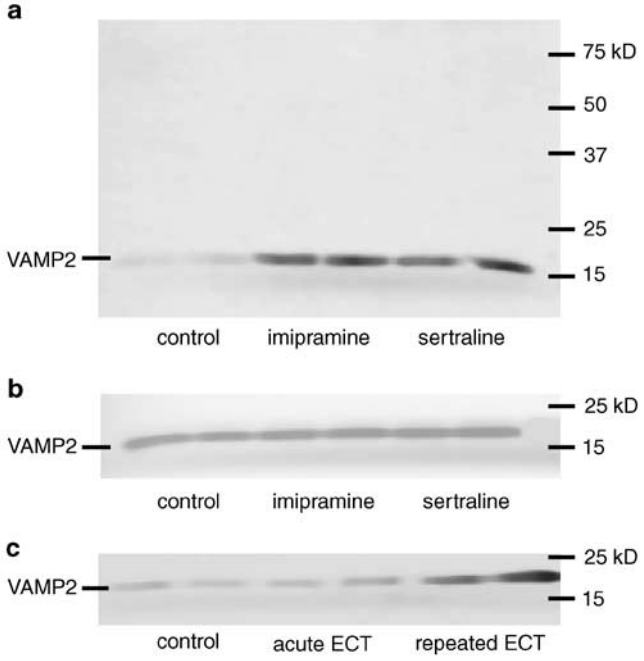


**Figure 3 Western blot analysis of VAMP2 after chronic antidepressant treatment (a), a single antidepressant treatment (b) or ECT (c). Protein sample was prepared from rat frontal cortex treated either with vehicle (control, lanes 1–2), 10 mg/kg of imipramine (lanes 3–4) or sertraline (lanes 5–6) and used for Western blot analysis as described in Materials and methods. In addition, protein sample was also prepared from rat frontal cortex treated either with sham operation (control, lanes 1–2), acute ECT (lanes 3–4) or repeated ECT (lanes 5–6). This figure represents a typical result of three independent experiments.**

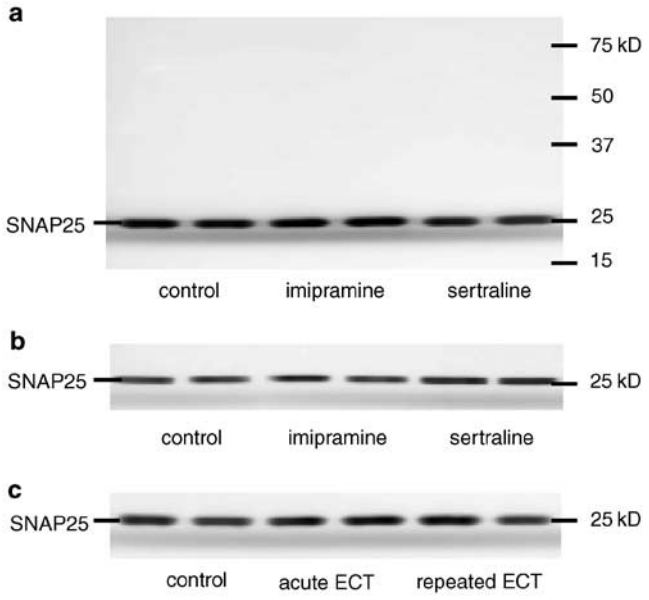


**Figure 4 Western blot analysis of SNAP25 after chronic antidepressant treatment (a), a single antidepressant treatment (b) or ECT (c). Protein sample was prepared from rat frontal cortex treated either with vehicle (control, lanes 1–2), 10 mg/kg of imipramine (lanes 3–4) or sertraline (lanes 5–6) and used for Western blot analysis as described in Materials and methods. In addition, protein sample was also prepared from rat frontal cortex treated either with sham operation (control, lanes 1–2), acute ECT (lanes 3–4) or repeated ECT (lanes 5–6). This figure represents a typical result of three independent experiments.**

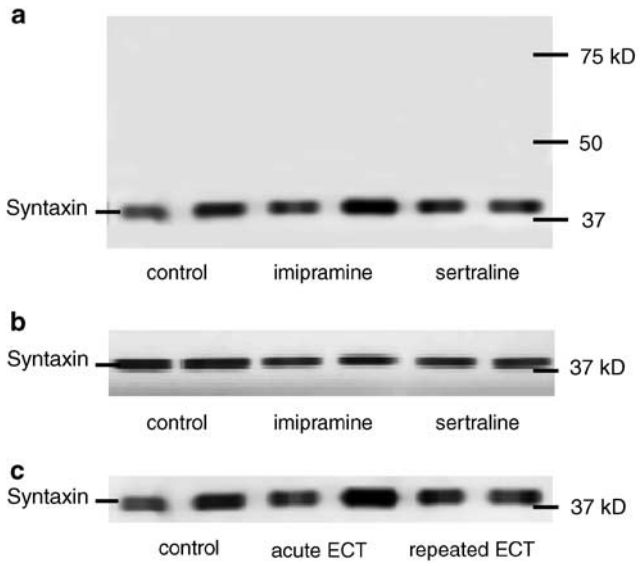


**Figure 5 Western blot analysis of syntaxin after chronic antidepressant treatment (a), a single antidepressant treatment (b) or ECT (c). Protein sample was prepared from rat frontal cortex treated either with vehicle (control, lanes 1–2), 10 mg/kg of imipramine (lanes 3–4) or sertraline (lanes 5–6) and used for Western blot analysis as described in Materials and methods. In addition, protein sample was also prepared from rat frontal cortex treated either with sham operation (control, lanes 1–2), acute ECT (lanes 3-4) or repeated ECT (lanes 5–6). This figure represents a typical result of three independent experiments.**

## DISCUSSION

In the present study, we focused on an EST, ADRG14, whose expression was increased after chronic antidepressant treatment and repeated ECT. Sequence and homology analysis of ADRG14 using the EMBL/GeneBank database showed significant matches to rat VAMP2.[16] Considerable evidence indicates that VAMP2 is an important component of the regulated secretory pathway at nerve terminals. It has been reported that VAMP2 interacts with syntaxin-1 and SNAP-25, constituting the SNARE complex, the biochemical intermediate essential for vesicular transport and/or fusion processes.[12–15] Fusion of vesicles with the plasma membrane leads to exocytosis, which mediates the release of neurotransmitter into the synaptic cleft. In this context, pharmacological modulation of VAMP2 gene expression would also be predicted to alter the secretory response of neurons. In the present study, we demonstrated a significant increase of both VAMP2 mRNA and protein levels in rat frontal cortex after chronic treatment with two different classes of antidepressants, imipramine and sertraline. Our data suggest that VAMP2 may be one of the common functional molecules induced after chronic antidepressant treatment. This altered pattern of VAMP2 gene expression was also observed after repeated ECT.

On the other hand, single administration of antidepressant or acute ECT did not induce VAMP2 expression, suggesting that the induction of VAMP2 is due to the long-term therapeutic action of these treatments. These findings suggest that genome-dependent alterations of the secretory behavior of neurons may be an important component of the therapeutic action of antidepressants and ECT. Interestingly, the work of others has shown that acute and chronic administration of antidepressants diminishes the release of glutamate and aspartate, and inhibits veratridine-evoked 5-HT release.[17]

An important feature of the action of antidepressants and ECT is that they did not globally alter the expression of other membrane-trafficking proteins. In contrast to the enhanced expression of VAMP2, we detected no significant change in the expression of other synaptic vesicle proteins, syntaxin-1 and SNAP-25. Although there are more than a dozen synaptic vesicle proteins,[18] we chose to investigate the expression of syntaxin-1 and SNAP-25 because they make a SNARE complex with VAMP2 and mediate the synaptic vesicle docking/fusion machinery. We reasoned that a coordinated change of VAMP2 and the expression of syntaxin-1 and SNAP-25 might signal a change in the overall number of SNARE complexes. An antidepressant-induced change in the expression of syntaxin-1 and SNAP-25, associated predominantly with the presynaptic plasma membrane, would have been indicative of more complex changes in the secretory pathway, such as an increase in the number of active zones. Instead, the absence of such a coordinated change in syntaxin-1 and SNAP-25 expression indicates that antidepressants or ECT produces a more selective modification of the regulated secretory machinery. Additional work will be necessary to understand the role of selective VAMP2 induction in rat frontal cortex.

Previously, we have demonstrated that a unique cysteine-rich protein, cysteine string protein (CSP), is clearly increased in the rat brain after chronic antidepressant treatment.[11] There are several reports indicating that the function of CSP in the central nervous system is to modulate the activity of presynaptic calcium channels, resulting in neurotransmitter release at the nerve terminal.[19,20] Similar to VAMP2, CSP is also localized to synaptic vesicle membranes and interacts with VAMP2 in rat brain.[21,22] Taken together, this coordinated induction of two presynaptic molecules may suggest that the number of secretory organelles, which includes both small clear vesicles as well as large dense-core granules, might be increased after chronic antidepressant treatment and repeated ECT. Interestingly, it is previously reported that the expression of VAMP2 and CSP was also enhanced by lithium ions, a well-established pharmacotherapy for the treatment of recurrent manic-depressive illness, in differentiated PC12 cells.[23,24] The coordinated induction of these genes may contribute to the therapeutic efficacy in mood disorders not only for depression, but also for manic-depressive illness.

Our findings suggest that gene-expression dependent alterations of the secretory behavior of neurons may be an important component of the pharmacological action of antidepressants and ECT. Thus, it will be of interest to determine whether these changes in the frontal cortex

contribute to clinical effects in patients treated with antidepressants and ECT. Indeed, the frontal cortex is one of the several brain regions that would be involved in the endocrine, emotional, cognitive, and vegetative abnormalities found in depressed patients. In the frontal cortex, glucose metabolism, blood flow, and electroencephalograph activity are altered in depressed patients.[25] It is reasonable to understand that alterations of mood, neurovegetative signs, or even social behavior of depressed patients reflect some changes in patterns of synaptic activity in the brain. Additional work would be necessary to test this hypothesis.

In conclusion, the current investigation has identified VAMP2 as a novel molecular target for antidepressants and ECT. Our findings offer novel insights into the actions of antidepressants and ECT that may be of both basic and clinical significance. Furthermore, the ADRG microarray we developed seemed to be a powerful tool for the discovery of novel therapeutic targets for future drug development with a new class of action in the brain.

## MATERIALS AND METHODS

### Experimental animals

Male Sprague-Dawley rats (age 7–10 weeks, Sankyo Labo Service Co. , Tokyo, Japan) were housed in a temperature-controlled environment with a 12 h light/12 h dark cycle and free access to food and water. Rats were randomly separated into control and treated groups. Six rats were used for each treatment group. Experimental animals for chronic treatment of antidepressants received either vehicle for 21 days, 10 mg/kg of imipramine (Sigma Chemical, MO, USA), or sertraline (Pfizer Pharmaceuticals Inc., NY, USA), dissolved in 1.5% Tween 80, by daily intraperitoneal injection. Rats for ECT were anesthetized with sevoflurane and received a 90 mA, 1.0 sec electric shock via ear-clip electrodes once (acute ECT group), or every other day for 14 days (repeated ECT group). ECT employed the Ugo Basile Model 7801 Unipolar square-wave electroconvulsive stimulation pulse generator (Stoelting Co., IL, USA). The control group was treated exactly the same as ECT-treated rats, but without the administration of the electric current. Animals were killed by decapitation 24 h after the final antidepressant administration or ECT treatment, and the brain was quickly removed, dissected and then frozen in liquid nitrogen immediately and stored at –80°C until use. All studies using animals were carried out in accordance with animal protocols approved by the National Institutes of Health, OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals.

### Identification of ADRG14

Fabrication of the ADRG microarray and fluorescence image analysis was done as described by our group previously.[10] Briefly, each of the cDNA inserts for ADRG were amplified by vector primers and negative controls, and 10 different kinds of housekeeping genes were spotted in duplicate on glass slides using a GMS417 Arrayer (Affymetrix, Inc., CA, USA). To make the fluorescence-labeled probe for hybridization, poly $A^+$ RNA was purified from total RNA that was pooled with three independent control or treated groups. One microgram of poly $A^+$ RNA from control or treated samples was converted to cDNA in the presence of Cy-5- or Cy-3-dUTP, respectively, to make fluorescence-labeled probes. Hybridization of probes to the microarray was done competitively in duplicate. The probes were mixed and placed on an array, overlaid with a coverslip, and hybridized for 16.5 h at 65°C. After the hybridization and washing procedure, each slide was scanned with a GMS418 Array Scanner (Affymetrix, Inc., CA, USA). Gene expression levels were quantified and analyzed using ImaGene software (Bio-Discovery Ltd. Swansea, UK). With our preliminary experiment (data not shown), the differences of fluorescence intensities ($\pm$2-fold) were regarded as significant.

Sequence analysis of ADRG14 was performed by dideoxy sequencing methods. Homology search and sequence alignment was done using the FASTA search servers at the National Center for Biotechnology Information.

### Messenger RNA Expression Analysis with RT-PCR

The first strand of cDNA was synthesized with reverse transcriptase and 1 μM of oligo-dT primer from 2 μg of total RNA samples treated with RNase-free DNase I and diluted to a final volume of 100 μL. One microliter of each cDNA sample was added to 24 μl of PCR reaction mixture containing 0.5 μM of a pair of primers for VAMP2 (5′-AGTCTAGTTTGCTTCCCTTACC-3′ and 5′-CAGTTTA-CATCTCCTTGGTTCC-3′) (Amersham Pharmacia Biotech, Tokyo, Japan). A pair of primers for a housekeeping gene, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH, 5′-TGAAGGTCGGTGTCAACGGATTTGGC-3′ and 5′-CATG-TAGGCCATGAGGTCCACCAC-3′) was also used for normalization. To ensure the fidelity of this analysis, we assayed several cycles of PCR to determine the linear range for the amplification of the PCR product. Amplification of VAMP2 was performed as follows: 3 min at 94°C for initial denaturation, 22 cycles of 94°C denaturing for 30 s, 51°C annealing for 30 s, and 72°C extension for 1 min, followed by a final extension at 72°C for 7 min. Amplification of GAPDH was performed as follows: 3 min at 94°C for initial denaturation, 18 cycles of 94°C denaturing for 30 s, 51°C annealing for 30 s, and 72°C extension for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were electrophoresed in a 1% agarose gel containing SYBR green, a nucleic acid gel stain reagent (Takara, Tokyo, Japan). The optical density of the digitized image was quantified using a fluorescence image analyzer, FM-bio II (Hitachi, Tokyo, Japan). Without reverse transcriptase, we found no PCR products in a gel, indicating that genomic DNA contamination was negligible.

### Western Blot Analysis

Frontal cortex from control and treated rats was homogenized in ice-cold Sucrose-Tris buffer (250 mM sucrose, 50 mM Tris-HCl, 5 mM EDTA, 10 mM EGTA, 0.3%. mercaptoethanol, pH 7.4). The protein concentration was deter-

mined by the Bradford method using the Bio-Rad protein assay kit. Each fraction (20 μg of protein) was separated by 10% SDS-PAGE after solubilization and boiling in Laemmli buffer. Electrophoretic protein was transferred from gels to nitrocellulose membranes using standard techniques. The membranes were blocked and incubated with antibody using Aurora Western Blotting kit (ICN Biomedicals Inc., CA, USA) following the manufacturer's instructions. The primary antibodies for VAMP2, SNAP-25 or syntaxin-1 (Wako, Osaka, Japan) were diluted 1:500, 1:1000, or 1:500, respectively, in blocking buffer followed by appropriate secondary antibodies. The immunoreactive bands were visualized on film by the ECL system. To ensure the fidelity of this analysis, we assayed the film exposed only in the linear range. The optical density of the digitized image was quantified using NIH image, software running on an Apple Computer. NIH Image is a public domain program (developed at the US National Institutes of Health) and available on the Internet at http://rsb.info.nih.gov/nih-image/).

### Statistical analysis

Data are given as mean ± SEM for the group. Differences were assessed using Analysis of Variance (ANOVA) followed by the Dunnett's test. A value of $P<0.05$ was regarded as significant.

## ACKNOWLEDGEMENTS

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## DUALITY OF INTEREST

None declared.

## REFERENCES

1 Wittchen HU, Knauper B, Kessler RC. Lifetime risk of depression. *Br J Psychiatry* 1994; **s26**: 16–22.
2 Dahmen N, Fehr C, Reuss S, Hiemke C. Stimulation of immediate early gene expression by desipramine in rat brain. *Biol Psychiatry* 1997; **42**: 317–323.
3 Torres G, Horowitz JM, Laflamme N, Rivest S. Fluoxetine induces the transcription of genes encoding c-fos, corticotropin-releasing factor and its type 1 receptor in rat brain. *Neuroscience* 1998; **87**: 463–477.
4 Johansson IM, Bjartmar L, Marcusson J, Ross SB, Seckl JR, Olsson T. Chronic amitriptyline treatment induces hippocampal NGFI-A glucocorticoid receptor and mineralocorticoid receptor mRNA expression in rats. *Brain Res Mol Brain Res* 1998; **62**: 92–95.
5 Bjartmar L, Johansson IM, Marcusson J, Ross SB, Seckl JR, Olsson T. Selective effects on NGFI-A, MR, GR and NGFI-B hippocampal mRNA expression after chronic treatment with different subclasses of antidepressants in the rat. *Psychopharmacology* 2000; **151**: 7–12.
6 Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S *et al*. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J Neurosci* 2000; **20**: 4030–4036.
7 Chen J, Nye HE, Kelz MB, Hiroi N, Nakabeppu Y, Hope BT *et al*. Regulation of delta FosB and FosB-like proteins by electroconvulsive seizure and cocaine treatments. *Mol Pharmacol* 1995; **48**: 880–889.
8 Reti IM, Baraban JM. Sustained increase in Narp protein expression following repeated electroconvulsive seizure. *Neuropsychopharmacology* 2000; **23**: 439–443.
9 Yamada M, Kiuchi Y, Nara K, Kanda Y, Morinobu S, Momose K *et al*. Identification of a novel splice variant of heat shock cognate protein 70 after chronic antidepressant treatment in rat frontal cortex. *Biochem Biophys Res Commun* 1999; **261**: 541–545.
10 Yamada M, Yamada M, Yamazaki S, Takahashi K, Nishioka G, Kudo K *et al*. Identification of a novel gene with RING-H2 finger motif induced after chronic antidepressant treatment in rat brain. *Biochem Biophys Res Commun* 2000; **278**: 150–157.
11 Yamada M, Yamada M, Yamazaki S, Takahashi K, Nara K, Ozawa H *et al*. Induction of cysteine string protein after chronic antidepressant treatment in rat frontal cortex. *Neurosci Lett* 2001; **301**: 183–186.
12 Trimble WS, Cowan DM, Scheller RH. VAMP-1: a synaptic vesicle-associated integral membrane protein. *Proc Natl Acad Sci USA* 1988; **85**: 4538–4542.
13 Oyler GA, Higgins GA, Hart RA, Battenberg E, Billingsley M, Bloom FE *et al*. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. *J Cell Biol* 1989; **109**: 3039–3052.
14 Bennett MK, Calakos N, Scheller RH. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science* 1992; **257**: 255–259.
15 Weis WI, Scheller RH. Membrane fusion. SNARE the rod, coil the complex. *Nature* 1998; **395**: 328–329.
16 Elferink LA, Trimble WS, Scheller RH. Two vesicle-associated membrane protein genes are differentially expressed in the rat central nervous system. *J Biol Chem* 1989; **264**: 11 061–11 064.
17 Golembiowska K, Dziubina A. Effect of acute and chronic administration of citalopram on glutamate and aspartate release in the rat prefrontal cortex. *Pol J Pharmacol* 2000; **52**: 441–448.
18 Sudhof TC. The synaptic vesicle cycle: a cascade of protein-protein interactions. *Nature* 1995; **375**: 645–653.
19 Gundersen CB, Umbach JA. Suppression cloning of the cDNA for a candidate subunit of a presynaptic calcium channel. *Neuron* 1992; **9**: 527–537.
20 Umbach JA, Zinsmaier KE, Eberle KK, Buchner E, Benzer S, Gundersen CB. Presynaptic dysfunction in Drosophila csp mutants. *Neuron* 1994; **13**: 899–907.
21 Leveque C, Pupier S, Marqueze B, Geslin L, Kataoka M, Takahashi M *et al*. Interaction of cysteine string proteins with the alpha1A subunit of the P/Q-type calcium channel. *J Biol Chem* 1998; **273**: 13 488–13 492.
22 Chamberlain LH, Burgoyne RD. Cysteine-string protein: the chaperone at the synapse. *J Neurochem* 2000; **74**: 1781–1789.
23 Cordeiro ML, Umbach JA, Gundersen CB. Lithium ions enhance cysteine string protein gene expression in vivo and in vitro. *J Neurochem* 2000; **74**: 2365–2372.
24 Cordeiro ML, Umbach JA, Gundersen CB. Lithium ions Up-regulate mRNAs encoding dense-core vesicle proteins in nerve growth factor-differentiated PC12 cells. *J Neurochem* 2000; **75**: 2622–2625.
25 Drevets W, Videen T, Price J, Preskorn S, Carmichael S, Raichle M. A functional anatomical study of unipolar depression. *J Neurosci* 1992; **12**: 3628–3641.