Full Paper

The Mechanisms of Electroconvulsive Stimuli in BrdU-Positive Cells of the Dentate Gyrus in ACTH-Treated Rats

Keiko Kuwatsuka¹, Hiromi Hayashi¹, Yuka Onoue¹, Ikuko Miyazaki², Toshihiro Koyama¹, Masato Asanuma², Yoshihisa Kitamura^{1,*}, and Toshiaki Sendo¹

¹Department of Clinical Pharmacy, ²Department of Brain Science, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

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Abstract. In clinical studies, electroconvulsive stimuli have been associated with improvements in both depression and treatment-resistant depression. In a previous study, treatment with adrenocorticotrophic hormone (ACTH) for 14 days decreased adult hippocampal cell proliferation. Furthermore, electroconvulsive stimuli significantly decreased the duration of immobility following repeated administration of ACTH for 14 days in rats. The present study was undertaken to further characterize the mechanism of treatment-resistant antidepressant effects of electroconvulsive stimuli by measuring cell proliferation, brain-derived neurotrophic factor (BDNF) levels, and phosphorylated and total cyclic adenosine monophosphate (cAMP) response element-binding protein (pCREB/CREB) levels in the hippocampus of ACTH-treated rats. Electroconvulsive stimuli increased cell proliferation in both saline-treated and ACTH-treated rats. Mature-BDNF protein levels showed a tendency to decrease in ACTH-treated rats. Electroconvulsive stimuli treatment increased mature-BDNF protein levels in the hippocampus of both saline-treated and ACTH-treated rats. Furthermore, electroconvulsive stimuli increased phospho-Ser133-CREB (pCREB) levels and the ratio of pCREB/CREB in both saline-treated and ACTH-treated rats. These findings suggest that the treatment-resistant antidepressant effects of electroconvulsive stimuli may be attributed, at least in part, to an enhancement of hippocampal cell proliferation.

Keywords: adrenocorticotrophic hormone, electroconvulsive stimuli, cell proliferation, brain-derived neurotrophic factor, cAMP response element binding protein

Introduction

Psychoendocrinological studies have focused on the regulation of the hypothalamic–pituitary–adrenal (HPA) axis in patients with depression (1). Multiple neuroendocrinological abnormalities appear in subjects with depressive disorders, including increases in cortisol secretion and the attenuation of cortisol suppression in response to dexamethasone (2). We previously reported that chronic administration of adrenocorticotrophic hormone (ACTH) to rats counteracted the decrease in immobility time induced by a tricyclic antidepressant, imipramine or desipramine, in the forced swim test,

which is widely used as a predictor of antidepressant activity (3). Chronic coadministration of lithium, an agent that potentiates the actions of antidepressants, and electroconvulsive therapy in patients with depression, including those with treatment-resistant depression, significantly decreased the duration of immobility, even when given concurrently with ACTH (3, 4). Chronic treatment of rats with ACTH may therefore be an effective model of tricyclic antidepressant treatment-resistant conditions.

Electroconvulsive therapy is a widely used and effective treatment for depressive patients, especially in severe intractable and antidepressant treatment-resistant depression (5). In a previous study, repeated electroconvulsive stimuli were found to be associated with decreased immobility time in the forced swim test in rats (4). In addition, electroconvulsive stimuli have been

*Corresponding author. kitamu-y@cc.okayama-u.ac.jp
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reported to increase brain-derived neurotrophic factor (BDNF) mRNA expression and BDNF protein levels in the rat hippocampus (6–8). It has also been reported that BDNF plays an important role in development, neural regeneration, synaptic transmission, synaptic plasticity and neurogenesis, and mossy fiber sprouting (9, 10). Furthermore, in humans, BDNF protein immunoreactivity was elevated in postmortem tissue from antidepressant-treated patients (11). In a study of the postmortem brains of suicide subjects, a reduced BDNF mRNA level was also reported in both the prefrontal cortex and hippocampus (12). These and related findings suggest a role for BDNF in the etiology and treatment of depression. On the other hand, chronic stress exposure decreases hippocampal cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) phosphorylation (pCREB) (13). CREB, in turn, regulates several targets involved in activity-dependent neuronal plasticity, including BDNF (13). Finally, we reported that repeated electroconvulsive stimuli treatment increased BDNF protein levels in the hippocampus in rats repeatedly treated with ACTH (4). These findings suggest that repeated electroconvulsive stimuli treatment has an antidepressant-like action in an animal model for tricyclic antidepressant-resistant depressive conditions through increased BDNF.

Chronic treatment with antidepressants may increase cell proliferation and cell survival and reverse stress-induced decreases in hippocampal cell proliferation and neurogenesis (14, 15). The ability to promote hippocampal neurogenesis is a feature of both classical antidepressants, such as tricyclic drugs, and selective serotonin re-uptake inhibitors (16, 17). Moreover, hippocampal cell proliferation and neurogenesis may be key factors in the actions of antidepressant drugs. However, it was also reported that electroconvulsive stimuli affect progenitors that exhibit a limited ability for cell proliferation (18). In a previous report, chronic administration of ACTH (100 μ g/rat, s.c., 14 days) was found to significantly decrease the number of 5-bromo-2'-deoxyuridine (BrdU)- and Ki-67 (an endogenous marker of cell proliferation)-positive cells compared with the control value in the subgranular zone (SGZ) of the hippocampal dentate gyrus (19, 20). This effect was not influenced by the chronic administration of imipramine, but was reversed by coadministration of imipramine and lithium for 14 days in ACTH-treated rats (20). Thus, these results suggest that imipramine and lithium may improve the efficacy of treatment for resistant depression by triggering neurogenesis.

In the present study, the ability of electroconvulsive stimuli to increase neurogenesis in ACTH-treated rats was examined. Additionally, the effect of electroconvul-

sive stimuli on pro- and mature-BDNF protein levels and CREB protein (which acts as a regulator of BDNF expression) in ACTH-treated rats was also investigated.

Materials and Methods

Animals

Wistar rats (Charles River, Yokohama) with an initial weight of 220–230 g were utilized. The rats were group-housed, 2 per cage, under a constant light–dark cycle (lights on 07:00–19:00) and fed standard laboratory food and tap water in an air-conditioned room (23°C $\pm$ 1°C, with approximately 60% humidity). All experiments were conducted according to the guidelines for animal experimentation at Okayama University Medical School. All efforts were made to minimize the number of animals used and their degree of suffering.

Drugs

Adrenocorticotrophic hormone-(1-24)-zinc (Cortrosyn[®]Z; Daiichi-Sankyo, Tokyo) was injected subcutaneously once a day at 100 μ g/rat (injection volume = 0.2 ml/rat) for 14 days. Control rats received an equivalent volume of saline. The thymidine-analog 5-bromo-2'-deoxyuridine (BrdU) (Sigma Aldrich, St. Louis, MO, USA) is incorporated into dividing cells during the S-phase and serves as a marker for cell proliferation. BrdU (50 mg/kg) was administered intraperitoneally 4 times at 6-h intervals after the last (14th) day of the electroconvulsive stimuli treatment period.

Electroconvulsive stimuli

Electroconvulsive stimuli (100 V, 50 mA, 0.2 s) were administered via ear electrodes before the administration of saline or ACTH. All rats receiving electroconvulsive stimuli showed typical tonic-clonic seizure lasting approximately 5–10 s. Sham electroconvulsive stimuli treatments were administered in the same manner without the application of electrical current.

Immunohistochemistry

Rats were transcardially perfused with ice-cold saline followed by a fixative containing 4% paraformaldehyde and 0.35% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under deep anesthesia with pentobarbital sodium (80 mg/kg) (Sigma Aldrich Japan, Tokyo) 24 h after the last BrdU injection. Following perfusion, brains were rapidly removed en bloc from skulls and post-fixed for 24 h in a fixative containing 4% paraformaldehyde in 0.1 M PB and stored in a cryoprotective solution containing 15% sucrose and 0.1% sodium azide in 0.1 M PB. Brains snap-frozen with powdered dry ice were dissected coronally on a cryostat into 20- μ m-thick sec-

tions containing the dentate gyrus of the hippocampus. Sections were collected in 10 mM phosphate-buffered saline (PBS) with 0.1% sodium azide for staining. Standard free-floating immunohistochemistry was used to detect BrdU-immunopositive signals in the dentate gyrus. Free-floating sections were subjected to deoxyribonucleic acid denaturation by incubation for 2 h in 50% formamide / $2 \times$ standard saline citrate (SSC) at 65°C followed by a $2 \times$ SSC rinse. Sections were incubated for 20 min in 2 N HCl, PBS and for 15 min in 0.1 M boric acid, pH 8.5. After incubation in 1% normal goat serum in PBST (10 mM PBS containing 0.2% Triton X-100) for 30 min, sections were exposed to a rat anti-BrdU monoclonal antibody (diluted 1:1000 in PBST; Abcam, Cambridge, MA, USA) for 18 h at 4°C. Sections were then washed in PBST (5×5 min) and incubated with a goat anti-rat IgG Alexa Fluor 488 antibody (diluted 1:1000 in PBST; Invitrogen, Carlsbad, CA, USA) for 2 h at room temperature. All slides were analyzed under a fluorescence microscope (Olympus BX50-FLA; Olympus, Tokyo) using a mercury lamp through a 470–490 nm band-pass filter to excite the Alexa Fluor 488. The light emitted from Alexa Fluor 488 was collected through a 515–550 nm band-pass filter or 470–490 nm long-pass filter. Stained cells were photographed at a magnification of $\times 200$. For each brain, serial sections were collected to generate five sets representative of the entire hippocampus (-2.3 mm to -4.3 mm from the bregma). Each set contained sections at least 20- μ m-thick (400- μ m apart) covering the entire anteroposterior extent of the hippocampus. The mean count obtained was multiplied by 5 (the number of series) to obtain an estimate of the total number of BrdU-positive cells throughout the dentate gyrus. The BrdU-positive cells of the dentate gyrus were counted by an investigator kept unaware of the treatment groups.

Western blot analysis

One day after the last ACTH and electroconvulsive stimuli treatment, nuclear protein was extracted from rat hippocampal tissues. Samples of homogenates were loaded on 1% sodium dodecyl sulfate-containing polyacrylamide gels and blotted onto polyvinylidene difluoride membranes (Hybond-P; Amersham, Buckinghamshire, UK). Blots were incubated with rabbit anti-BDNF polyclonal antibody (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-phospho CREB (Ser133) monoclonal antibody (1:1000 dilution, Santa Cruz Biotechnology), or rabbit anti-CREB polyclonal antibody (1:250 dilution, Santa Cruz Biotechnology) and then reacted with the corresponding secondary antibody conjugated to horseradish peroxidase. After

washing with 20 mM Tris-buffered saline containing 0.1% Tween 20, blots were developed using the ECL Western blotting detection system (Amersham). Images were obtained and quantified using a FUJIFILM Luminescent Image Analyzer LAS-3000 (FUJIFILM, Tokyo) and Multi Gauge (v3.0) software. For quantitative analysis, the signal ratio of a specific protein (relative chemiluminescence unit) to that of constitutively expressed β -actin protein was calculated to normalize for loading and transfer artifacts.

Statistical analyses

Quantifications were performed blindly with regard to treatment. Quantitative data were analyzed by one-way analysis of variance, followed by Tukey's test. Data are expressed as the mean $\pm$ S.E.M. Values of $P < 0.05$ were considered statistically significant.

Results

Effects of repeated electroconvulsive stimuli on cell proliferation in the SGZ of the hippocampal dentate gyrus in saline-treated and ACTH-treated rats

Immunohistochemistry was performed to examine whether electroconvulsive stimuli change the number of BrdU-positive cells in the SGZ of the hippocampal dentate gyrus. Treatment with ACTH for 14 days significantly decreased the number of BrdU-positive cells in the SGZ of the hippocampal dentate gyrus. Repeated electroconvulsive stimuli increased the number of BrdU-positive cells in the SGZ of both saline-treated and ACTH-treated rats (Fig. 1A).

Effects of repeated electroconvulsive stimuli on pro- and mature-BDNF protein in saline-treated and ACTH-treated rats

Electroconvulsive stimuli have been reported to increase BDNF mRNA expression and BDNF protein levels in the rat hippocampus (6–8). To confirm these findings and further examine pro- and mature-BDNF protein levels after electroconvulsive stimuli, we collected hippocampal tissue after exposure to electroconvulsive stimuli in ACTH-treated rats and performed western blot analyses. Figure 2 depicts the effects of repeated electroconvulsive stimuli treatment on pro- and mature-BDNF protein levels in the hippocampus in saline- or ACTH-treated rats. Pro- and mature-BDNF protein levels tended to decrease in ACTH-treated rats. Repeated electroconvulsive stimuli treatment increased mature-BDNF protein levels in the hippocampus of both saline-treated and ACTH-treated rats (Fig. 2B). However, pro-BDNF protein levels did not change as a result of treatment with electroconvulsive stimuli (Fig. 2A).

Fig. 1. Effects of repeated electroconvulsive stimuli for 14 days on the number of BrdU-positive cells in the subgranular zone of the hippocampal dentate gyrus in saline-treated and ACTH-treated rats. Representative fluorescence photomicrographs of the effects of chronic electroconvulsive stimuli for 14 days on the number of BrdU-positive cells of the dentate gyrus in saline-treated and ACTH-treated rats (B). BrdU-positive cells on either side were counted manually and the average of the immunopositive cell number was calculated. Each column was expressed as the mean $\pm$ S.E.M.; ECS, electroconvulsive stimuli; $n = 4 - 5$ per group; * $P < 0.05$ (vs. control), # $P < 0.05$ (vs. ACTH).

Effects of repeated electroconvulsive stimuli on tropomyosin-related kinase B (TrkB) and p75^{NTR} protein levels in saline-treated and ACTH-treated rats

We next examined whether electroconvulsive stimuli affected TrkB and p75^{NTR} protein levels, which are both known to be receptors for pro- and mature-BDNF. Figure 3 displays the effects of repeated electroconvulsive stimuli treatment on TrkB and p75^{NTR} protein levels in the hippocampus in saline- or ACTH-treated rats. Levels of these proteins did not change as a result of treatment with ACTH and electroconvulsive stimuli.

Effects of repeated electroconvulsive stimuli on total CREB and phospho-Ser133-CREB proteins in saline-treated and ACTH-treated rats

CREB regulates several targets involved in BDNF expression. In addition, phosphorylation of CREB at serine133 is essential for neuronal plasticity following

repeated exposure to drugs. The effects of repeated electroconvulsive stimuli treatment on total and phospho-Ser133-CREB levels in the hippocampus in saline or ACTH-treated rats are displayed in Fig. 4. Repeated electroconvulsive stimuli increased phospho-Ser133-CREB levels in both saline-treated and ACTH-treated rats (Fig. 4A). Additionally, the ratio of pCREB/CREB increased as a result of electroconvulsive stimuli in saline- and ACTH-treated rats (Fig. 4B). However, total CREB did not change as a result of treatment with ACTH and electroconvulsive stimuli (Fig. 4A).

Discussion

Electroconvulsive therapy is considered to be the most effective biological treatment for depression, especially in severe intractable depression or treatment-resistant major depression (5). Previous studies using a behavioral

Fig. 2. Effects of repeated electroconvulsive stimuli for 14 days on pro-BDNF (A) and mature-BDNF protein levels (B) in the hippocampus in saline-treated and ACTH-treated rats. Each column was expressed as the mean $\pm$ S.E.M.; ECS, electroconvulsive stimuli; $n = 6 - 8$ per group; ** $P < 0.01$ (vs. control), ## $P < 0.01$ (vs. ACTH).

screening method for antidepressant activity in rodents have shown that repeated treatment with electroconvulsive stimuli has an antidepressant-like action in rats in the forced swim test (4, 21). Conversely, results from a previous experiment demonstrated that a decrease in immobility induced by single and repeated administrations of imipramine and desipramine was blocked by the repeated administration of ACTH for 14 days (3). In addition, the use of lithium has been shown to be effective in the treatment of several forms of affective disorders such as treatment-resistant depression. Therefore, using lithium in combination with tricyclic antidepressants may be a promising way to improve efficacy in the treatment of resistant depression. Furthermore, results from a previous study demonstrated that repeated

Fig. 3. Effects of repeated electroconvulsive stimuli for 14 days on TrkB (A) and p75^{NTR} (B) protein levels in the hippocampus in saline-treated and ACTH-treated rats. Each column was expressed as the mean $\pm$ S.E.M.; ECS, electroconvulsive stimuli; $n = 6 - 8$ per group.

electroconvulsive stimuli for 6 days and 14 days decreased the duration of immobility in the forced swim test in rats chronically treated with ACTH (4). These findings suggest that ACTH-treated rats may serve as an animal model for tricyclic antidepressant treatment-resistant depression conditions.

Chronic treatment with ACTH was observed to result in a decrease in cell proliferation in the SGZ of the hippocampal dentate gyrus (20). In the present study, electroconvulsive stimuli increased neurogenesis in ACTH-treated rats. The results from several studies strongly suggest that BDNF plays a role in the survival of neurons during hippocampal development and that

Fig. 4. Effects of repeated electroconvulsive stimuli for 14 days on CREB and pCREB protein levels (A) and the pCREB/CREB ratio (B) in the hippocampus in saline-treated and ACTH-treated rats. Each column was expressed as the mean $\pm$ S.E.M.; ECS, electroconvulsive stimuli; $n = 6-8$ per group; ** $P < 0.01$ (vs. control), ## $P < 0.01$ (vs. ACTH).

this action may relate to its putative role in depression, particularly via the hippocampus. A role for BDNF in the action of antidepressant treatments has been supported by several lines of evidence. BDNF mRNA expression is decreased by stress and glucocorticoids in the hippocampus (22, 23), while a range of antidepressants increased BDNF protein levels (6). Conversely, pretreatment with antidepressants results in chronic blockade of stress-induced decreases in BDNF mRNA expression in the hippocampus (6). Furthermore, intracerebral BDNF infusion results in antidepressant-like activity in animal

models of depression (24). Lastly, heterozygous BDNF knockout mice were shown to be unresponsive to antidepressants in the forced swim test (25). In a previous study, the finding of significantly decreased BDNF mRNA levels following chronic treatment with ACTH was confirmed. Decreased levels of BDNF may therefore contribute to the observed suppression of cell proliferation in the hippocampus of rats treated with ACTH.

BDNF is translated as precursor protein (pro-BDNF) and then proteolytically cleaved to generate a small mature protein (mature-BDNF). The results of the present study demonstrated that repeated treatment with electroconvulsive stimuli significantly increased mature-BDNF protein levels in the hippocampus of ACTH-treated rats. This finding suggests that enhanced hippocampal neurogenesis by electroconvulsive stimuli may contribute to increased levels of mature-BDNF. BDNF binds to high-affinity TrkB receptors and also to common low-affinity p75 receptors (p75^{NTR}). Initially, TrkB, a protein tyrosine kinase receptor expressed at high levels in the brain, was identified (26) and was subsequently identified as a receptor for BDNF (27). TrkB receptors exert trophic effects, whereas p75^{NTR} belongs to the tumor necrosis factor family and has a death domain that plays a role in apoptosis (28). Among the mature-BDNF and its specific receptors, TrkB receptors are highly expressed in the adult brain and are essential for survival of neurons and neurotransmission (29). Results from the present study indicated no changes in TrkB or p75^{NTR} protein levels. It was reported that acute electroconvulsive stimuli attenuated TrkB phosphorylation (activation) in the prefrontal cortex. However, protein levels of TrkB were not altered by electroconvulsive stimuli (30). Furthermore, electroconvulsive stimuli rapidly and robustly induced BDNF mRNA expression in the frontal cortex and hippocampus (6). These findings suggest that electroconvulsive stimuli induce a rapid activation of TrkB signaling, which is likely a consequence of the stimulatory effect of electroconvulsive stimuli on BDNF synthesis.

The genomic analysis of the structure of the BDNF promoter indicates the presence of several cAMP response element sites that bind the transcription factor CREB when phosphorylated at Ser133. CREB is involved in both the adaptive response to stress and in the physiological and pharmacological regulation of BDNF expression (31, 32). In a previous study, electroconvulsive stimuli consistently up-regulated several components of this pathway in rat and mouse brain tissue, including CREB and pCREB levels (33, 34). These effects may elucidate elements of the mechanism of action of electroconvulsive stimuli. Studies of downstream targets of the cAMP pathway revealed that

BDNF, which regulates neuronal survival and synaptic plasticity, was also increased by electroconvulsive stimuli in the cerebral cortex and hippocampus (6, 35). In the present study, electroconvulsive stimuli significantly increased pCREB levels and the pCREB/CREB ratio in ACTH-treated rats. Therefore, the observed antidepressant-like effects may have been related to enhanced cell proliferation in the hippocampus via the activation of the BDNF-CREB pathway.

In conclusion, the results from the present study demonstrate that electroconvulsive stimuli increase cell proliferation in the hippocampal dentate gyrus in ACTH-treated rats. In addition, alterations in BDNF expression and phosphorylation of the Ser133 site on CREB, a fundamental step in CREB activation, were stimulated by electroconvulsive stimuli in the hippocampus of normal and ACTH-treated rats. When combined with the results of previous studies, these results support the assertion that electroconvulsive stimuli may improve the efficacy of treatment for treatment-resistant depression by triggering cell proliferation via BDNF.

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References

- Carroll BJ, Curtis GC, Mendels J. Neuroendocrine regulation in depression. II. Discrimination of depressed from nondepressed patients. *Arch Gen Psychiatry*. 1976;33:1051–1058.
- Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, et al. A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. *Arch Gen Psychiatry*. 1981;38:15–22.
- Kitamura Y, Araki H, Suemaru K, Gomita Y. Effects of imipramine and lithium on wet-dog shakes mediated by the 5-HT_{2A} receptor in ACTH-treated rats. *Pharmacol Biochem Behav*. 2002;72:397–402.
- Li B, Suemaru K, Cui R, Kitamura Y, Gomita Y, Araki H. Repeated electroconvulsive stimuli increase brain-derived neurotrophic factor in ACTH-treated rats. *Eur J Pharmacol*. 2006;529:114–121.
- Fink M. How does convulsive therapy work? *Neuropsychopharmacology*. 1990;3:73–82.
- Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci*. 1995;15:7539–7547.
- Altar CA, Laeng P, Jurata LW, Brockman JA, Lemire A, Bullard J, et al. Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. *J Neurosci*. 2004;24:2667–2677.
- Segawa M, Morinobu S, Matsumoto T, Fuchikami M, Yamawaki S. Electroconvulsive seizure, but not imipramine, rapidly up-regulates pro-BDNF and t-PA, leading to mature BDNF production, in the rat hippocampus. *Int J Neuropsychopharmacol*. 2013;16:339–350.
- Schinder AF, Berninger B, Poo M. Postsynaptic target specificity of neurotrophin-induced presynaptic potentiation. *Neuron*. 2000;25:151–163.
- Pencea V, Bingaman KD, Wiegand SJ, Luskin MB. Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. *J Neurosci*. 2001;21:6706–6717.
- Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry*. 2001;50:260–265.
- Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry*. 2003;60:804–815.
- Gourley SL, Kiraly DD, Howell JL, Olausson P, Taylor JR. Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. *Biol Psychiatry*. 2008;64:884–890.
- Fuchs E, Czeh B, Flugge G. Examining novel concepts of the pathophysiology of depression in the chronic psychosocial stress paradigm in tree shrews. *Behav Pharmacol*. 2004;15:315–325.
- Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology*. 2003;28:1562–1571.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*. 2003;301:805–809.
- Kodama M, Fujioka T, Duman RS. Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. *Biol Psychiatry*. 2004;56:570–580.
- Nakamura K, Ito M, Liu Y, Seki T, Suzuki T, Arai H. Effects of single and repeated electroconvulsive stimulation on hippocampal cell proliferation and spontaneous behaviors in the rat. *Brain Res*. 2013;1491:88–97.
- Doi M, Miyazaki I, Nagamachi T, Shinomiya K, Matsunaga H, Sendo T, et al. Effects of imipramine and lithium on the suppression of cell proliferation in the dentate gyrus of the hippocampus in adrenocorticotrophic hormone-treated rats. *Acta Med Okayama*. 2010;64:219–223.
- Kitamura Y, Doi M, Kuwatsuka K, Onoue Y, Miyazaki I, Shinomiya K, et al. Chronic treatment with imipramine and lithium increases cell proliferation in the hippocampus in adrenocorticotrophic hormone-treated rats. *Biol Pharm Bull*. 2011;34:77–81.
- Kawashima K, Araki H, Uchiyama Y, Aihara H. Amygdaloid catecholaminergic mechanisms involved in suppressive effects of electroconvulsive shock on duration of immobility in rats forced to swim. *Eur J Pharmacol*. 1987;141:1–6.
- Smith MA, Zhang LX, Lyons WE, Mamounas LA. Anterograde transport of endogenous brain-derived neurotrophic factor in hippocampal mossy fibers. *Neuroreport*. 1997;8:1829–1834.
- Vaidya VA, Marek GJ, Aghajanian GK, Duman RS. 5-HT_{2A} receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J Neurosci*.

- 1997;17:2785–2795.
- 24 Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem Behav.* 1997;56:131–137.
- 25 Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, et al. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J Neurosci.* 2003;23:349–357.
- 26 Middlemas DS, Lindberg RA, Hunter T. trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. *Mol Cell Biol.* 1991;11:143–153.
- 27 Soppet D, Escandon E, Maragos J, Middlemas DS, Reid SW, Blair J, et al. The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell.* 1991;65:895–903.
- 28 Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci.* 2005;25:5455–5463.
- 29 Minichiello L. TrkB signalling pathways in LTP and learning. *Nat Rev Neurosci.* 2009;10:850–860.
- 30 Hansen HH, Rantamaki TP, Larsen MH, Woldbye DP, Mikkelsen JD, Castren EH. Rapid activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway by electroconvulsive shock in the rat prefrontal cortex is not associated with TrkB neurotrophin receptor activation. *Cell Mol Neurobiol.* 2007;27:585–594.
- 31 Alfonso J, Frick LR, Silberman DM, Palumbo ML, Genaro AM, Frasch AC. Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. *Biol Psychiatry.* 2006;59:244–251.
- 32 Nair A, Vadodaria KC, Banerjee SB, Benekareddy M, Dias BG, Duman RS, et al. Stressor-specific regulation of distinct brain-derived neurotrophic factor transcripts and cyclic AMP response element-binding protein expression in the postnatal and adult rat hippocampus. *Neuropsychopharmacology.* 2007;32:1504–1519.
- 33 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.
- 34 Tanis KQ, Duman RS, Newton SS. CREB binding and activity in brain: regional specificity and induction by electroconvulsive seizure. *Biol Psychiatry.* 2008;63:710–720.
- 35 Fujimaki K, Morinobu S, Duman RS. Administration of a cAMP phosphodiesterase 4 inhibitor enhances antidepressant-induction of BDNF mRNA in rat hippocampus. *Neuropsychopharmacology.* 2000;22:42–51.