Superoxide dismutase overexpression protects against glucocorticoid-induced depressive-like behavioral phenotypes in mice

Yuki Uchihara¹, Ken-ichiro Tanaka¹, Teita Asano¹, Fumiya Tamura¹, Tatsuya Hoshino¹, Tohru Mizushima²

¹Department of Drug Discovery and Development, Faculty of Pharmacy, Keio University, Tokyo 105-8512, Japan.

²LTT Bio-Pharma Co., Ltd, Shiodome Building 3F, 1-2-20 Kaigan, Minato-ku, Tokyo 105-0022, Japan.

Corresponding author:

Dr. Ken-ichiro Tanaka, Department of Drug Discovery and Development, Faculty of Pharmacy, Keio University, 1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, Japan. TEL & FAX: 81-3-5400-2620, E-mail: tanaka-kn@pha.keio.ac.jp

Abstract

In the stress response, activation of the hypothalamic-pituitary-adrenal axis, and particularly the release of glucocorticoids, plays a critical role. However, dysregulation of this system and sustained high plasma levels of glucocorticoids can result in depression. Recent studies have suggested the involvement of reactive oxygen species (ROS), such as superoxide anion, in depression. However, direct evidence for a role of ROS in the pathogenesis of this disorder is lacking. In this study, using transgenic mice expressing human Cu/Zn-superoxide dismutase (SOD1), an enzyme that catalyzes the dismutation of superoxide anions, we examined the effect of SOD1 overexpression on depressive-like behavioral phenotypes in mice. Depressive-like behaviors were induced by daily subcutaneous administration of the glucocorticoid corticosterone for 4 weeks, and was monitored with the social interaction test, the sucrose preference test and the forced swim test. These tests revealed that transgenic mice overexpressing SOD1 are more resistant to glucocorticoid-induced depressive-like behavioral disorders than wild-type animals. Furthermore, compared with wild-type mice, transgenic mice showed a reduction in the number of 8-hydroxy-2'-deoxyguanosine (a marker of oxidative stress)-positive cells in the hippocampal CA3 region following corticosterone administration. These results suggest that overexpression of SOD1 protects mice against glucocorticoid-induced depressive-like behaviors by decreasing cellular ROS levels.

Abbreviations: ANOVA, analysis of variance; EC-SOD, extracellular SOD; HPA axis, hypothalamic-pituitary-adrenal axis; PC-SOD, lecithinized SOD; ROS, reactive oxygen species; SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

1. Introduction

Chronic and uncontrollable psychological stress causes psychiatric diseases, including major depression [1]. WHO has estimated that depression affects more than 121 million people worldwide, and the continuous increase in patients with depression is a major medical and social problem, with an economic burden estimated at approximately 83 billion dollars in the United States alone [2].

Decreased monoaminergic neurotransmission appears to play a major role in the pathogenesis of depression, based on observations that brain levels of serotonin and noradrenaline are reduced in patients with major depression [3]. Drugs that stimulate monoaminergic neurotransmission by inhibiting monoamine reuptake (such as selective serotonin reuptake inhibitors and serotonin and noradrenaline reuptake inhibitors) or by inhibiting monoamine degradation (such as tranylcypromine) are widely used for patients with major depression [3]. However, these drugs have several drawbacks, such as low remission rates, delayed response times and adverse side effects [4]. Thus, the development of better antidepressants is of major clinical importance.

It was previously thought that depression involved only a dysfunction of synaptic (monoaminergic) neurotransmission. However, new studies indicate that structural changes also play a major role in the pathogenesis of depression [5]. Indeed, atrophy of brain regions involved in mood and cognition has been observed in patients with major depression, and decreased neural synaptogenesis and adult neurogenesis in the hippocampus has been found in animals subjected to chronic psychological stress [6-9]. Furthermore, it was recently reported that the inhibition or enhancement of adult neurogenesis in the hippocampus by transgenic techniques induces or suppresses, respectively, depressive-like phenotypes in mice [10].

In the systemic response to psychological stress, activation of the hypothalamic-pituitary-adrenal (HPA) axis, especially the release of glucocorticoids, plays a critical role [11]. The acute response to stress mediated by the HPA axis has various homeostatic functions, including reducing energy consumption and controlling glucocorticoid levels through negative feedback regulation [11]. However, excessively high or long-lived psychological stress impairs negative feedback regulation, resulting in sustained high plasma glucocorticoid levels [12]. This hypercortisolemia is thought to play an important role in depression, because chronic glucocorticoid administration to animals causes depressive-like behavioral disorders, neural synaptic loss and inhibition of adult neurogenesis [13-15]. Furthermore, high plasma glucocorticoid levels are observed in patients with depression [16]. Thus, animals given glucocorticoid (such as corticosterone) administration are good models of depression [17].

To protect against reactive oxygen species (ROS), the body has a well-developed antioxidant system, with catalase, superoxide dismutase (SOD) and glutathione peroxidase the main antioxidant enzymes. SOD, which has three isoforms (Cu/Zn-SOD (SOD1), manganese-SOD and extracellular (EC)-SOD), catalyzes the dismutation of the superoxide anion to hydrogen peroxide [18]. We previously reported that PC-SOD, a lecithinized derivative of SOD1 with a higher stability in plasma and a greater affinity for tissue, has therapeutic effects in animal models of various diseases, such as idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, ulcerative colitis, focal cerebral ischemic injury and spinal cord injury-induced motor dysfunction [19-23].

Recent studies suggest that ROS, such as superoxide anions, play an important role in the pathogenesis of depression by causing neural structural damage. Chronic glucocorticoid administration increases levels of oxidative stress markers and decreases levels of endogenous antioxidants in the brains of animals. Furthermore, increased levels of oxidative

stress markers have been observed in the brains of depressive patients [24-26]. It has been reported that reducing ROS levels by genetic deletion of nicotinamide adenine dinucleotide phosphate oxidase suppresses depressive-like phenotypes in mice [27]. These observations suggest that antioxidant proteins such as SOD may be able to alleviate the symptoms of depression. However, there is currently no report providing experimental support for this concept.

In this study, we found that compared with wild-type mice, transgenic mice expressing human SOD1 showed resistance to depressive-like behavioral disorders induced by chronic glucocorticoid administration. Based on these findings, we propose that SODs are promising therapeutic candidates for depression.

2. Materials and methods

2.1. Materials

Sucrose was obtained from Nacalai Tesque (Kyoto, Japan). Peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulins was from Abcam (Cambridge, MA, USA). An antibody against 8-hydroxy-2'-deoxyguanosine (8-OHdG) was from the Japan Institute for the Control of Aging (Shizuoka, Japan). Corticosterone was obtained from Tokyo Chemical Industry (Tokyo, Japan). The assay kit for SOD was from Cayman Chemical (Ann Arbor, MI). Olive oil was from Wako Pure Chemical Industries (Osaka, Japan). The DV-Track Video Tracking System was from Muromachi Kikai (Tokyo, Japan). Malinol was from Muto Pure Chemicals (Tokyo, Japan). Mayer's hematoxylin was from Muto Pure Chemicals (Tokyo, Japan).

2.2. Animals and corticosterone administration

Transgenic mice expressing the human SOD1 transgene (C57BL/6, hSOD1^{+/-}) were gifts from Dr. T. Araki (Department of Peripheral Nervous System Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan). All experiments in this study were performed on 8–10-week-old male mice. C57BL/6 mice (wild-type) were from Japan Charles River (Yokohama, Japan). The experiments and procedures described here were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and were approved by the Animal Care Committee of Keio University.

To induce depressive-like behavior, corticosterone (0.5 mg in 200 µl olive oil) was subcutaneously injected daily for 4 weeks. The vehicle group was injected with olive oil only.

2.3. Social interaction, sucrose preference and forced swim tests

The social interaction test was performed as described previously with minor modification [28]. For habituation to the environment, the mouse was kept for 150 s in an open field chamber (50 cm × 50 cm) with an empty wire mesh circle cage (diameter, 8 cm) located along one of the walls (Fig. 1A). Then, the mouse was kept for 150 s in the same chamber, but with an unfamiliar C57BL/6 mouse enclosed in the mesh cage. An open field was divided into 5 zones, 1 interaction zone and 4 corner zones (Fig. 1A), and the time spent in the interaction zone and corner zones was measured by a computer based video-tracking system.

The sucrose preference test was performed as described previously with some minor modifications [29]. Mice were habituated to drink 1% sucrose solution for 4 h. The next day, after a 6-h water deprivation period, mice were placed with identical bottles of 1% sucrose and water for 4 h, and the drinking bottles were switched position every 2 h to avoid location preference. After the test, the consumption of each fluid was determined, and the sucrose preference index (%) was calculated as $([\text{sucrose solution consumption}]/[\text{sucrose solution consumption} + \text{water consumption}])$.

The forced swim test was conducted as described previously [30]. Mice were subjected to forced swimming for 5 min, and the immobility time was calculated.

2.4. Measurement of SOD activity

SOD activity in the hippocampal homogenate was measured using an assay kit according to the manufacturer's instructions.

2.5. Immunohistochemical analysis

The brains were fixed in 4% buffered paraformaldehyde for 48 h and then embedded in paraffin before being cut into 6- μ m-thick sections. Sections were treated for antigen retrieval using a microwave in 0.01 M citrate buffer for 15 min, and then incubated with 0.3% hydrogen peroxide in methanol for removal of endogenous peroxidase. Sections were blocked with 2.5% goat serum for 30 min and then incubated for 12 h with antibody against 8-OHdG (1:200 dilution) at 4°C. After blocking with 3% BSA for 10 min, sections were incubated with peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulin for 1 h. After addition of 3,3'-diaminobenzidine, sections were incubated with Mayer's hematoxylin. Samples were mounted with malinol and analyzed with a NanoZoomer-XR digital slide scanner (Hamamatsu Photonics, Shizuoka, Japan). The number of 8-OHdG-positive cells was determined using Definiens Tissue Studio software (Definiens AG, Munich, Germany).

2.6. Statistical analysis

All values are expressed as the mean $\pm$ SEM. One or two-way analysis of variance (ANOVA) followed by the Tukey test was used to evaluate differences between more than three groups. Student's *t*-test was used to evaluate differences between more than two groups. Differences were considered to be significant at $P < 0.05$.

3. Results and Discussion

3.1. Effect of SOD1 expression on corticosterone administration-induced behavioral disorders

Rodents subjected to repeated corticosterone administration exhibit social avoidance behavior [8], which we used here as an animal model of depression. To evaluate the effect of SOD1 overexpression on depressive-like behavior, we used the social interaction test, in which avoidance behavior to an unfamiliar mouse can be quantitatively assessed. Using this test, we examined the difference between transgenic mice overexpressing SOD1 and wild-type mice after daily corticosterone administration. The time spent in the social interaction zone (an area around the mesh cage where an unfamiliar C57BL/6 mouse is located, Fig. 1A) was significantly decreased, while time spent in the social avoidance zones (corner zones in Fig. 1A) was significantly increased, in wild-type mice following daily corticosterone administration for 4 weeks (Fig. 1B). Furthermore, time in the social interaction zone was significantly greater, while time in the social avoidance zones was significantly lower, in transgenic mice expressing SOD1 compared with wild-type mice after daily corticosterone administration. There were no significant differences between these two genotypes in animals not administered corticosterone (Fig. 1B). These results suggest that daily corticosterone administration induces social avoidance behavior, and that SOD1 overexpression can abrogate this effect of the glucocorticoid.

Anhedonia (decreased pleasure seeking behavior) is a hallmark symptom of depression, and can be monitored in mice based on a decreased preference for sucrose solution compared with water [31]. The sucrose preference index (volume of sucrose intake relative to the combined intake of sucrose and water) was significantly decreased in wild-type mice given daily corticosterone administration (Fig. 2A). This index was higher in the SOD1 transgenic

mice than in the wild-type mice after daily corticosterone administration. There was no difference in this index between these two genotypes without corticosterone administration (Fig. 2A). These results suggest that daily corticosterone administration induces anhedonia, and that SOD1 overexpression can block this effect.

Immobility time in the forced swim test is an indicator of despair and depression in mice, and chronic administration of corticosterone has been reported to increase immobility time [14]. Immobility time was increased by daily corticosterone administration in wild-type mice, but not in the SOD1 transgenic mice (Fig. 2B). Taken together, our findings suggest that SOD1 overexpression protects against corticosterone administration-induced depressive-like behavioral disorders.

3.2. Effects of corticosterone administration and SOD1 overexpression on oxidative stress levels

The preceding results suggest that SOD1 overexpression protects against corticosterone administration-induced behavioral disorders by decreasing oxidative stress. Therefore, we examined the effects of corticosterone administration and SOD1 overexpression on oxidative stress in the brain. We first examined the effect of corticosterone administration on SOD activity. Chronic corticosterone administration decreased SOD activity in the hippocampus (Fig. 3A). Furthermore, SOD activity in the hippocampus was higher in the transgenic mice compared with wild-type mice, whether they were given corticosterone administration or not (Fig. 3A).

8-OHdG is a marker of oxidative damage to DNA, and can be used to assess oxidative stress levels in cells. We compared the number of 8-OHdG-positive cells in the hippocampal CA3 region (a region with an important role in the pathogenesis of depression [32]) in transgenic and wild-type mice after corticosterone administration. Immunohistochemical

analysis with an antibody against 8-OHdG showed that the number of 8-OHdG-positive cells in the hippocampal CA3 region was increased in wild-type mice following daily corticosterone administration (Fig. 3B); however, this increase was not statistically significant ($P = 0.058$) (Fig. 3C). The number of 8-OHdG-positive cells was lower in SOD1 transgenic mice compared with wild-type mice after daily corticosterone administration. There was no significant difference in the number of 8-OHdG-positive cells between these two genotypes without corticosterone administration (Fig. 3B, C). These results suggest that SOD1 overexpression protects against the corticosterone administration-induced increase in oxidative stress in the hippocampus.

Collectively, our findings suggest that SOD1 overexpression alleviates corticosterone administration-induced depressive-like behavioral disorders. This study is the first to show that SOD overexpression impacts depressive-like behaviors in animals. We also found that chronic administration of corticosterone decreases SOD activity in the hippocampus, and that SOD1 overexpression decreases oxidative stress in mice subjected to daily corticosterone administration. These results suggest that SOD1 overexpression protects against corticosterone administration-induced behavioral disorders by decreasing oxidative stress in the brain. Previous reports indicate that psychological stress or persistently high plasma levels of corticosterone inhibits adult neurogenesis and synaptogenesis through ROS-induced cellular injury [33, 34]. Therefore, although the mechanisms underlying the effects of SOD1 overexpression in the present study are unknown, we speculate that SOD1 overexpression suppresses the corticosterone administration-induced inhibition of adult neurogenesis and synaptogenesis.

In conclusion, our findings suggest that SOD1 overexpression protects mice against corticosterone administration-induced depressive-like behavioral disorders by decreasing

oxidative stress in the brain. Accordingly, we propose that SODs and their derivatives (such as PC-SOD) may have therapeutic potential in the treatment of depression.

Acknowledgements

We thank Dr. T. Araki (Department of Peripheral Nervous System Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan) for providing transgenic mice expressing SOD1. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan; the Center of Innovation Program from Japan Science and Technology Agency; Scientific Technique Research Promotion Program for Agriculture, Forestry and Food Industry; and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

- [1] B.S. McEwen, Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders, *Ann N Y Acad Sci*, 1032 (2004) 1-7.
- [2] P.E. Greenberg, R.C. Kessler, H.G. Birnbaum, S.A. Leong, S.W. Lowe, P.A. Berglund, P.K. Corey-Lisle, The economic burden of depression in the United States: how did it change between 1990 and 2000?, *J Clin Psychiatry*, 64 (2003) 1465-1475.
- [3] R.H. Belmaker, G. Agam, Major depressive disorder, *N Engl J Med*, 358 (2008) 55-68.
- [4] D. Chancellor, The depression market, *Nat Rev Drug Discov*, 10 (2011) 809-810.
- [5] R.S. Duman, G.K. Aghajanian, Synaptic dysfunction in depression: potential therapeutic targets, *Science*, 338 (2012) 68-72.
- [6] Y.I. Sheline, P.W. Wang, M.H. Gado, J.G. Csernansky, M.W. Vannier, Hippocampal atrophy in recurrent major depression, *Proc Natl Acad Sci U S A*, 93 (1996) 3908-3913.
- [7] M. Vythilingam, C. Heim, J. Newport, A.H. Miller, E. Anderson, R. Bronen, M. Brummer, L. Staib, E. Vermetten, D.S. Charney, C.B. Nemeroff, J.D. Bremner, Childhood trauma associated with smaller hippocampal volume in women with major depression, *Am J Psychiatry*, 159 (2002) 2072-2080.
- [8] I. Goshen, T. Kreisel, O. Ben-Menachem-Zidon, T. Licht, J. Weidenfeld, T. Ben-Hur, R. Yirmiya, Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression, *Mol Psychiatry*, 13 (2008) 717-728.
- [9] M.L. Lehmann, R.A. Brachman, K. Martinowich, R.J. Schloesser, M. Herkenham, Glucocorticoids orchestrate divergent effects on mood through adult neurogenesis, *J Neurosci*, 33 (2013) 2961-2972.

- [10] J.S. Snyder, A. Soumier, M. Brewer, J. Pickel, H.A. Cameron, Adult hippocampal neurogenesis buffers stress responses and depressive behaviour, *Nature*, 476 (2011) 458-461.
- [11] B.S. McEwen, Physiology and neurobiology of stress and adaptation: central role of the brain, *Physiol Rev*, 87 (2007) 873-904.
- [12] F. Holsboer, U. von Bardeleben, K. Wiedemann, O.A. Muller, G.K. Stalla, Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression. Implications for pathophysiology of DST nonsuppression, *Biol Psychiatry*, 22 (1987) 228-234.
- [13] D.J. David, B.A. Samuels, Q. Rainer, J.W. Wang, D. Marsteller, I. Mendez, M. Drew, D.A. Craig, B.P. Guiard, J.P. Guilloux, R.P. Artymyshyn, A.M. Gardier, C. Gerald, I.A. Antonijevic, E.D. Leonardo, R. Hen, Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression, *Neuron*, 62 (2009) 479-493.
- [14] S.L. Gourley, D.D. Kiraly, J.L. Howell, P. Olausson, J.R. Taylor, Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone, *Biol Psychiatry*, 64 (2008) 884-890.
- [15] S.Y. Yau, B.W. Lau, J.B. Tong, R. Wong, Y.P. Ching, G. Qiu, S.W. Tang, T.M. Lee, K.F. So, Hippocampal neurogenesis and dendritic plasticity support running-improved spatial learning and depressive-like behaviour in stressed rats, *PLoS One*, 6 (2011) e24263.
- [16] B.J. Carroll, The dexamethasone suppression test for melancholia, *Br J Psychiatry*, 140 (1982) 292-304.
- [17] M.R. Levinstein, B.A. Samuels, Mechanisms underlying the antidepressant response and treatment resistance, *Front Behav Neurosci*, 8 (2014) 208.
- [18] V.L. Kinnula, J.D. Crapo, Superoxide dismutases in the lung and human lung diseases, *Am J Respir Crit Care Med*, 167 (2003) 1600-1619.

- [19] T. Ishihara, K. Tanaka, Y. Tasaka, T. Namba, J. Suzuki, S. Okamoto, T. Hibi, M. Takenaga, R. Igarashi, K. Sato, Y. Mizushima, T. Mizushima, Therapeutic effect of lecithinized superoxide dismutase against colitis, *J Pharmacol Exp Ther*, 328 (2009) 152-164.
- [20] M. Takenaga, Y. Ohta, Y. Tokura, A. Hamaguchi, M. Nakamura, H. Okano, R. Igarashi, Lecithinized superoxide dismutase (PC-SOD) improved spinal cord injury-induced motor dysfunction through suppression of oxidative stress and enhancement of neurotrophic factor production, *J Control Release*, 110 (2006) 283-289.
- [21] T. Tsubokawa, V. Jadhav, I. Solaroglu, Y. Shiokawa, Y. Konishi, J.H. Zhang, Lecithinized superoxide dismutase improves outcomes and attenuates focal cerebral ischemic injury via antiapoptotic mechanisms in rats, *Stroke*, 38 (2007) 1057-1062.
- [22] K. Tanaka, Y. Tanaka, Y. Miyazaki, T. Namba, K. Sato, K. Aoshiba, A. Azuma, T. Mizushima, Therapeutic effect of lecithinized superoxide dismutase on pulmonary emphysema, *The Journal of pharmacology and experimental therapeutics*, 338 (2011) 810-818.
- [23] K. Tanaka, A. Azuma, Y. Miyazaki, K. Sato, T. Mizushima, Effects of lecithinized superoxide dismutase and/or pirfenidone against bleomycin-induced pulmonary fibrosis, *Chest*, 142 (2012) 1011-1019.
- [24] Y. Che, J.F. Wang, L. Shao, T. Young, Oxidative damage to RNA but not DNA in the hippocampus of patients with major mental illness, *J Psychiatry Neurosci*, 35 (2010) 296-302.
- [25] H. Sato, T. Takahashi, K. Sumitani, H. Takatsu, S. Urano, Glucocorticoid Generates ROS to Induce Oxidative Injury in the Hippocampus, Leading to Impairment of Cognitive Function of Rats, *J Clin Biochem Nutr*, 47 (2010) 224-232.

- [26] D. Gupta, M. Radhakrishnan, Y. Kurhe, Effect of a novel 5-HT₃ receptor antagonist 4i, in corticosterone-induced depressive-like behavior and oxidative stress in mice, *Steroids*, 96 (2015) 95-102.
- [27] J.S. Seo, J.Y. Park, J. Choi, T.K. Kim, J.H. Shin, J.K. Lee, P.L. Han, NADPH oxidase mediates depressive behavior induced by chronic stress in mice, *J Neurosci*, 32 (2012) 9690-9699.
- [28] S.A. Golden, H.E. Covington, 3rd, O. Berton, S.J. Russo, A standardized protocol for repeated social defeat stress in mice, *Nat Protoc*, 6 (2011) 1183-1191.
- [29] S. Uchida, K. Hara, A. Kobayashi, M. Fujimoto, K. Otsuki, H. Yamagata, T. Hobara, N. Abe, F. Higuchi, T. Shibata, S. Hasegawa, S. Kida, A. Nakai, Y. Watanabe, Impaired hippocampal spinogenesis and neurogenesis and altered affective behavior in mice lacking heat shock factor 1, *Proc Natl Acad Sci U S A*, 108 (2011) 1681-1686.
- [30] H.J. Kang, B. Voleti, T. Hajszan, G. Rajkowska, C.A. Stockmeier, P. Licznarski, A. Lepack, M.S. Majik, L.S. Jeong, M. Banasr, H. Son, R.S. Duman, Decreased expression of synapse-related genes and loss of synapses in major depressive disorder, *Nat Med*, 18 (2012) 1413-1417.
- [31] D.H. Overstreet, Modeling depression in animal models, *Methods Mol Biol*, 829 (2012) 125-144.
- [32] E.J. Nestler, M. Barrot, R.J. DiLeone, A.J. Eisch, S.J. Gold, L.M. Monteggia, Neurobiology of depression, *Neuron*, 34 (2002) 13-25.
- [33] Y. Xu, S. Li, M.M. Vernon, J. Pan, L. Chen, P.A. Barish, Y. Zhang, A.P. Acharya, J. Yu, S.S. Govindarajan, E. Boykin, X. Pan, J.M. O'Donnell, W.O. Ogle, Curcumin prevents corticosterone-induced neurotoxicity and abnormalities of neuroplasticity via 5-HT receptor pathway, *J Neurochem*, 118 (2011) 784-795.

[34] W.Y. Lee, S.W. Jang, J.S. Lee, Y.H. Kim, H.G. Kim, J.M. Han, D.W. Kim, M.H. Yi, M.K. Choi, C.G. Son, Uwhangchungsimwon, a traditional herbal medicine, protects brain against oxidative injury via modulation of hypothalamus-pituitary-adrenal (HPA) response in a chronic restraint mice model, *J Ethnopharmacol*, 151 (2014) 461-469.

FIGURE LEGENDS

Figure 1. Effect of SOD1 expression on corticosterone administration-induced social avoidance behavior. The apparatus for the social interaction test (A). Wild-type mice (WT) and transgenic mice expressing human SOD1 (SOD1 Tg) were subcutaneously administered corticosterone (CORT) (0.5 mg/mouse) or vehicle for 4 weeks. The social interaction test was performed, and the times spent in the interaction zone and corner zones were measured (B). Values are given as mean $\pm$ SEM. * $P < 0.05$; n.s., not significant.

Figure 2. Effect of SOD1 expression on corticosterone administration-induced behavioral disorders. WT and SOD1 Tg mice were subcutaneously administered corticosterone (CORT) (0.5 mg/mouse) or vehicle for 4 weeks. The sucrose preference test (A) and forced swim test (B) were performed. Values are given as mean $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$; n.s., not significant.

Figure 3. Effect of corticosterone administration on SOD activity and 8-OHdG-positive cells in the hippocampal CA3 region. WT and SOD1 Tg mice were subcutaneously administered corticosterone (CORT) (0.5 mg/mouse) or vehicle for 4 weeks, and the brains were removed. SOD activity in the hippocampal homogenates was determined. Data in the left and right panels for wild-type mice are the same (A). Sections were prepared and subjected to Mayer's hematoxylin staining and immunohistochemical analysis with an antibody against 8-OHdG (scale bar, 100 μ m) (B). The number of 8-OHdG positive cells was determined (C). Values are given as mean $\pm$ SEM. ** $P < 0.01$; * $P < 0.05$; n.s., not significant.

Figure 1

A

B

Interaction zone

Corner zone

Figure 2

A

B

Figure 3

A

B

C