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Minocycline produced antidepressant-like effects on the learned helplessness rats with alterations in levels of monoamine in the amygdala and no changes in BDNF levels in the hippocampus at baseline

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ABSTRACT

Previous studies have indicated that minocycline might function as an antidepressant drug. The aim of this study was to evaluate the antidepressant-like effects of minocycline, which is known to suppress activated microglia, using learned helplessness (LH) rats (an animal model of depression). Infusion of minocycline into the cerebral ventricle of LH rats induced antidepressant-like effects. However, infusion of minocycline into the cerebral ventricle of naïve rats did not produce locomotor activation in the open field tests, suggesting that the antidepressant-like effects of minocycline were not attributed to the enhanced locomotion. LH rats showed significantly higher serotonin turnover in the orbitofrontal cortex and lower levels of brain-derived neurotrophic factor (BDNF) in the hippocampus than control rats. However, these alterations in serotonin turnover and BDNF expression remained unchanged after treatment with minocycline. On the contrary, minocycline treatment of LH rats induced significant increases in the levels of dopamine and its metabolites in the amygdala when compared with untreated LH rats. Taken together, minocycline may be a therapeutic drug for the treatment of depression.

1. Introduction

Minocycline, the second-generation tetracycline antibiotic drug, has powerful anti-inflammatory and neuroprotective effects. The action of minocycline is assumed to be exerted through the inhibition of cytochrome c release from the mitochondria, the inhibition of caspase expression, and the suppression of microglial activation (Domercq and Matute, 2004; Kim and Suh, 2009). Minocycline thereby reduces transcription of the downstream pro-inflammatory nitric oxide synthase and cyclooxygenase-2 and the subsequent release of interleukin1β (IL-1β), nitric oxide (NO), and prostaglandin E2.

Minocycline is currently receiving attention as a potential new agent for the treatment of major depression (Hashimoto, 2009; Pae et al., 2008). A previous case report documented the antidepressant effects of minocycline in a patient with bipolar disorder (Levine et al., 1996). In animal studies, minocycline reduced immobility by increasing climbing and enhanced the anti-immobility effect of subthreshold doses of desipramine in the forced swimming test (an antidepressant-screening model) (Molina-Hernandez et al., 2008). Furthermore, minocycline attenuated lipopolysaccharide (LPS)-induced expression of pro-inflammatory cytokines, and prevented LPS-induced development of depressive-like behaviors in mice (O'Connor et al., 2009). These lines of evidence suggest that minocycline is a potential antidepressant drug.

The prefrontal cortex, nucleus accumbens, hippocampus, and amygdala are candidates for the locus of depression, and their involvement in the pathophysiology of depression is well documented. Dysfunctional changes within these interconnected limbic regions have been implicated in depression and the actions of antidepressants (Berton and Nestler, 2006; Krishnan and Nestler, 2008). Postmortem and neuroimaging studies of depressed patients have revealed reductions in gray-matter volume and glial density in the prefrontal cortex and hippocampus (Drevets, 2001; Harrison, 2002; Sheline et al., 2003). Activity in the amygdala and anterior cingulate cortex is strongly correlated with dysphoric emotions: indices of neuronal activity within these regions are chronically increased in depressed individuals, but revert to normal levels after successful treatment (Drevets, 2001; Ressler and Mayberg, 2007).

The networks described above are significantly modulated by monoamine projections from the midbrain and brainstem nuclei. Abnormal monoamine metabolism is also observed in animal models of depression such as olfactory bulbectomized rats (Zhou et al., 1998), Wistar–Kyoto rats (De La Garza and Mahoney, 2004) and Flinders

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Sensitive Line rats (Zangen et al., 1997, 1999), and treatment with antidepressants improves these monoaminergic dysfunctions (Zangen et al., 1997, 1999). Olfactory bulbectomized rats showed increased serotonin (5-HT) turnover in the frontal cortex (Zhou et al., 1998). Flinders Sensitive Line rats, a genetic model of depression, also showed increased dopamine turnover in the prefrontal cortex and decreased serotonin turnover in the nucleus accumbens (Zangen et al., 1997, 1999). Serotonergic neurons are known to be associated with depression-related neuropsychological functions including stress responsiveness, motivation, working memory, and anxiety (Jans et al., 2007). In support of this, a previous clinical study demonstrated that depressed patients exhibited significantly higher 5-HT turnover in plasma levels than normal controls (Mitani et al., 2006).

The monoamine hypothesis of depression posits that depression is caused by decreased monoamine function in the brain (Berton and Nestler, 2006). It is assumed that initial increases in the levels of synaptic monoamines (5-HT and norepinephrine (NE)) induced by antidepressant drugs produce secondary neuroplastic changes that involve transcriptional and translational changes, mediating molecular and cellular plasticity (Nestler et al., 2002; Pittenger and Duman, 2008). Although monoamine-based antidepressants remain the first line of therapy for depression, therapeutic delays and low remission rates have encouraged the search for more effective agents (Berton and Nestler, 2006; Mathew et al., 2008; Trivedi et al., 2006).

Brain-derived neurotrophic factor (BDNF) is implicated in neuronal plasticity and plays an important role in learning and memory. It has been reported that stress reduced the expression of BDNF in the hippocampus of rats, and that treatment with antidepressants or electroconvulsive therapy restored the reduced hippocampal BDNF levels in stressed rats. It is well known that subchronic treatments with antidepressants increase the BDNF expression in the hippocampus of animals (Duman and Monteggia, 2006; Nibuya et al., 1995). Direct infusion of BDNF into the hippocampus induces an anti-depressive effect in learned helplessness (LH) rats (Shirayama et al., 2002). Furthermore, treatments with antidepressants did not improve the depressive-like behavior in the forced swim test in mice whose expression of BDNF in the dentate gyrus of hippocampus was selectively attenuated (Adachi et al., 2008). Clinical studies including a recent meta-analysis study have reported that the concentration of serum BDNF was decreased in depressed patients, and that subsequent treatment with antidepressants increased the concentration of serum BDNF (Brunoni et al., 2008; Sen et al., 2008; Shimizu et al., 2003). Furthermore, external stressors activate cyclooxygenase enzymes that enable the production of prostaglandins, increasing the secretion and synthesis of BDNF (Toyomoto et al., 2004). Moreover, pro-inflammatory cytokines such as IL-1β, which are increased in clinical depression, impaired BDNF signal transduction (Tong et al., 2008).

LH is a widely used animal model of depression. In this model, application of an uncontrollable and unpredictable stressor such as inescapable shock leads to a helpless state in a variety of animals and humans (Overmier and Seligman, 1967; Maier and Seligman, 1976; Breier et al., 1987). Helpless animals lose weight, appear agitated, and have sleep disturbances, libido reduction, and associative-cognitive deficits (Henn and Vollmayr, 2005). LH animals are responsive to tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and electroconvulsive treatment (Sherman et al., 1982; Shirayama et al., 2002). LH rats show changes in the NE and 5-HT systems. Thus, the NE-β receptor and 5-HT-1B receptor were up-regulated in the hippocampus of LH rats, and the neurochemical and behavioral changes were reversed with subchronic treatment with antidepressants (Henn and Vollmayr, 2005).

We examined whether minocycline could recover the behavioral deficits observed in LH rats. The focus of this investigation was to determine the mechanism of the antidepressant-like effects of minocycline on LH rats. Therefore, we examined the effects of minocycline on levels of monoamine and their metabolites after LH paradigm and after subsequent treatment with minocycline in the medial prefrontal cortex, orbitofrontal cortex, nucleus accumbens, striatum, hippocampus, and amygdala. These regions are possibly involved in the pathophysiology of depression (Pittenger and Duman, 2008). Moreover, we examined the BDNF level in the LH paradigm and after subsequent treatment with minocycline in the hippocampus.

2. Materials and methods

2.1. Animals and treatments

The animal procedures were in accordance with the Chiba University Graduate School of Medicine Guide for the Care and Use of Laboratory Animals and were approved by the Chiba University Graduate School of Medicine Animal Care and Use Committee. Male Sprague–Dawley rats (190–220 g) were housed under a 12-h light/12-h dark cycle at room temperature (22 ± 2 °C) with free access to food and water.

Surgery was performed using a stereotaxic apparatus (Kopf, Tujunga, CA) under anesthesia with pentobarbital sodium solution (50 mg/kg, intraperitoneal injection; Abbott Laboratories, Abbott Park, IL) 1 day after the acquisition of LH. The coordinates for the cerebral ventricle relative to the bregma according to the atlas of Paxinos and Watson (Paxinos and Watson, 1997) were as follows: −0.3 anteroposterior (AP), ±1.2 lateral, −3.4 dorsoventral (DV) from the dura. Minocycline hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in physiological saline. Rats received bilateral microinjection of different amounts of minocycline (160 or 20 μg/side) or saline (control) into the cerebral ventricle. A total volume of 4.0 μl was infused into each side over 10 min, and the injection syringe was left in place for an additional 5 min to allow for diffusion.

2.2. LH paradigm

LH behavioral tests were performed using the Gemini Avoidance System (San Diego, CA, USA). This apparatus was divided into two compartments by a retractable door. On days 1 and 2, rats were subjected to 30 inescapable electric footshocks [0.65 mA, 30 s duration, at random intervals (averaging 18–42 s)]. On day 3, a two-way conditioned avoidance test was performed as a post-shock test to determine if the rats would show the predicted escape deficits. This screening session consisted of 30 trials in which the electric footshocks [0.65 mA, 6 s duration, at random intervals (mean of 30 s)] were preceded by a 3 s conditioned stimulus tone that remained on until the shock was terminated. Rats with more than 25 escape failures in the 30 trials were regarded as having reached the criterion. Approximately 65% of the rats reached this criterion.

On day 4, rats received bilateral microinjections of minocycline into the ventricle.

On day 8, a two-way conditioned avoidance test was performed. This test session consisted of 30 trials in which electric footshock [0.65 mA, 30 s duration, at random intervals (mean of 30 s, averaging 18–42 s)] was preceded by a 3 s conditioned stimulus tone that remained on until the shock was terminated. The numbers of escape failures and latency to escape in each of 30 trials were recorded by the Gemini Avoidance System.

2.3. Open field test

Four days after the surgery, locomotor activity was measured in the open field test in a square area (76.5 × 76.5 × 49 cm) using a standard procedure (Lacroix et al., 1998). This experiment was performed separately from the two-way conditioned avoidance test using different animals. The open field was divided into two areas, a peripheral area and a square center (40 × 40 cm). The test room was dimly

illuminated (60 W lights, indirect). Rats were allowed to explore for 45 min. The computer software (BeTrace: Behavioral and Medical Sciences Research Consortium, Hyogo, Japan) calculated the velocity of movement, the distance traveled, and time spent in the center of the open field. These parameters are assumed to reflect locomotor activity and fear or anxiety, respectively.

2.4. Measurement of monoamines

On day 8, animals were decapitated and the brains were immediately removed. These animals had not been subjected to the two-way conditioned avoidance test or open field test. The prefrontal cortex, nucleus accumbens, striatum, amygdala, and hippocampus were dissected and stored at −80 °C until used for the assay. Tissue samples were homogenized in 0.2 M perchloric acid ($HClO_4$) containing 100 μM disodium EDTA and 100 ng/ml isoproterenol (internal standard), and were then centrifuged at 20,000×*g* for 15 min at 4 °C. The supernatants were filtered through a 0.45 μm pore membrane (Millex-LH, 4 mm; Millipore, Tokyo, Japan) and were analyzed for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by high-performance liquid chromatography (HPLC) coupled with electrochemical detection. The HPLC system consisted of a liquid chromatograph pump (EP-300, Eicom, Kyoto, Japan), degasser (DG-300, Eicom), reversed phase column (Eicompak SC-5ODS 3.0×150 mm; Eicom), ECD-300 electrochemical detector (Eicom), and data processor (EPC-300, Eicom). The mobile phase consisted of 0.1 M acetate–citric acid buffer (pH 3.5) containing 13% methanol, 5 mg/l disodium EDTA, and 190 mg/l sodium octyl sulfate.

2.5. Measurements of BDNF protein levels

On day 8, animals were decapitated and the hippocampus was dissected out. These rats had not been subjected to the two-way conditioned avoidance test or open field test. The samples were homogenized by a Polytron in 3 ml of buffer containing 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 4 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM Na_3VO_4, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml leupeptin. The homogenized samples were spun at 15,000 rpm for 30 min, and the supernatants were analyzed for BDNF using a two-site enzyme-linked immunosorbent assay (ELISA). BDNF proteins were quantified by using the BDNF Emax immunoassay system (Promega Co., Madison, WI, USA). Data were expressed as percent of control and are the means with S.E.M.

2.6. Statistical analysis

Statistical differences among three groups were determined by one-way ANOVA, followed by post hoc analysis (Tukey's test). For comparison of the mean values between the two groups, statistical evaluation was done using the two-tailed Student's *t*-test. Differences were considered to be significant when the P values were less than 0.05.

3. Results

3.1. LH and conditioned avoidance test

LH rats that received bilateral microinjections of minocycline into the cerebral ventricle demonstrated a significant improvement on the conditioned avoidance test relative to saline-treated controls (Fig. 1).

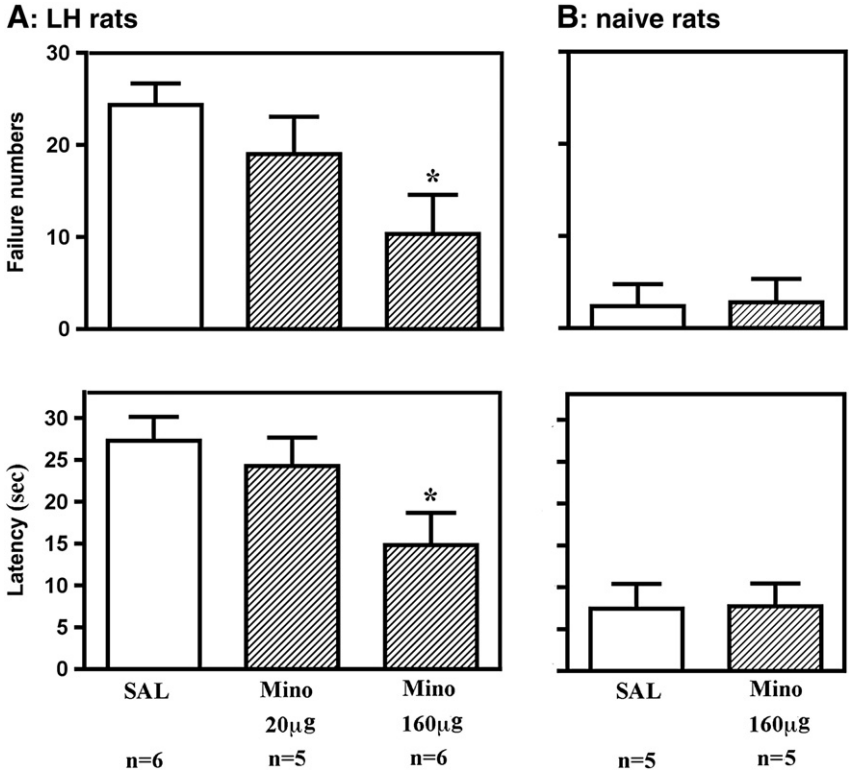

Fig. 1. Minocycline decreased escape failure in the LH paradigm. Minocycline (Mino) or saline (SAL) was administered via bilateral infusion into the cerebral ventricle, and animals were subjected to a conditioned avoidance test 4 days later. Escape failure and latency to escape were determined. The results were expressed as mean ± S.E.M. The number of animals is listed under each column. Shown on the right are the results of minocycline-injection into naïve rats for comparison. Left top, $F(2, 14) = 4.052$, $p = 0.0409$; left bottom, $F(2, 14) = 3.861$, $p = 0.0462$; right top, $t = 0.114$, $p = 0.9120$; right bottom, $t = 0.072$, $p = 0.9442$. *$p < 0.05$ when compared with saline-treated controls (ANOVA followed by Tukey's test).

Meanwhile, injection of minocycline into the cerebral ventricle of naïve rats failed to induce the antidepressant-like effects in the conditioned avoidance test (Fig. 1).

3.2. Locomotor activity

Infusions of minocycline into the cerebral ventricle of naïve rats failed to affect the time spent in the center and distance traveled, but decreased velocity in the open field test (Fig. 2). This is not the result expected if a general increase in locomotor activity contributed to the effect of minocycline on conditioned avoidance in the LH models of depression.

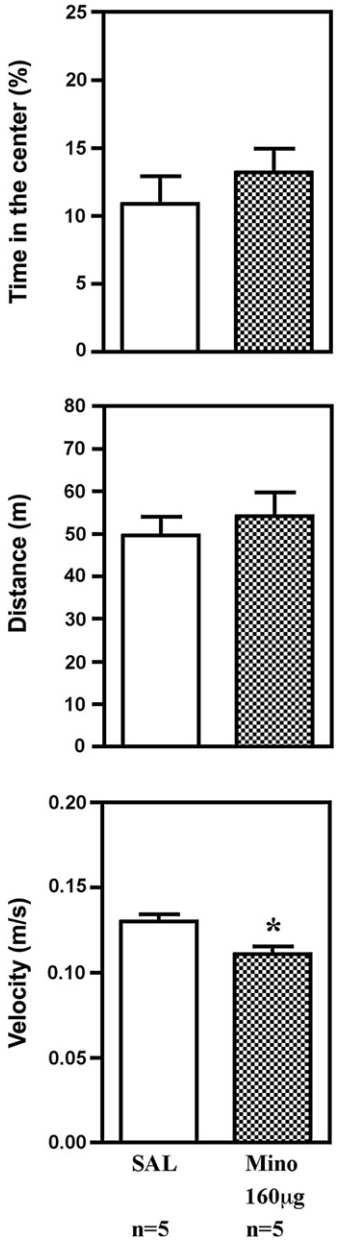

Fig. 2. Effects of minocycline infusion into the cerebral ventricle of naïve rats on locomotor activity. Minocycline (Mino) or saline (SAL) was administered via bilateral infusion into the cerebral ventricle, and 4 days later, the time spent in the center, distance traveled, and velocity in an open field were determined. The results were expressed as mean ± S.E.M. The number of animals is listed under each column. Top, $t = 0.873$, $p = 0.4079$; middle, $t = 0.642$, $p = 0.5389$; bottom, $t = 3.058$, $p = 0.0156$. *$p < 0.05$ when compared to saline-injected controls (Student's *t*-test).

3.3. Monoamines and their metabolites

LH rats showed a significant increase in 5-HT turnover in the orbitofrontal cortex, and the alteration remained unchanged after treatment with minocycline ($F(2,26) = 5.542$, $P = 0.0099$; Table 1). No alterations were found in 5-HT levels, 5-HIAA levels, and 5-HIAA/5-HT ratio in the medial prefrontal cortex, nucleus accumbens, striatum, hippocampus, or amygdala (Table 1).

No changes in the levels of DA, DOPAC, or HVA, or in the (DOPAC + HVA)/DA ratio were seen in the medial prefrontal cortex, orbitofrontal cortex, nucleus accumbens or striatum (Table 2). On the contrary, subsequent treatment with minocycline significantly increased levels of DA and DOPAC in the amygdala when compared with LH rats (DA, $F(2,25) = 4.189$, $P = 0.0270$; DOPAC, $F(2,25) = 5.290$, $P = 0.0121$; Table 2).

LH rats did not show any alterations in the NE levels, MHPG levels or MHPG/NE ratios in the medial prefrontal cortex, orbitofrontal cortex or nucleus accumbens (Table 3).

3.4. BDNF levels

LH rats showed a significantly decreased level of BDNF in the hippocampus compared with control rats (Fig. 3). However, subsequent treatment with minocycline did not result in any improvement in the decreased expression of BDNF (Fig. 3).

4. Discussion

The primary finding of the present study is that infusion of minocycline into the cerebral ventricle produced antidepressant-like effects in LH rats, an animal model of depression. The open field test showed a decrease in velocity and no alterations in distance traveled

Table 1
Levels of serotonin metabolism and its turnover in brain regions.

		5-HT	5-HIAA	5-HIAA/5-HT
<*Medial prefrontal cortex*>				
Control	n = 11	0.330 ± 0.021	0.455 ± 0.021	1.414 ± 0.080
LH	n = 10	0.315 ± 0.024	0.436 ± 0.015	1.449 ± 0.114
LH + Mino	n = 9	0.340 ± 0.021	0.496 ± 0.023	1.478 ± 0.057
<*Orbitofrontal cortex*>				
Control	n = 11	0.463 ± 0.021	0.371 ± 0.013	0.762 ± 0.030
LH	n = 10	0.430 ± 0.027	0.418 ± 0.020	0.920 ± 0.042*
LH + Mino	n = 10	0.455 ± 0.015	0.402 ± 0.016	0.886 ± 0.033*
<*Nucleus accumbens*>				
Control	n = 11	0.395 ± 0.025	0.700 ± 0.024	1.825 ± 0.101
LH	n = 10	0.439 ± 0.047	0.756 ± 0.056	1.793 ± 0.092
LH + Mino	n = 10	0.391 ± 0.037	0.738 ± 0.020	1.900 ± 0.180
<*Striatum*>				
Control	n = 11	0.342 ± 0.019	0.628 ± 0.028	1.857 ± 0.067
LH	n = 10	0.358 ± 0.033	0.644 ± 0.041	1.843 ± 0.076
LH + Mino	n = 10	0.333 ± 0.028	0.667 ± 0.025	2.082 ± 0.116
<*Hippocampus*>				
Control	n = 11	0.289 ± 0.023	0.509 ± 0.031	1.891 ± 0.086
LH	n = 10	0.311 ± 0.013	0.512 ± 0.019	1.667 ± 0.083
LH + Mino	n = 10	0.267 ± 0.018	0.485 ± 0.017	1.964 ± 0.149
<*Amygdala*>				
Control	n = 10	0.665 ± 0.058	0.776 ± 0.024	1.242 ± 0.099
LH	n = 9	0.629 ± 0.042	0.700 ± 0.019	1.146 ± 0.068
LH + Mino	n = 10	0.617 ± 0.041	0.782 ± 0.046	1.291 ± 0.068

Monoamine level (ng/mg tissue) and turnover are indicated as mean ± SEM.
Sample numbers are indicated in each row.
5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.
* $P < 0.05$ when compared to control animals (ANOVA followed by Tukey's test).

Table 2
Levels of dopamine metabolism in brain regions.

		DA	DOPAC	HVA	(DOPAC + HVA)/DA
<*Medial prefrontal cortex*>					
Control	n = 11	0.168 ± 0.011	0.072 ± 0.007	0.106 ± 0.007	1.066 ± 0.054
LH	n = 10	0.140 ± 0.017	0.062 ± 0.005	0.092 ± 0.002	1.110 ± 0.080
LH ± Mino	n = 9	0.134 ± 0.012	0.056 ± 0.006	0.110 ± 0.011	1.248 ± 0.084
<*Orbitofrontal cortex*>					
Control	n = 11	0.331 ± 0.088	0.101 ± 0.025	0.139 ± 0.020	1.063 ± 0.142
LH	n = 10	0.252 ± 0.079	0.098 ± 0.022	0.132 ± 0.014	1.420 ± 0.271
LH + Mino	n = 10	0.274 ± 0.076	0.081 ± 0.016	0.129 ± 0.015	1.135 ± 0.183
<*Nucleus accumbens*>					
Control	n = 11	7.239 ± 0.245	2.664 ± 0.188	0.953 ± 0.064	0.503 ± 0.034
LH	n = 10	6.908 ± 0.452	2.780 ± 0.223	0.908 ± 0.063	0.537 ± 0.021
LH + Mino	n = 10	7.492 ± 0.442	3.013 ± 0.202	1.108 ± 0.117	0.553 ± 0.033
<*Striatum*>					
Control	n = 11	11.176 ± 0.384	2.799 ± 0.168	1.127 ± 0.037	0.351 ± 0.012
LH	n = 10	9.901 ± 0.619	2.424 ± 0.188	1.038 ± 0.073	0.348 ± 0.010
LH + Mino	n = 10	10.235 ± 0.456	2.548 ± 0.141	1.169 ± 0.065	0.363 ± 0.011
<*Amygdala*>					
Control	n = 10	1.001 ± 0.102	0.293 ± 0.031	0.157 ± 0.012	0.494 ± 0.059
LH	n = 9	0.679 ± 0.131	0.176 ± 0.029	0.116 ± 0.015	0.501 ± 0.101
LH + Mino	n = 9	1.385 ± 0.250[#]	0.346 ± 0.048[#]	0.186 ± 0.035	0.411 ± 0.025

Monoamine level (ng/mg tissue) and turnover are indicated as mean ± SEM.
Sample numbers are indicated in each row.
DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.
[#] $P<0.05$ when compared to LH rats (ANOVA followed by Tukey's test).

or time spent in the center, suggesting that the antidepressant-like effects of minocycline may not be attributed to enhanced locomotion.

Second, LH rats showed decreased levels of DA and DOPAC in the amygdala, and minocycline significantly increased the levels of DA and DOPAC in the amygdala when compared with untreated LH rats. Previous studies showed that manipulation of the amygdala exerted antidepressant-like effects (Wallace et al., 2004; Shirayama et al., 2011). Therefore, the mechanism of minocycline could be attributable to a significant alteration in DA and DOPAC in the amygdala.

Third, serotonin turnover (5-HIAA/5-HT ratios) was statistically increased in the orbitofrontal cortex of LH rats when compared with control rats, but the increases in 5-HT turnover remained unchanged after treatment with minocycline. This is in partial agreement with the recent study in which depressed patients exhibited higher 5-HT turnover levels in plasma than normal controls (Mitani et al., 2006). It demonstrates that LH contributed to alteration of the 5-HT systems in the orbitofrontal cortex. The orbitofrontal cortex is involved in motivation, which is lowered in depression. This is compatible with a working hypothesis that antidepressant drugs, especially selective serotonin uptake inhibitors, exert their beneficial effects through activating serotonergic neural transmission (Jans et al., 2007). Further study will be needed to elucidate the role of 5-HT in the antidepressant effects of minocycline.

We did not find statistically significant results for NE. However, a recent study showed that minocycline administration reduced immobility in the forced swim test (an antidepressant-screening model) by increasing climbing (Molina-Hernandez et al., 2008), indicating that minocycline exerts an antidepressant-like effect through the NE system because a previous study on antidepressants indicated that increased climbing reflects the NE system whereas increased swimming reflects the 5-HT system in the forced swim test (Lucki, 1997). Further studies will be needed to elucidate the involvement of NE systems in LH rats during stressful conditions.

A previous study showed that Wistar–Kyoto rats, which are prone to develop stress-induced anhedonia, exhibited increased DA and 5-

Table 3
Levels of norepinephrine in brain regions.

		NE	MHPG	MHPG/NE
<*Medial prefrontal cortex*>				
Control	n = 11	0.334 ± 0.009	0.199 ± 0.012	0.599 ± 0.038
LH	n = 10	0.323 ± 0.008	0.192 ± 0.013	0.603 ± 0.050
LH + Mino	n = 9	0.310 ± 0.019	0.233 ± 0.025	0.691 ± 0.067
<*Orbitofrontal cortex*>				
Control	n = 11	0.263 ± 0.008	0.177 ± 0.011	0.682 ± 0.053
LH	n = 10	0.274 ± 0.005	0.176 ± 0.016	0.635 ± 0.051
LH + Mino	n = 10	0.253 ± 0.011	0.209 ± 0.022	0.751 ± 0.061
<*Nucleus accumbens*>				
Control	n = 10	0.335 ± 0.029	0.181 ± 0.019	0.565 ± 0.081
LH	n = 9	0.354 ± 0.038	0.194 ± 0.028	0.595 ± 0.130
LH + Mino	n = 9	0.388 ± 0.069	0.199 ± 0.028	0.549 ± 0.135

Monoamine level (ng/mg tissue) and turnover are indicated as mean ± SEM.
Sample numbers are indicated in each row.
NE, norepinephrine; MHPG, 3-methoxy-4-hydroxyphenylglycol.

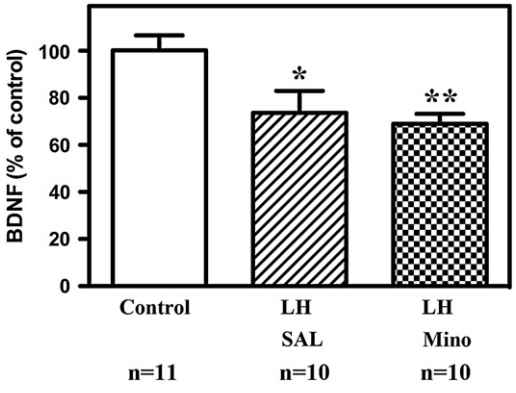

Fig. 3. Effects of minocycline on the BDNF expression in the hippocampus of LH rats. Minocycline (Mino) or saline (SAL) was administered via bilateral infusion into the cerebral ventricle of LH rats, and 4 days later, BDNF expression was examined. BDNF level (% control) are indicated as mean ± SEM. Sample numbers are indicated in each row. $F(2, 28) = 6.042$, $p = 0.0066$. *$p<0.05$, **$p<0.01$ when compared with controls (ANOVA followed by Tukey's test).

HT turnover in the nucleus accumbens under the steady state and in the prefrontal cortex under a stressful condition, although normal control rats did not show any alterations in DA or 5-HT turnover in the steady state or under a stressful condition (De La Garza and Mahoney, 2004). Therefore, LH rats might show further alterations in the levels of monoamines, metabolites and turnover under stressful conditions, and treatment with minocycline might block the monoaminergic changes induced by the stressful condition. Future studies will be needed to examine this question.

Finally, BDNF levels in the hippocampus of LH rat were lower than those of control rats, but the reduction in BDNF expression remained unchanged after treatment with minocycline. A reduction of BDNF in the hippocampus of LH rats was the expected result. A recent study on the effects of minocycline during in vitro hypoxia showed that minocycline suppressed the microglial activation and up regulation of pro-inflammatory mediators, but did not affect the hypoxic activation of BDNF (Lai and Todd, 2006). Microglia may supply neurons with BDNF (Kempermann and Neumann, 2003). Considering these results together, we may reasonably exclude the involvement of BDNF in the antidepressant-like effect of minocycline.

In a recent study, minocycline was effective as an antidepressant drug in an animal model of inflammatory-associated depressive disorders induced by lipopolysaccharide (LPS) (O'Connor et al., 2009). Pro-inflammatory cytokines, mainly interferonγ (IFN-γ) and TNF-α, induce Indoleamine 2,3-dioxygenase (IDO), which degrades tryptophan along the kynurenine pathway. Minocycline blocks IFN-γ-mediated protein kinase C phosphorylation and nuclear translocation of protein kinase C, which is necessary for IDO activation. The relationship between depression and inflammation remains to be elucidated. Future studies need to address the involvement of microglia in the antidepressant-like effect of minocycline.

In conclusion, infusion of minocycline into the cerebral ventricle of LH rats produced antidepressant-like effects, although infusion of minocycline into the cerebral ventricle of naïve rats did not increase locomotor activity in the open field tests. LH rats showed significant increased 5-HT turnover in the orbitofrontal cortex and decreased levels of BDNF in the hippocampus compared with control rats. However, these alterations in 5-HT turnover and BDNF expression remained unchanged after treatment with minocycline. Taken together, these results suggest that minocycline may be a therapeutic drug for the treatment of depression.

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