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Minocycline improves recognition memory and attenuates microglial activation in Gunn rat: A possible hyperbilirubinemia-induced animal model of schizophrenia

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ABSTRACT

Background: Accumulating evidence indicates that neuroinflammation plays a significant role in the pathophysiology of schizophrenia. We previously reported evidence of schizophrenia-like behaviors and microglial activation in Gunn rats. We concluded that the Gunn rat, which exhibits a high concentration of unconjugated bilirubin, may be useful as an animal model of schizophrenia. On the other hand, there have been numerous reports that minocycline is effective in treating schizophrenia.
Methods: In the present study, we investigated the effects of minocycline on performance of behavioral tests (prepulse inhibition (PPI) and novel object recognition test (NORT)) after animals received either 40 mg/kg/d of minocycline or vehicle by intraperitoneal (i.p.) injection for 14 consecutive days. Furthermore, we examined the effects of minocycline on microglial activation in the hippocampal dentate gyrus of Gunn rats and Wistar rats.
Results: We found that administration of minocycline for 14 days significantly increased the exploratory preference in retention sessions and tended to improve the PPI deficits in Gunn rats. Immunohistochemistry analysis revealed that microglial cells in the minocycline-treated Gunn rat group showed less expression of CD11b compared to vehicle-treated Gunn and Wistar groups.
Conclusions: Our findings suggest that minocycline improves recognition memory and attenuates microglial activation in the hippocampal dentate gyrus of Gunn rats. Therefore, minocycline may be a potential therapeutic drug for schizophrenia.

1. Introduction

Schizophrenia is one of the most mysterious and costliest mental disorders in terms of human suffering and societal expenditures (van Os and Kapur, 2009). Despite extensive research and remarkable advances in the neurobiological, neurochemical and genetic aspects of this disabling mental illness, the underlying etiological processes remain a challenge for clinicians and basic researchers alike (Meyer, 2013). Moreover, this imperfect understanding of the etiology has been associated with poor treatment outcomes for many individuals (Dean et al., 2012). Therefore, a novel insight into the underlying mechanism of this illness is an urge to allow new treatment targets to be explored.

An accumulating body of evidence points to the significant role of neuroinflammation and the immune system in the pathophysiology of schizophrenia (Takahashi and Sakurai, 2013). There are also numerous reports that support the hypothesis that infection/inflammation is a risk for developing schizophrenia later in life (Kneeland and Fatemi, 2013; Mednick et al., 1988; Miller et al., 2013). Moreover, evidence from genomic (Ripke et al., 2011; Schwab et al., 2002), blood (Erbagci et al., 2001; Miller et al., 2011), postmortem (Fisman, 1975; Radewicz et al., 2000; Steiner et al., 2008), and in vivo imaging (Doorduin et al., 2009; van Berckel et al., 2008) studies are leading toward a greater consensus that immune activation is involved in the pathophysiology of

Abbreviations: UCB, unconjugated bilirubin; PPI, prepulse inhibition; NORT, novel object recognition test; i.p., intraperitoneal; Iba1, ionized calcium binding adaptor molecule 1; ITGAM, integrin alpha M; CD11b, cluster of differentiation molecule 11B; DG, dentate gyrus; SGZ, subgranular zone; GL, granular layer; ML, molecular layer; PB, phosphate buffer; PBS, phosphate buffer saline; ABC, avidin–biotin peroxidase complex; ANOVA, analysis of variance.

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schizophrenia. Interestingly, some anti-psychotics may have anti-inflammatory effects (Bian et al., 2008; Kato et al., 2007) and some anti-inflammatory agents may have anti-psychotic effects (Laan et al., 2010; Muller and Schwarz, 2008).

Minocycline is a second generation of the antibiotic, tetracycline, and is one of the most promising neuroprotective and anti-inflammatory agents currently in clinical and experimental trials (Miyaoka, 2012). Our previous clinical studies showed the potential of minocycline as an adjunctive treatment in schizophrenia patients (Miyaoka et al., 2007, 2008). Similar results were also found in various animal models of psychosis or schizophrenia. Minocycline was reported to attenuate behavioral impairment as well as neurotoxicity after administration of methamphetamine (Mizoguchi et al., 2008; Zhang et al., 2006a), 3,4-methylenedioxymethamphetamine (MDMA) (Zhang et al., 2006b), N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (Levkovitz et al., 2007; Zhang et al., 2007), and viral mimetic polyriboinosinic-polyribocytidilid acid (PolyI:C) (Juckel et al., 2011).

Our previous studies generated Gunn rat (Gunn, 1944) as a possible animal model of schizophrenia. First, we reported behavioral abnormalities, deficits in prepulse inhibition (PPI), and neuropathological changes in Gunn rats that are similar to the characteristics of schizophrenia (Hayashida et al., 2009). Then, we demonstrated evidence of activated microglial cells in the hippocampal dentate gyrus (DG) of Gunn rats (Liaury et al., 2012). Furthermore, anti-psychotic medications affected Gunn rat behaviors in a way similar to their effect on schizophrenic behaviors (Tsuchie et al., 2013). We proposed that hyperbilirubinemia (a high level of unconjugated bilirubin, UCB) leads to chronic neuroinflammation and has a pathogenic effect on the development of the brain and concluded that the Gunn rat may be used as an animal model of schizophrenia.

In the present study, we sought to investigate the effects of minocycline on Gunn rat, a possible hyperbilirubinemia-induced animal model of schizophrenia. We hypothesized that minocycline may improve behavioral disturbances and attenuate microglial activation in Gunn rats. First, we performed behavior tests (prepulse inhibition (PPI) and novel object recognition test (NORT)). Then, using immunohistochemistry analysis, we examined the microglial activation in hippocampal DG of Gunn rats.

2. Methods

2.1. Animals

The animals were male homozygous (j/j) Gunn rats (6 weeks old, 160–200 g body weight) and male Wistar rats (6 weeks old, 200–240 g body weight) at the beginning of the experiment (Japan SLC, Inc., Shizuoka, Japan). Animals were housed under standard conditions with a room temperature of 23 ± 2°C, humidity of 55 ± 5%, and 12 h light/12 h dark cycle (light phase 7:00 to 19:00) with free access to food and water. All procedures were performed with the approval of the Shimane University Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Japan.

2.2. Drug administration

Minocycline hydrochloride (Sigma-Aldrich, St. Louis, Mo., USA) was freshly prepared as a stock solution of 5 mg/ml (10 mM) in filtered PBS and stored frozen at −80 °C every 7 days. We adjusted the acidic pH (pH 4.0) of minocycline hydrochloride to neutrality by adding sodium hydroxide [as described by (Ferretti et al., 2012)]. As the vehicle, we use the filtered PBS, pH 7.4.

Animals were divided into four groups: Wistar + vehicle (WV) group, Wistar + minocycline (WM) group, Gunn + vehicle (GV) group, and Gunn + minocycline (GM) group. Each animal received either 40 mg/kg/d of minocycline or vehicle by intraperitoneal (i.p.) injection for 14 consecutive days. The dose (40 mg/kg) of minocycline was selected based on the fact that this dose was effective in improving the methamphetamine-induced impairment of recognition memory (Mizoguchi et al., 2008) as well as prepulse inhibition deficits after the administration of the NMDA receptor antagonist dizocilpine (Zhang et al., 2007). To avoid stress or pain due to the intraperitoneal injection, animals were anesthetized by putting them into a halothane inhalation chamber for less than 1 min before the daily injection.

2.3. Prepulse inhibition (PPI) of startle response

A startle response system was used (SR-LAB, San Diego Instrument) as reported previously (Hayashida et al., 2009). Briefly, each rat was placed in a Plexiglass cylinder where it was exposed to white background noise at 65 dB for a 5 min acclimatization period. This was followed by four types of trials: (1) pulse (P) alone, consisting of a 20 ms burst of white noise at 120 dB; (2) a 20 ms burst of white noise at 70 dB followed by a 20 ms white noise at 120 dB (70 PP + P); (3) a 20 ms burst of white noise at 80 dB followed by a 20 ms white noise at 120 dB (80 PP + P); and (4) background noise only (no stimulus). Our pre-experiment (unpublished data) found that Gunn rat showed a sensitive response and behavior, therefore the use of three or four different prepulse intensities affected the behavior test and biased the result. In consequence, we decided to use just 2 prepulse intensities (70 dB and 80 dB). The interval between prepulse and pulse was set at 100 ms. Trials were given in a pseudo-random order with variable intervals (20 s–60 s, average 40 s) between each trial. Startle response was measured in sessions consisting of 50 trials. The percentage of PPI (%PPI) was defined as the magnitude of inhibition due to the startle amplitude that was induced by the prepulse. %PPI = (1 − (startle magnitude after prepulse–pulse pair / startle magnitude after pulse-only)) × 100.

2.4. Novel object recognition test (NORT)

The NORT procedure consisted of three sessions: habituation, training, and retention. The experimental apparatus consisted of a Plexiglas open field box (42 × 42 × 42 cm) with a sawdust-covered floor located in a sound attenuated room. Each animal was individually habituated to the box, with 15 min of exploration in the absence of objects for two consecutive days (habituation session, days 1–2). During the training session (day 3), two identical objects were symmetrically fixed to the floor of the box, 5 cm from the walls, and each animal was allowed to explore freely for 2 × 5 min. An animal was considered to be exploring the object when its head was facing the object (a distance between the head and object of approximately 1 cm or less) or it was touching or sniffing the object. In the retention session (day 4), one of the familiar objects used during the training session had been replaced with a novel object. The novel object was different in shape and color, but similar in size to the familiar object. The animal was then allowed to explore freely for 5 min. All sessions were videotaped for later examination. The exploratory preference index in the retention session, a ratio of the amount of time spent exploring the novel object over the total time exploring both objects, was used to measure cognitive function. In the training session, the exploratory preference index was calculated as the ratio of time spent exploring the object that was replaced by the novel object in the retention session over the total exploration time.

2.5. Immunohistochemistry for light and confocal laser scanning microscopy

Soon after finishing the behavior test, animals underwent deep intraperitoneal anesthesia with sodium pentobarbital (80 mg/kg body weight) and were perfused transcardially with 500 ml of physiological

saline, followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brain was quickly removed, post fixed in a solution of 4% paraformaldehyde at room temperature for 4 h, and then immersed overnight in a cold solution of 20% sucrose. Later, the brains were cut at 50 μm thickness in the frontal plane using a freezing microtome.

The immunohistochemistry procedure was carried out as described previously (Liaury et al., 2012). After incubation in 1% H_2O_2 solution, free-floating sections were preincubated with 1.5% normal goat serum and 0.2% Triton-X in 0.1 M PB (pH 7.4) for 1 h rotating at room temperature (RT), and then incubated overnight with the primary antibody, mouse anti-CD11b (1:500, AbD Serotec, Oxford, UK). Subsequently, the sections were incubated for 1 h in biotinylated anti-mouse IgG (1:200, Standard ABC Kit, Vector Labs, Burlingame, CA, USA), then incubated for 1 h in PBS containing avidin–biotin peroxidase complex (ABC). Later, the sections were developed by incubating in PBS containing 10 mg diaminobenzidine (DAB) and 5 μl of 30% hydrogen peroxide for 10 min.

For double immunofluorescent labeling, sections were incubated in the primary antibody, rabbit anti-ionized calcium binding adaptor molecule 1 (Iba1, 1:4000, Wako Ltd., Osaka, Japan), followed by Cy3-conjugated anti-rabbit IgG (1:1000, Amersham Bioscience Ltd., Piscataway, NJ, USA). For the secondary antibody, we used mouse anti-CD11b (1:500), followed by Alexa488-conjugated anti-mouse IgG (1:1000, Invitrogen, Carlsbad, CA, USA).

2.6. Measuring CD11b-labeled microglial cells immunoreactive area

The immunoreactive area was measured using a computer-assisted image analysis program (ImageTool V. 3.0, Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). Images were captured from four areas within the hippocampal DG: hilus, subgranular zone (SGZ), granular layer (GL), and molecular layer (ML). Sections containing CD11b-labeled cells were examined under a light microscope (Nikon, ECLIPSE E800). Images (N = 80 per region, per time point) were randomly captured from the same region of interest from interaural 5.88 mm, bregma −3.12 mm, to interaural 4.20 mm, bregma −4.80 mm, based on the rat brain atlas (Paxinos and Watson, 2007). The images were analyzed using the software that automates converting all immunolabeled elements that fall within a threshold range into pure black pixels and the rest of the image into pure white pixels. The software then quantifies the total numbers and percentages of black and white pixels for statistical analysis of the data.

2.7. Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM). The PPI data were analyzed by three-way analysis of variance (ANOVA) using R version 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria) which analyzed the interaction between three factors (strain × treatment × prepulse intensity). When appropriate, group means at individual levels were compared by post-hoc Bonferroni analysis to determine the differences among groups. The NORT and immunohistochemistry data were analyzed by two-way ANOVA (strain × treatment) and followed by the post hoc Bonferroni test. This analysis was performed with SPSS software (Dr. SPSS II for Windows V. 11.0, SPSS Inc., Chicago, IL, USA). Significance for the result was set at $p < 0.05$.

3. Results

3.1. Effects of minocycline on PPI deficits

The PPI test was conducted with two different prepulse stimulus intensities, 70 dB and 80 dB. The three-way ANOVA revealed that there was no significant three-way interaction between strain, treatment and prepulse intensity ($F_{(1,48)} = 0.025$, $p > 0.05$). As for two-way interaction, only interaction between strain and prepulse intensity was significant ($F_{(1,48)} = 4.792$, $p < 0.05$). We found significant main effects for all factors included. The %PPI results were significantly affected by strain, treatment and prepulse intensity. %PPI was significantly lower for Gunn rats than Wistar rats ($F_{(1,48)} = 34.433$, $p < 0.001$). Treatment with minocycline produced significantly higher %PPI result than treatment with saline ($F_{(1,48)} = 4.413$, $p < 0.05$). As shown in Fig. 1, post hoc Bonferroni test revealed that the GV group had a significant lower %PPI compared to the WV group in both 70 dB ($F_{(1,24)} = 20.886$, $p < 0.001$) and 80 dB ($F_{(1,24)} = 9.040$, $p = 0.006$) tests. Treatment with minocycline for 14 days significantly increased the %PPI in the GM group compared to the GV group at 80 dB ($F_{(1,24)} = 4.360$, $p = 0.048$) but produced no significant difference at 70 dB ($F_{(1,24)} = 3.545$, $p = 0.072$). Meanwhile, no significant difference was found in WM compared to WV groups both at 70 dB ($F_{(1,24)} = 0.006$, $p = 0.941$) and at 80 dB ($F_{(1,24)} = 0.044$, $p = 0.835$).

3.2. Effects of minocycline on recognition memory

As shown in Fig. 2, in the training session, two-way ANOVA revealed that here were no significant interaction between strain × treatment in both total time ($F_{(3,38)} = 2.272$, $p = 0.913$) and in exploratory preference ($F_{(3,38)} = 2.945$, $p = 0.412$). In the retention session, two-way ANOVA revealed significant differences among the groups studied in total time ($F_{(3,38)} = 0.257$, $p = 0.005$) and in exploratory preference ($F(3,38) = 0.224$, $p = 0.004$). Post hoc analysis indicated that GV group showed significant reduction in total time ($F_{(1,38)} = 4.701$, $p = 0.036$) and exploratory preference ($F_{(1,38)} = 10.975$, $p = 0.002$) compared to the WV group. Treatment with minocycline in the GM group significantly improved the exploratory preference ($F_{(1,38)} = 8.126$, $p = 0.007$) without affecting the total exploration time ($F_{(1,38)} = 0.121$, $p = 0.730$) compared to the GV group. Meanwhile, treatment with minocycline showed no significant difference in the WM group compared to the WV group on total exploration time and exploratory preference in both the training or retention sessions.

3.3. Effects of minocycline on microglial activation

Next, we examined the effects of minocycline treatment on microglial activation in the hippocampal DG. CD11b expression in the hippocampal DG of the GV group was consistently high. In many cases, the CD11b immunoreactivity was detected not only in the thick processes, but also strongly expressed within the cell bodies of the Iba1-labeled microglial cells (Fig. 3-1b, -1c). Statistical analysis by two-way ANOVA revealed a significant interaction between strain × treatment in hilus ($F_{(3,20)} = 1.977$, $p = 0.006$), SGZ ($F_{(3,20)} = 1.902$, $p = 0.003$), granular layer ($F_{(3,20)} = 7.135$, $p = 0.045$), but not in molecule layer ($F_{(3,20)} = 6.986$, $p = 0.644$). Post hoc analysis indicated a significant increase in percentage of CD11b immunoreactivity of the GV group in the hilus ($F_{(1,20)} = 43.506$, $p < 0.001$), SGZ ($F_{(1,20)} = 57.156$, $p < 0.001$), granular layer ($F_{(1,20)} = 18.619$, $p < 0.001$), and molecular layer ($F_{(1,20)} = 11.323$, $p = 0.003$) compared to the WV group (Fig. 4a–d).

The consecutive administration of minocycline (40 mg/kg) for 14 days decreased the CD11b expression in the DG of the GM group. In this case, most CD11b immunoreactivity was expressed only on the thin ramified processes with a slight expression within the cell bodies of the Iba1-labeled microglial cells (Fig. 3-2b, 3-2c). A post hoc Bonferroni test revealed that minocycline significantly decreased the percentage of CD11b immunoreactivity in the hilus ($F_{(1,20)} = 23.510$, $p < 0.001$), SGZ ($F_{(1,20)} = 18.473$, $p < 0.001$), and granular layer ($F_{(1,20)} = 8.907$, $p = 0.007$) compared to the GV group (Fig. 4a–c). No significant difference was found between WV and WM groups.

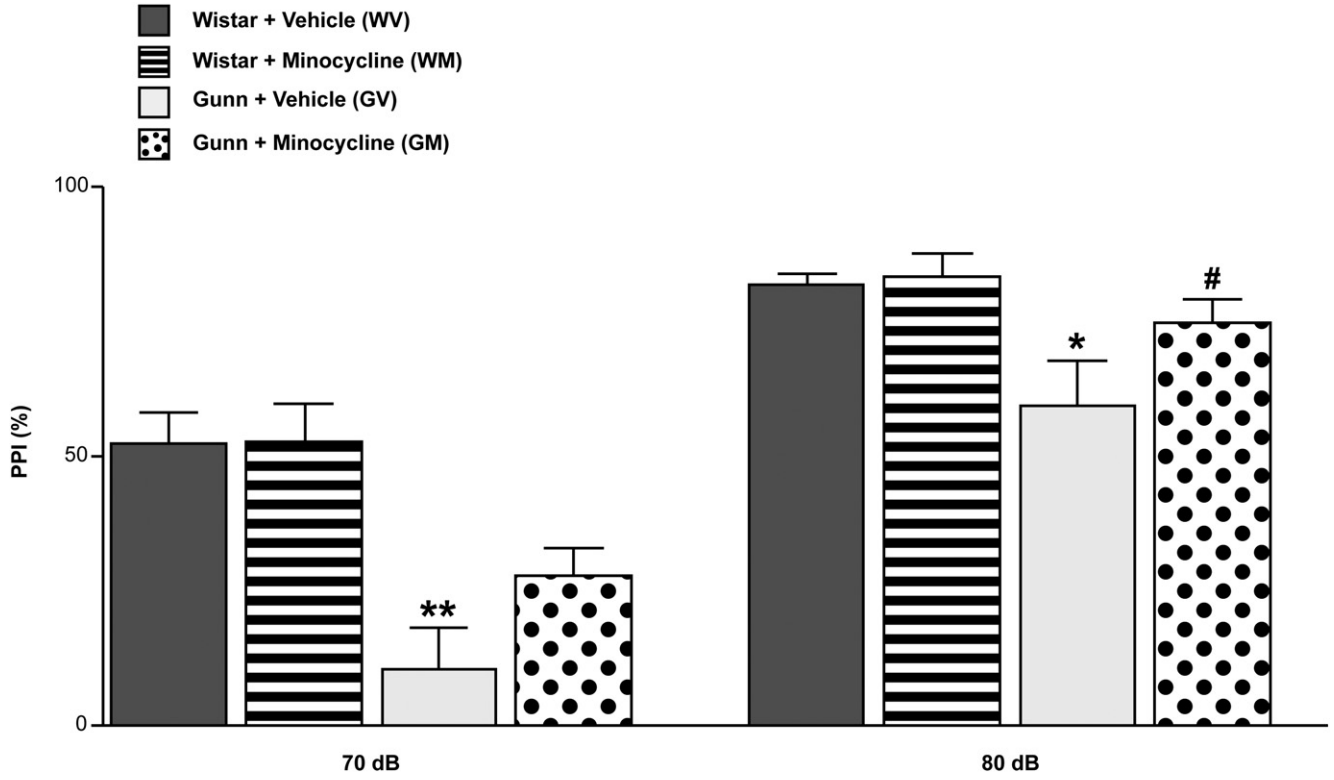

Fig. 1. Effects of minocycline on PPI test at 70 dB and 80 dB. Each value is the mean ± S.E.M. (n = 7 per group). $^{*}p < 0.01$, $^{**}p < 0.001$ as compared to WV control group; $\#p < 0.05$ as compared to GV group.

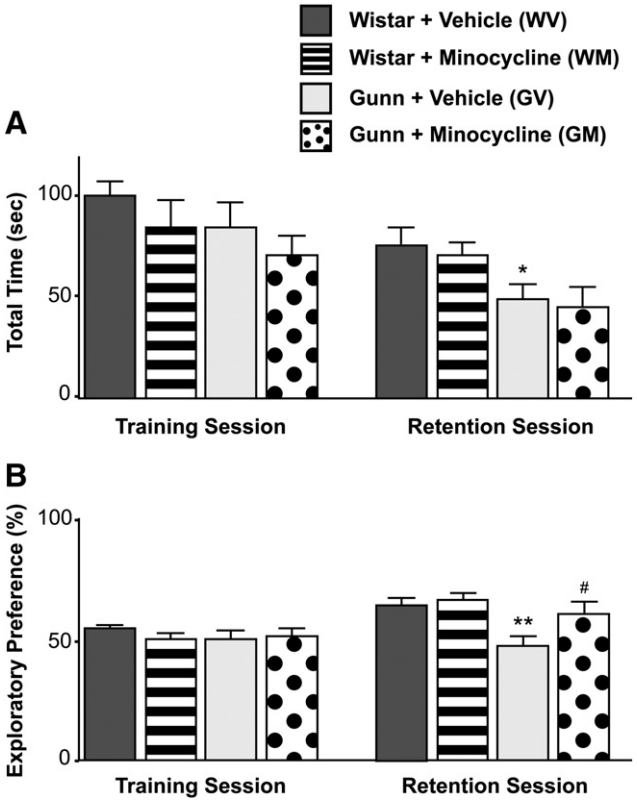

Fig. 2. Effects of minocycline on novel object recognition test (NORT). A. Total exploration time; B. exploratory preference. Each value is the mean ± S.E.M. (n = 9 each in Wistar rat group, n = 12 each in Gunn rat group). $^{*}p < 0.05$, $^{**}p < 0.005$ as compared to WV control group; $\#p < 0.01$ as compared to GV group.

4. Discussion

The major findings of the present study are that minocycline significantly improved the impairment of recognition memory in Gunn rats, and that minocycline significantly attenuated the activation of microglial cells in the hippocampal DG. To our knowledge, this is the first report demonstrating the effects of minocycline on behavioral changes and on microglial activation in the Gunn rat, a possible hyperbilirubinemia-induced animal model of schizophrenia.

Our results showed that Gunn rats had lower %PPI compared to Wistar control rats, indicating that the Gunn rat has impaired sensorimotor gating. Moreover, Gunn rats also showed a reduction of exploratory preference after long-term retention interval (24 h) during NORT, indicating that the Gunn rat has impaired recognition memory. Our findings are in agreement with other animal models of schizophrenia on the association with PPI deficits (Dieckmann et al., 2007; Geyer et al., 1990) and NORT impairment (Kamei et al., 2006; Mizoguchi et al., 2008). Taking these facts together, we herein propose the Gunn rat as a potential animal model in relation to the cognitive deficits in schizophrenia.

To detect and measure the microglial activation in the hippocampal DG, we used a marker of integrin alpha M (ITGAM) cluster of differentiation molecule 11B (CD11b), which is expressed on all types of microglia, and expression of which is significantly increased on activated microglial cells (He et al., 1997). Furthermore, one of the earliest changes in microglia cells after facial nerve trisection was the up-regulation of CD11b (Graeber et al., 1988). Therefore, CD11b expression appears to be a sensitive marker of microglial activation. The hippocampal region was our main interest due to its potential role in learning and memory with the dentate gyrus (DG) as the primary gateway for inputs into the hippocampus with a continuously developing structure with birth of new neurons — 'neurogenesis' — throughout mammalian life (Gage, 2002).

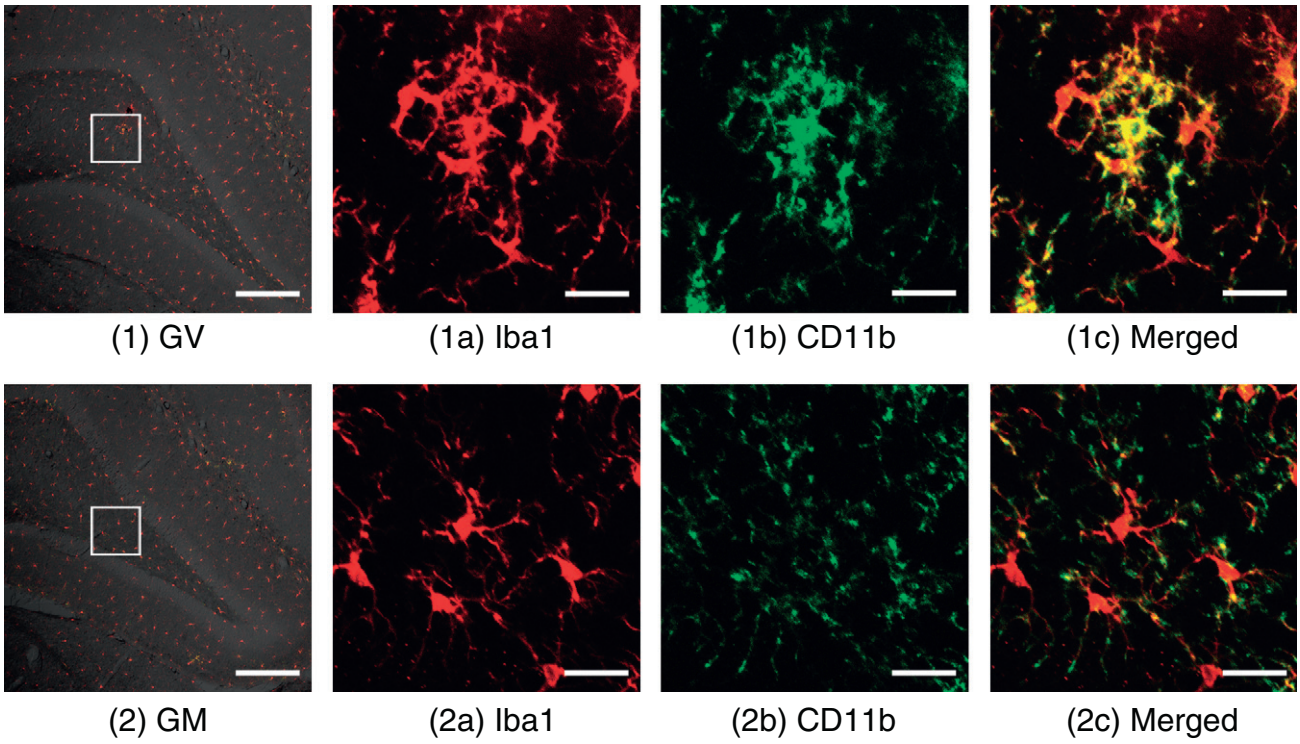

Fig. 3. CD11b expression in ionized calcium binding adaptor molecule 1 (Iba1)-labeled microglial cells. A confocal Z stack image depicting Iba1-labeled (red), CD11b expression (green), and merged (yellow) cells of vehicle-treated Gunn rat (GV) group (1a–1c) compared to those of minocycline-treated Gunn rat (GM) group. Microglial cells in the GM (2b,c) group expressed low levels of CD11b immunoreactivity compared to the GS group (1b,c). Scale bars: 200 μm (1,2), 20 μm (1a–1c, 2a–2c).

Moreover, hippocampus is also one of the brain regions whose function is altered in schizophrenia (Tamminga et al., 2010).

Consistent with our previous report, we found a significant high level of CD11b immunoreactivity in all areas of the hippocampal DG, including the hilus, SGZ, granular layer, and molecular layer in Gunn rats. The high level of CD11b immunoreactivity was accompanied by altered morphology, suggesting that microglial cells in Gunn rat were in an activated state. Microglial cell is well-known as a key player in the reaction of the cerebral innate immune system to pathological changes. Threats to CNS homeostasis can trigger a rapid transformation from a normally "ramified" state into alerted and "activated" states. Microglial cells primarily serve in tissue defense and protection when participating in the mechanisms of innate and adaptive immunity. Conversely, excessive acute or chronic microglial activation can provoke severe neuronal and glial damage by carrying or fuelling destructive cascades [for review, see (Kettenmann et al., 2011; van Rossum and Hanisch, 2004)]. Microglial cells may also contribute to synaptic modulation, learning, and memory processes. Disturbance of the functions or alterations in

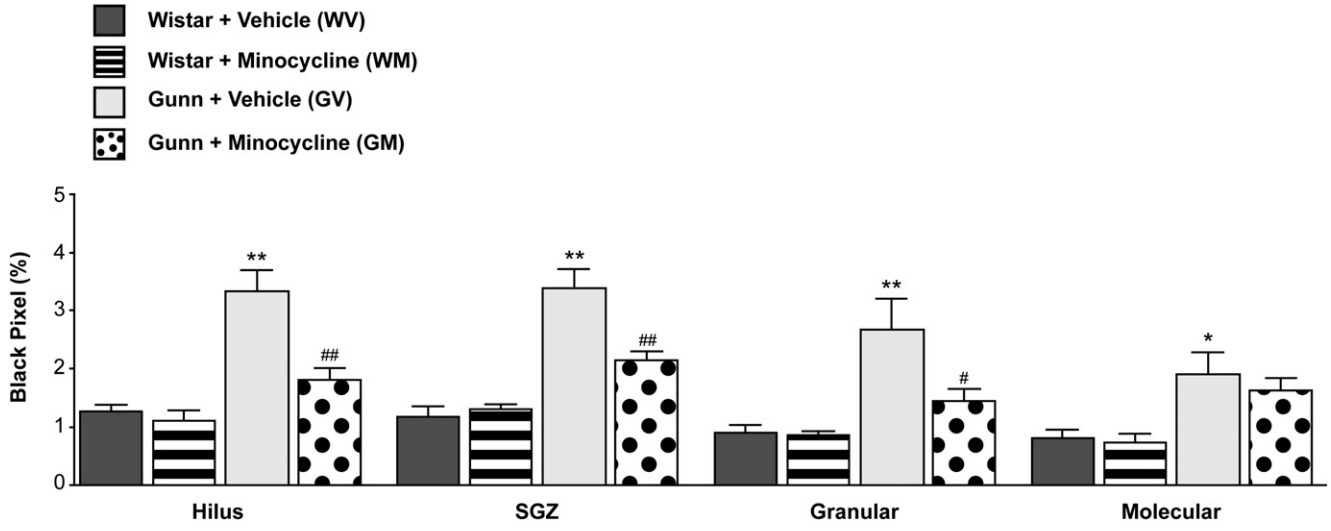

Fig. 4. Mean percentage of black pixels indicating CD11b immunoreactivity in the hippocampal dentate gyrus (DG). Each value is the mean ± S.E.M. (n = 6 per group). *$p < 0.005$, **$p < 0.001$ as compared to WV control group; #$p < 0.01$, ##$p < 0.001$ as compared to GV group.

the number and/or morphology of microglial cells in brain regions involved in cognitive and emotional aspects of behavior may represent an etiological factor in the onset or progression of memory impairment and neuropsychiatric disorders (Blank and Prinz, 2013). Accordingly, we assumed that the chronic microglial activation in adult Gunn rat is potentially more maladaptive than neuroprotective (Liaury et al., 2012), and based on our present results, we suggest that the microglial activation in the hippocampal DG in Gunn rat may be involved in promoting cognitive impairment, as was shown in the behavior test.

Consecutive administration of minocycline for 14 days significantly improved the impaired recognition memory in Gunn rats, which was indicated by the increase of exploratory preference in the retention session during NORT. Moreover, minocycline significantly attenuated the activation of microglial cells in the hippocampal DG of Gunn rats, which was indicated by the decreased immunoreactivity of CD11b marker. According to previous studies showing about the neuroprotective effects of minocycline (Miyaoka, 2012; Yrjanheikki et al., 1998), we assume that this phenomenon is part of the mechanism underlying minocycline's anti-inflammatory properties through inhibition of microglial activation. Similar results were reported that minocycline significantly improved the recognition memory of mice treated with methamphetamine (Mizoguchi et al., 2008) and phencyclidine (Fujita et al., 2008). Minocycline was also reported to significantly attenuate the microglial activation in mouse brains induced by methamphetamine and 3,4-methylenedioxymethamphetamine (Zhang et al., 2006a, 2006b). Taking these facts and our results into consideration, we speculate that the inhibition of microglial activation by minocycline in the hippocampal DG may, in part, be implicated in the mechanism of minocycline action to improve the recognition memory in Gunn rats. Although we demonstrated that minocycline attenuated microglial activation, we did not evaluate its effects on inflammatory markers, such as pro-inflammatory cytokines and chemokines. Therefore, it may be of interest to further study and establish the anti-inflammatory effects of minocycline in Gunn rats. On the other hand, the PPI deficit in Gunn rats was not fully rescued by minocycline administration. While the results at the 80 dB showed a significant improvement in the PPI deficits, later we only found a tendency toward improvement at the 70 dB. We speculate that this may be due to the small number of animals used for the PPI test ($n = 7$, per group). Other possibility is that it may be due to the incomplete inhibition of microglial activation, since minocyline did not reduce the immunoreactivity of CD11b in the molecular layer of the hippocampal DG in Gunn rats. Another study also reported the partial improvement of minocycline on olfactory bulbectomized (OBX) rats, a model of cognitive and behavioral impairments arising from neurodegenerative processes (Borre et al., 2012). Minocycline normalized OBX-induced hyperactivity in the open field, protected against hippocampal dependent spatial memory deficit, but failed to prevent fear memory loss. They suggested that minocycline may have ameliorated the OBX-induced cognitive deficits in a region specific manner and that treatment with minocycline may be effective in the early phase of a neurodegenerative disease.

However, the exact cellular and molecular mechanisms underlying this complex UCB–neuron–glia interaction in Gunn rats still remain elusive. We presume that another mechanism may also be involved, which is mediated through the glutamatergic system in the brain. Exposure of glia cells to UCB increases the extracellular concentration of glutamate by decreased uptake (Silva et al., 1999) and/or enhanced secretion (Fernandes et al., 2004), engendering overstimulation of glutamate and N-methyl-D-aspartate (NMDA) receptor (Ostrow et al., 2004). Moreover, high levels of bilirubin can lower the brain's threshold and enhance its vulnerability to NMDA-triggered excitotoxic brain injury (McDonald et al., 1998). Release of pro-inflammatory cytokines may affect gliogenesis and neurogenesis, and may lead to deficits in learning and memory. Dysregulation of glutamate metabolism and over-expression of tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β are consistent with schizophrenia neuropathology. Increased proinflammatory status of the brain also interacts with glutamatergic and dopaminergic neurotransmission, which can induce or aggravate positive, negative, and cognitive symptoms of schizophrenia (Muller and Schwarz, 2006). Interestingly, some studies have shown that minocycline has properties that affect glutamatergic pathways. Administration of minocycline to neurons in-vitro and to mice in-vivo increased GluR1 phosphorylation and membrane insertion (Imbesi et al., 2008). GluR1 receptors, which appear to be critical for cognitive processes that are impaired in schizophrenia, may be crucially involved in the pathobiology of schizophrenia (Wiedholz et al., 2008). Moreover, minocycline attenuated behavioral changes after administration of a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (Levkovitz et al., 2007; Zhang et al., 2007).

Inhibition of chronic neuroinflammation, particularly of microglial activation, has been suggested to be a practical strategy in the treatment of many psychiatry diseases, including schizophrenia (Dean et al., 2012). Two studies reported the effectiveness of minocycline adjunctive therapy in early schizophrenia for negative symptoms (Chaudhry et al., 2012; Levkovitz et al., 2010). In addition, Levkovitz et al. also reported the improvement on cognitive function. Another report showed two cases with persistent schizophrenia symptoms despite long term clozapine treatment that were treated successfully with adjunct minocyline (Kelly et al., 2011). Moreover, long term treatment with minocycline is generally safe and well tolerated in humans (Bonelli et al., 2003; Stone et al., 2003). Taken together, it might be worthwhile to further explore the effects of minocycline in schizophrenia.

5. Conclusion

The present study indicates that minocycline improves recognition memory and attenuates the activation of microglial cells in the hippocampal DG of Gunn rat, a possible hyperbilirubinemia-induced animal model of schizophrenia. Therefore, minocycline may be a potential therapeutic drug for schizophrenia. Our results may also provide crucial information to elucidate the etiology of schizophrenia and support the possibility of using the Gunn rat as an animal model of schizophrenia.

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