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Research article

Comparison of rapid and long-lasting antidepressant effects of negative modulators of α5-containing $GABA_A$ receptors and (*R*)-ketamine in a chronic social defeat stress model

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ABSTRACT

The negative allosteric modulators (NAMs: L-655,708 and MRK-016) of α5 subunit-containing $GABA_A$ receptors are reported to show rapid-acting antidepressant effects in rodents. However, there are no reports comparing these NAMs and (*R*)-ketamine, (*R*)-enantiomer of the rapid-acting antidepressant ketamine, in a chronic social defeat stress (CSDS) model. Here we measured expression of α5 $GABA_A$ receptor in the brain regions from CSDS susceptible mice and postmortem brain samples from depressed patients. Expression of α5 $GABA_A$ receptor in the prefrontal cortex and hippocampus from CSDS susceptible mice was significantly higher than that of control mice. Furthermore, expression of α5 $GABA_A$ receptor in the parietal cortex from depressed patients was also higher than that of control subjects. In the tail suspension and forced swimming tests, (*R*)-ketamine and MRK-016 significantly attenuated the increased immobility time in the susceptible mice, compared with the vehicle-treated group. In the sucrose preference test, (*R*)-ketamine and MRK-016 significantly enhanced the reduced preference in CSDS susceptible mice two days after a single injection. Unlike (*R*)-ketamine, MRK-016 did not attenuate the reduced sucrose preference in susceptible mice 7 days after a single injection. In contrast, L-655,708 did not show antidepressant effects in the same model. In conclusion, this study shows that increased levels of α5 $GABA_A$ receptors in the PFC and hippocampus may play a role in depression-like phenotype after CSDS. It is unlikely that MRK-016 has long-lasting antidepressant effects although it elicits rapid-acting antidepressant effects.

1. Introduction

Depression is one of the most common psychiatric disorders. Although the currently available antidepressants are generally effective in the treatment of depression, there is a significant time lag of weeks to months (Duman, 2018). Despite the efficacy of standard treatments, approximately first-thirds of depressed patients fail to respond to the current pharmacotherapy. Therefore, the development of novel drugs capable of inducing rapid and robust antidepressant responses in treatment-resistant depressed patients is unmet need (Chaki, 2017; Duman, 2018; Garay et al., 2018; Hashimoto, 2015; Krystal et al., 2013; Monteggia and Zarate, 2015; Murrough et al., 2017; Witkin et al., 2018).

The discovery of rapid-acting and sustained antidepressant effects of *N*-methyl-D-aspartate receptor (NMDAR) antagonist (*R*,*S*)-ketamine in treatment-resistant depression is the greatest breakthrough in the field of depression in over 60 years (Duman, 2018). A single sub-anesthetic dose of (*R*,*S*)-ketamine produces a rapid and robust antidepressant response in two-thirds of patients with treatment-resistant depression, which can last for over a week (Aan Het Rot et al., 2012; Berman et al., 2000; Diazgranados et al., 2010; Zarate et al., 2006, 2012). (*R*,*S*)-ketamine is a racemic mixture containing equal parts of (*R*)-ketamine (arketamine) and (*S*)-ketamine (esketamine). (*S*)-ketamine has an approximately 4-fold greater affinity for the NMDAR than (*R*)-ketamine (Domino, 2010). Preclinical studies demonstrated that (*R*)-ketamine showed greater potency and longer lasting antidepressant effects than (*S*)-ketamine in different animal models of depression (Zhang et al., 2014; Yang et al., 2015b; Zanos et al., 2016; Fukumoto et al., 2017; Yang et al., 2017a, 2017b, 2018). Importantly, unlike (*S*)-ketamine, (*R*)-ketamine may not induce psychotomimetic side effects or exhibit

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abuse potential in rodents and monkeys (Hashimoto et al., 2017; Tian et al., 2018; Yang et al., 2015b; Yang et al., 2016). Collectively, (*R*)-ketamine could be a safer antidepressant effect than (*S*)-ketamine and (*R*,*S*)-ketamine (Hashimoto, 2014, 2016a, 2016b, 2016c). However, a clinical study of (*R*)-ketamine in depressed patients has not yet been reported.

The $GABA_A$ (γ-aminobutyric acid, type A) receptors play a role in a number of psychiatric disorders including depression since the regulation of $GABA_A$ receptors is known to influence glutamate neurotransmission (Kalueff and Nutt, 2007; Luscher et al., 2011; Rudolph and Knoflach, 2011; Rudolph and Möhler, 2014). Recent studies showed that two negative allosteric modulators (NAMs: L-655,708 and MRK-016) (Atack et al., 2005, 2006, 2009) of α5 subunit-containing $GABA_A$ receptors produced rapid antidepressant effects in chronic restraint stress (CRS) and chronic unpredictable stress (CUS) models (Fischell et al., 2015). Unlike (*R*,*S*)-ketamine, MRK-016 produced no impairment of rota-rod performance, no reduction of prepulse inhibition, no conditioned-place preference, and no change in locomotion (Zanos et al., 2017). However, there are no reports showing the comparison of (*R*)-ketamine and these two NAMs in animal models of depression. In addition, there are no reports showing alterations in the expression of α5 $GABA_A$ receptors in susceptible mice or postmortem brain samples from depressed patients.

The purpose of this study was undertaken to study the role of α5 subunit-containing $GABA_A$ receptors in the pathophysiology of depression and in the therapeutic target for this disease. First, we examined whether the protein expression of α5 subunit-containing $GABA_A$ receptors was altered in the brain regions from susceptible mice after chronic social defeat stress (CSDS). Second, we examined whether the protein expression of α5 subunit-containing $GABA_A$ receptors was altered in the postmortem brain samples from depressed patients. Finally, we compared the antidepressant effects of (*R*)-ketamine and two NAMs (L-655,708 and MRK-016) of α5 subunit-containing $GABA_A$ receptors in a CSDS model.

2. Methods

2.1. Animals

Male adult C57BL/6 mice, aged 8 weeks (body weight 20–25 g, Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 mice, aged 13–15 weeks (body weight > 40 g, Japan SLC, Inc., Hamamatsu, Japan) were used in experiments. Animals were housed under controlled temperatures and 12 hour light/dark cycles (lights on between 07:00 and 19:00 h), with ad libitum food and water. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee (permission number: 30-323). All efforts were made to minimize suffering.

2.2. Drugs and drug administration

(*R*)-ketamine hydrochloride was prepared by recrystallization of racemic ketamine (Ketalar®, ketamine hydrochloride, Daiichi Sankyo Pharmaceutical Ltd., Tokyo, Japan) and D-(−)-tartaric acid, as described previously (Zhang et al., 2014). Vehicle (10 ml/kg; 25% dimethyl sulfoxide (DMSO)), (*R*)-ketamine (10 mg/kg as hydrochloride salt), L-655,708 (1.0 mg/kg, Tocris Bioscience, Bristol, UK), or MRK-016 (3.0 mg/kg, Tocris Bioscience, Bristol, UK) was administered intraperitoneally (i.p.) into susceptible mice after CSDS. The doses of (*R*)-ketamine (10 mg/kg), L-655,708 (1.0 mg/kg) and MRK-016 (3.0 mg/kg) were selected as reported previously (Fischell et al., 2015; Yang et al., 2015b, 2016, 2017a, 2017b, 2018; Zanos et al., 2017).

2.3. Chronic social defeat stress (CSDS)

CSDS model was performed as previously reported (Golden et al., 2011: Ma et al., 2016; Ren et al., 2016; Yang et al., 2015b, 2016, 2017a, 2017b, 2018; Zhang et al., 2015b). Every day the C57BL/6 mice were exposed to a different CD1 aggressor mouse for 10 min, total for 10 days. When the social defeat session ended, the resident CD1 mouse and the intruder mouse were housed in one half of the cage separated by a perforated Plexiglas divider to allow visual, olfactory, and auditory contact for the remainder of the 24-h period. At 24 h after the last session, all mice were housed individually. On day 11, a social interaction test was performed to identify subgroups of mice that were susceptible and unsusceptible to social defeat stress. This was accomplished by placing mice in an interaction test box (42 × 42 cm) with an empty wire-mesh cage (10 × 4.5 cm) located at one end. The movement of the mice was tracked for 2.5 min, followed by 2.5 min in the presence of an unfamiliar aggressor confined in the wire-mesh cage. The duration of the subject's presence in the “interaction zone” (defined as the 8-cm-wide area surrounding the wiremesh cage) was recorded by a stopwatch. The interaction ratio was calculated as time spent in an interaction zone with an aggressor/time spent in an interaction zone without an aggressor. An interaction ratio of 1 was set as the cutoff: mice with scores < 1 were defined as “susceptible” to social defeat stress and those with scores ≥ 1 were defined as “unsusceptible”. Only susceptible mice were used in the subsequent experiments.

2.4. Behavioral tests

Behavioral tests were performed as reported previously (Ma et al., 2016; Ren et al., 2016; Yang et al., 2015b, 2016, 2017a, 2017b, 2018; Zhang et al., 2015b).

2.4.1. Locomotion

The locomotor activity was measured by an animal movement analysis system SCANETMV-40 (MELQUEST Co., Ltd., Toyama, Japan), the mice were placed in experimental cages (length × width × height: 560 × 560 × 330 mm). The cumulative exercise was recorded for 60 min. Cages were cleaned between testing session.

2.4.2. Tail suspension test (TST)

A small piece of adhesive tape placed approximately 2 cm from the tip of the tail for mouse. A single hole was punched in the tape and mice were hung individually, on a hook. The immobility time was recorded for 10 min. Mice were considered immobile only when they hung passively and completely motionless.

2.4.3. Forced swimming test (FST)

The FST was tested by an automated forced-swim apparatus SCANET MV-40 (MELQUEST Co., Ltd., Toyama, Japan). The mice were placed individually in a cylinder (diameter: 23 cm; height: 31 cm) containing 15 cm of water, maintained at 23 ± 1 °C. Immobility time from activity time as (total) − (active) time was calculated by the apparatus analysis software. The immobility time for mouse was recorded for 6 min.

2.4.4. Sucrose preference test (SPT)

Mice were exposed to water and 1% sucrose solution for 48 h, followed by 4 h of water and food deprivation and a 1 hour exposure to two identical bottles, one is water, and another is 1% sucrose solution. The bottles containing water and sucrose were weighed before and at the end of this period and the sucrose preference was determined.

2.5. Western blot analysis

Western blot analysis was performed by one observer who was blinded to the four groups. Brain regions of prefrontal cortex (PFC),

Table 1
Characteristics of the postmortem samples from Neuropathology Consortium of the Stanley Medical Research Institute.

Characteristics	Control (n = 15)	MDD (n = 15)	P value
Age at death (years)	48.1 ± 10.7 (29–68)	46.5 ± 9.3 (30–65)	0.678[a]
Gender (male/female)	9/6	9/6	1.000[b]

The data are shown the mean ± SD. MDD: major depressive disorder.
[a] Unpaired *t*-test.
[b] x^2 test for independence.

CA1, CA3, and dentate gyrus (DG) from hippocampus, nucleus accumbens (NAc), and cerebellum were collected from control (no CSDS) mice and CSDS susceptible mice 9 days after social interaction test. Human postmortem brain samples from Stanley Research Foundation (Bethesda, MD, USA) (Torrey et al., 2000) were stored at −80 °C until biochemical analyses. We used the postmortem brain samples from MDD patients (n = 15) and age- and gender-matched controls (n = 15) (Table 1). Tissue samples were homogenized in Laemmli lysis buffer, then centrifuged at 3000 ×*g* at 4 °C, for 10 min to obtain the supernatants. Protein concentrations were determined using a BCA method assay kit (Bio-Rad, Hercules, CA), then samples were incubated for 5 min at 95 °C, with an equal volume of 125 mM Tris/HCl, pH 6.8, 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol and 4% sodium dodecyl sulfate. Proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, on 10% mini-gels (Mini-PROTEAN® TGX™ Precast Gel; Bio-Rad). Separated proteins were then transferred onto polyvinylidene difluoride membranes using a Trans Blot Mini Cell (Bio-Rad). For immunodetection, blots were blocked with 2% BSA in TBST (TBS + 0.1% Tween-20) for 1 h at room temperature (RT), then incubated with primary antibodies overnight, at 4 °C. The following primary antibodies were used: anti-$GABA_A$ receptor subunit α5 (1:1000,Cat #: ab10098, Abcam, Tokyo, Japan) and β-actin (1:10,000, Sigma-Aldrich Co., Ltd., St Louis, MO, USA). The next day, blots were washed three times in TBST and incubated with horseradish peroxidase conjugated anti-rabbit or anti-mouse antibody (1:5000) for 1 h, at RT. After three washes in TBST, bands were detected using enhanced chemiluminescence (ECL), plus the Western Blotting Detection system (GE Healthcare Bioscience). Finally, blots were washed three times in TBST and incubated with a primary antibody directed against β-actin. Images were captured with a Fuji LAS3000-mini imaging system (Fujifilm, Tokyo, Japan), and immunoreactive bands were quantified.

2.6. Statistical analysis

The data show as the mean ± standard error of the mean (S.E.M.). Analysis was performed using PASW Statistics 20 (formerly SPSS Statistics; SPSS). The data of two groups were analyzed using Student *t*-test. The behavioral data were analyzed using the one-way analysis of variance (ANOVA), followed by *post-hoc* Dunnet test. The P-values of < 0.05 were considered statistically significant.

3. Results

3.1. Altered expression of α5 $GABA_A$ receptors in the brain regions from CSDS susceptible mice

Protein expression of α5 $GABA_A$ receptors in the PFC and hippocampus from CSDS susceptible mice was significantly higher than that of control mice (Fig. 1). In contrast, protein expression of α5 $GABA_A$ receptors in the NAc from CSDS susceptible mice was significantly lower than that of control mice. Furthermore, there is no change in the expression of α5 $GABA_A$ receptors in the cerebellum between control mice and CSDS susceptible mice (Fig. 1).

3.2. Increased expression of α5 $GABA_A$ receptors in the parietal cortex from depressed patients

Western blot analysis using postmortem brain samples showed that the protein expression of α5 $GABA_A$ receptors in the parietal cortex from depressed patients was significantly higher than that of control subjects (Fig. 2).

3.3. Effects of (R)-ketamine, L-655,708, and MRK-016 in a CSDS model

Two compounds (L-655,708 and MRK-016) are reported to have antidepressant effects in a chronic restraint stress (CRS) model and a chronic unpredictable stress (CUS) model (Fischell et al., 2015). In this study, we compared the rapid-acting and sustained antidepressant effects of (*R*)-ketamine, L-655,708 and MRK-016 in a CSDS model (Fig. 3A).

Locomotion showed no difference ($F_{4,76} = 0.143$, $P = 0.966$) among the five groups (Fig. 3B). In the TST and FST, (*R*)-ketamine (10 mg/kg) and MRK-016 (3.0 mg/kg), but not L-655,708 (1.0 mg/kg), significantly attenuated the increased immobility times in susceptible mice (Fig. 3C and D). One-way ANOVA detected statistical significance in both the TST and FST (TST: $F_{4,76} = 5.970$, $P < 0.001$; FST: $F_{4,76} = 5.097$, $P = 0.001$) among the five groups (Fig. 3C and D). In the SPT, the sucrose preference of mice after a single injection of (*R*)-ketamine or MRK-016 was significantly higher ($F_{4,76} = 4.307$, $P = 0.003$) than that of the vehicle-treated group (Fig. 3E). In contrast, the sucrose preference of mice 7 days after a single injection of MRK-016 did not show antidepressant effects although (*R*)-ketamine showed antidepressant effects 7 days after a single injection (Fig. 3F). These behavioral data suggest that both (*R*)-ketamine and MRK-016 promote rapid antidepressant effects in a CSDS model, and that (*R*)-ketamine produces longer lasting antidepressant effects compared to MRK-016. Unexpectedly, L-655,708 (1.0 mg/kg) did not show antidepressant effects in a CSDS model, inconsistent with the previous report (Fischell et al., 2015).

4. Discussion

The findings of this study are as follows: Protein expression of α5 $GABA_A$ receptors in the PFC and hippocampus from CSDS susceptible mice was higher than that of control mice. In contrast, protein expression of α5 $GABA_A$ receptors in the NAc from CSDS susceptible mice was lower than that of control mice. Furthermore, protein expression of α5 $GABA_A$ receptors in the parietal cortex from depressed patients was higher than that of control subjects. Collectively, it is likely that increased expression of α5 $GABA_A$ receptors in the PFC and hippocampus as well as decreased expression of α5 $GABA_A$ receptors in the NAc may play a role in depression-like behaviors after CSDS. Interestingly, a single dose of (*R*)-ketamine and MRK-016 promoted a rapid antidepressant response in a CSDS model, although (*R*)-ketamine produced longer-lasting antidepressant effects than MRK-016. In contrast, L-655,708 did not show antidepressant effects in a CSDS model, inconsistent with previous report (Fischell et al., 2015). To the best of our knowledge, this is the first report showing a comparison of antidepressant effects for (*R*)-ketamine and NAMs at α5 $GABA_A$ receptors (L-655,708 and MRK-016) in a CSDS model of depression.

We previously reported a marked reduction of BDNF protein in the PFC and hippocampus of inflammation-treated mice (Zhang et al., 2015a), CSDS susceptible mice (Yang et al., 2015b; Zhang et al., 2015b) and learned helplessness rats (Shirayama et al., 2015; Yang et al., 2015a). In contrast, rodents with depression-like phenotype induced a marked increase in BDNF protein within the NAc (Zhang et al., 2015a, 2015b; Yang et al., 2015a; Shirayama et al., 2015). Thus, it is likely that decreased levels of BDNF in DG and CA3 of hippocampus and PFC, as well as increased levels of BDNF in the NAc may promote depression-like behavior in rodents (Nestler and Carlezon, 2006; Ren et al., 2015;

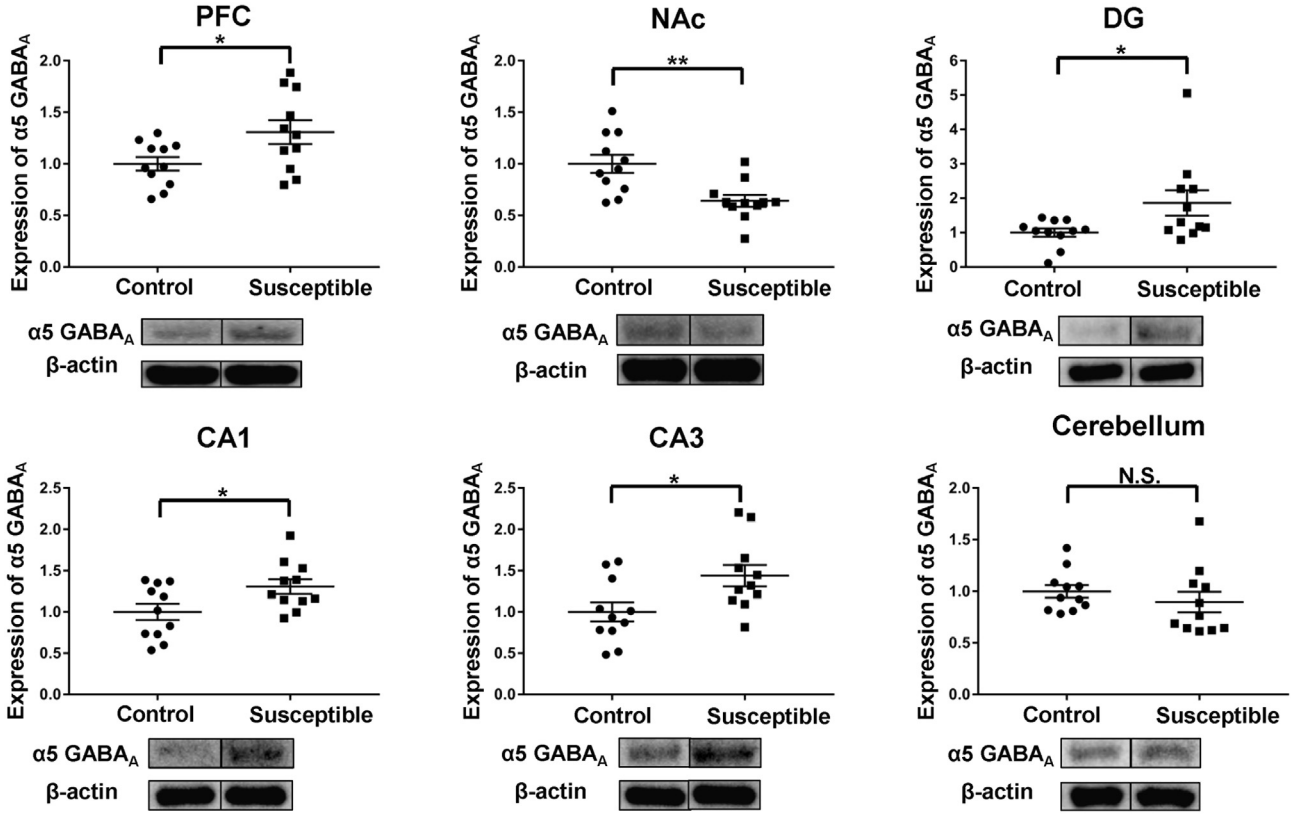

Fig. 1. Protein expression of α5 subtype of $GABA_A$ receptor in the mouse brain after CSDS.
Expression of α5 subtype of $GABA_A$ receptor in the PFC, CA1, CA3, DG of hippocampus from CSDS susceptible mice was significantly higher than that of control (no CSDS) mice. In contrast, expression of α5 subtype of $GABA_A$ receptor in the NAc from CSDS susceptible mice was significantly lower than that of control (no CSDS) mice. The values represent the mean ± S.E.M. (n = 11). *P < 0.05, **P < 0.01 compared to control group. N.S.: not significant.

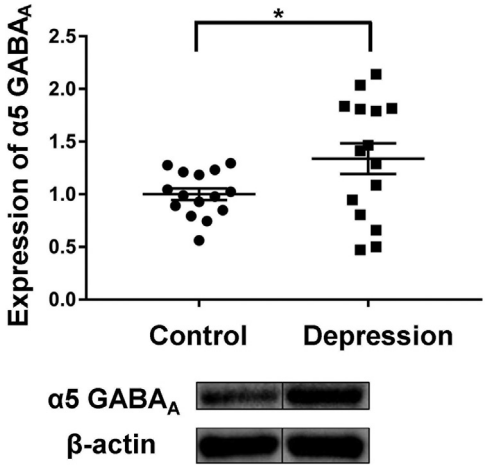

Fig. 2. Increased protein expression of α5 subtype of $GABA_A$ receptor in the parietal cortex from depressed patients.
Expression of α5 subtype of $GABA_A$ receptor in the parietal cortex from depressed patients was significantly higher than that of control subjects. The values represent the mean ± S.E.M. (n = 15). *P < 0.05 compared to control group.

Shirayama et al., 2015; Yang et al., 2015a; Zhang et al., 2015a, 2015b, 2016). In this study, we found a marked increase of α5 $GABA_A$ receptors in the PFC and hippocampus of CSDS susceptible mice whereas the expression of α5 $GABA_A$ receptors in the NAc of CSDS susceptible mice was decreased compared with control mice. Collectively, it is probable that CSDS causes increased α5 $GABA_A$ receptors in the hippocampus and PFC, but decreased α5 $GABA_A$ receptors in the NAc, resulting in depression-like behavior in mice. Interestingly, we also found increased expression of α5 $GABA_A$ receptors in the parietal cortex from depressed patients. In contrast, there was no change in the expression of α5 $GABA_A$ receptors in the lateral cerebella from depressed patients (Fatemi et al., 2013). The discrepancy may be due to the difference of brain regions (parietal cortex vs. lateral cerebella). Taken together, it is likely that increased expression of α5 $GABA_A$ receptors in the prefrontal cortex may play a role in the pathophysiology of depression although further study is needed. It is also of interest to study whether α5 $GABA_A$ receptors can modulate BDNF-TrkB signaling in these brain regions.

It is reported that L-655,708 (0.7 mg/kg) and MRK-016 (3.0 mg/kg) rapidly reversed loss of sucrose preference and social interaction behaviors in rats after CRS or CUS (Fischell et al., 2015), and that MRK-016 (3.0 mg/kg) reversed reduced sucrose preference in mice after CRS (Zanos et al., 2017). In contrast, we found that, like (*R*)-ketamine, MRK-016 (3.0 mg/kg) showed rapid-acting antidepressant effects in CSDS susceptible mice although L-655,708 (1.0 mg/kg) did not show antidepressant effects in the same model. Furthermore, L-655,708 (3 mg/kg) is reported to show sustained (1 week) antidepressant-like effects in the forced swimming test in control rats without depression-like phenotype (Carreno et al., 2017). Although the reasons underlying this discrepancy are currently unknown, the different models (CRS or CUS rat models for Fischell et al., 2015, control naïve rats for Carreno et al., 2017 vs. CSDS mouse model for this study) may contribute to the discrepancy of antidepressant actions of two studies (Hashimoto and Shirayama, 2018). Importantly, it is also known that MRK-016 is a full inverse agonist at α5 subtype whereas L-655,708 is a very weak inverse agonist at α5 subtype (Rudolph and Knoflach, 2011). Therefore, it seems that the different pharmacology (MRK-016: full inverse agonist at α5 subtype vs. L-655,708: very weak inverse agonist at α5 subtype)

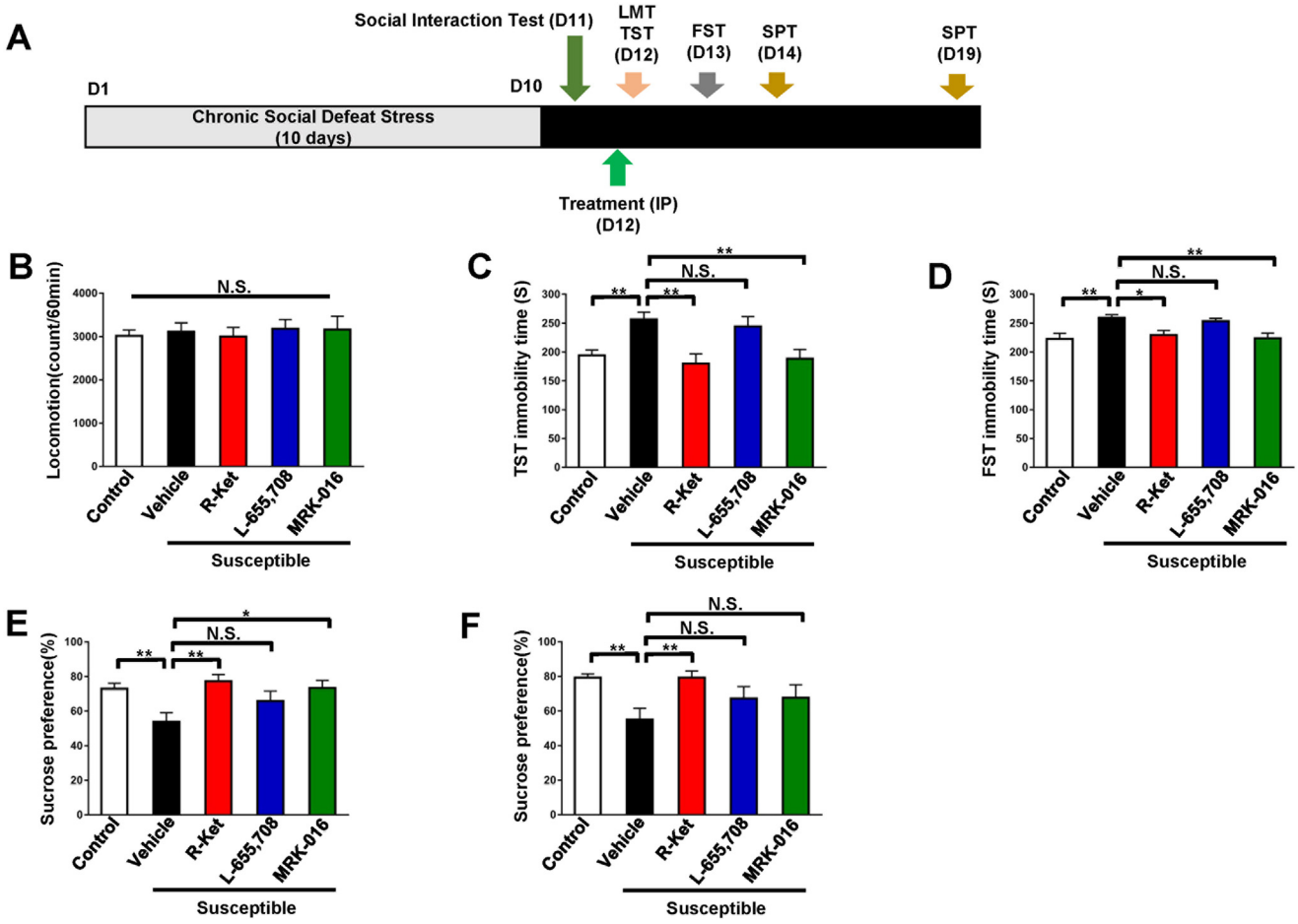

Fig. 3. Comparison of the rapid and long-lasting antidepressant effects of (*R*)-ketamine, L-655,708, and MRK-016 in a CSDS model.
(A): CSDS was performed from day 1 to day 10, and the social interaction test (SIT) was performed on day 11. Vehicle (25% DMSO, 10 ml/kg), (*R*)-ketamine (10 mg/kg), L-655,708 (1.0 mg/kg), or MRK-016 (3.0 mg/kg) was administered i.p. in the susceptible mice on day 12. Locomotion and tail suspension test (TST) were performed 2 and 4 h after injection, respectively. Forced swimming test (FST) was performed 1 day after injection. One % sucrose preference test (SPT) was performed 2 (day 14) and 7 days (day 19) after injection. (B): Locomotion. (C): TST. (D): FST. (E): SPT on day 14. (F): SPT on day 19. The values represent the mean ± S.E.M. (n = 15–18). *P < 0.05, **P < 0.01 compared to vehicle-treated susceptible group N.S.: not significant. LMT: locomotion test; TST: tail suspension test; R-Ket: (*R*)-ketamine; SPT: 1% sucrose preference test.

of two compounds may contribute to the discrepancy of antidepressant actions of these two NAMs in a CSDS model. In contrast, α5 $GABA_A$ receptor antagonist S44819 did not show antidepressant-like effects in the tail suspension and forced swimming tests (Gacsályi et al., 2017). Collectively, it is likely that full inverse agonists at α5 subtype of $GABA_A$ receptors may have more potent antidepressant actions than weak inverse agonists although further detailed study is needed.

It is also reported that MRK-016 (3.0 mg/kg) showed long-lasting (7 days) antidepressant effects in CRS rat model (Fischell et al., 2015). However, we did not find long-lasting (7 days) antidepressant effects of MRK-016 (3.0 mg/kg) in a CSDS model although (*R*)-ketamine showed long-lasting (7 days) antidepressant effects in the same model. Although the reasons underlying this discrepancy are currently unknown, the different animal models (CRS rat model for Fischell et al., 2015 vs. CSDS mouse model for this study) may contribute to the discrepancy of antidepressant actions of two studies.

The endogenous NMDAR agonist D-serine and glycine transporter-1 inhibitor sarcosine have antidepressant effects in rodent (Kawaura et al., 2015; Chen et al., 2015, 2017; Wei et al., 2017) and depressed patients (Huang et al., 2013). Interestingly, it is reported that serum levels of D-serine and L-serine in depressed patients were significantly higher than those of healthy controls, and that the ratio of L-serine to glycine in depressed patients was higher than that of healthy controls (Hashimoto et al., 2016), suggesting abnormality in the D-serine-L-serine-glycine cycle in depression. Therefore, it is of interest to compare antidepressant effects of D-serine (or sarcosine) and $GABA_A$ NAMs in animal models of depression.

In conclusion, this study shows that a single dose of (*R*)-ketamine or MRK-016 (full inverse agonist at α5 subtype) can produce rapid antidepressant effects in a CSDS model of depression, and that (*R*)-ketamine elicits a longer-lasting antidepressant effect than MRK-016. In contrast, L-655,708 (very weak inverse agonist at α5 subtype) did not show antidepressant effects in the same model. Collectively, full inverse agonists at α5 subtype of $GABA_A$ receptor may have ketamine-like rapid-acting, but not longer-lasting, antidepressant effects.

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Conflict of interest

Dr. Hashimoto is an inventor on a filed patent application on “The use of (*R*)-ketamine in the treatment of psychiatric diseases” by Chiba

University. Dr. Hashimoto has received research support from Dainippon-Sumitomo, Otsuka, and Taisho. Other authors declare no conflict of interest.

References

Aan Het Rot, M., Zarate Jr., C.A., Charney, D.S., Mathew, S.J., 2012. Ketamine for depression: where do we go from here? Biol. Psychiatry 72, 537–547.

Atack, J.R., Alder, L., Cook, S.M., Smith, A.J., McKernan, R.M., 2005. *In vivo* labelling of α5 subunit-containing GABAA receptors using the selective radioligand [^{3}H] L-655,708. Neuropharmacology 49, 220–229.

Atack, J.R., Bayley, P.J., Seabrook, G.R., Wafford, K.A., McKernan, R.M., Dawson, G.R., 2006. L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for α5-containing GABAA receptors. Neuropharmacology 51, 1023–1029.

Atack, J.R., Maubach, K.A., Wafford, K.A., O'Connor, D., Rodrigues, A.D., Evans, D.C., Tattersall, F.D., Chambers, M.S., MacLeod, A.M., Eng, W.S., Ryan, C., Hostetler, E., Sanabria, S.M., Gibson, R.E., Krause, S., Burns, H.D., Hargreaves, R.J., Agrawal, N.G., McKernan, R.M., Murphy, M.G., Gingrich, K., Dawson, G.R., Musson, D.G., Petty, K.J., 2009. *In vitro* and *in vivo* properties of 3-tert-butyl-7-(5-methylisoxazol-3-yl)-2-(1-methyl-1H-1, 2, 4-triazol-5-ylmethoxy)-pyrazolo [1, 5-d]-[1, 2, 4] triazine (MRK-016), a GABAA receptor α5 subtype-selective inverse agonist. J. Pharmacol. Exp. Ther. 331, 470–484.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351–354.

Carreno, F.R., Collins, G.T., Frazer, A., Lodge, D.J., 2017. Selective pharmacological augmentation of hippocampal activity produces a sustained antidepressant-like response without abuse-related or psychotomimetic effects. Int. J. Neuropsychopharmacol. 20, 504–509.

Chaki, S., 2017. mGlu2/3 receptor antagonists as novel antidepressants. Trends Pharmacol. Sci. 38, 569–580.

Chen, K.T., Tsai, M.H., Wu, C.H., Jou, M.J., Wei, I.H., Huang, C.C., 2015. AMPA receptor-mTOR activation is required for the antidepressant-like effects of sarcosine during the forced swim test in rats: insertion of AMPA receptor may play a role. Front. Behav. Neurosci. 9, 162.

Chen, K.T., Wu, C.H., Tsai, M.H., Wu, Y.C., Jou, M.J., Huang, C.C., Wei, I.H., 2017. Antidepressant-like effects of long-term sarcosine treatment in rats with or without chronic unpredictable stress. Behav. Brain Res. 316, 1–10.

Diazgranados, N., Ibrahim, L., Brutsche, N.E., Newberg, A., Kronstein, P., Khalife, S., Kammerer, W.A., Quezado, Z., Luckenbaugh, D.A., Salvadore, G., Machado-Vieira, R., Manji, H.K., Zarate Jr., C.A., 2010. A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. Arch. Gen. Psychiatry 67, 793–802.

Domino, E.F., 2010. Taming the ketamine tiger. Anesthesiology 113, 678–684.

Duman, R.S., 2018. Ketamine and rapid-acting antidepressants: a new era in the battle against depression and suicide. F1000Research 7.

Fatemi, S.H., Folsom, T.D., Rooney, R.J., Thuras, P.D., 2013. Expression of GABA A α2-, β1-and ε-receptors are altered significantly in the lateral cerebellum of subjects with schizophrenia, major depression and bipolar disorder. Transl. Psychiatry 3, e303.

Fischell, J., Van Dyke, A.M., Kvarta, M.D., LeGates, T.A., Thompson, S.M., 2015. Rapid antidepressant action and restoration of excitatory synaptic strength after chronic stress by negative modulators of alpha5-containing GABA A receptors. Neuropsychopharmacology 40, 2499–2509.

Fukumoto, K., Toki, H., Iijima, M., Hashihayata, T., Yamaguchi, J.I., Hashimoto, K., Chaki, S., 2017. Antidepressant potential of (*R*)-ketamine in rodent models: comparison with (*S*)-ketamine. J. Pharmacol. Exp. Ther. 361, 9–16.

Gacsályi, I., Móricz, K., Gigler, G., Wellmann, J., Nagy, K., Ling, I., Barkóczy, J., Haller, J., Lambert, J.J., Szénási, G., Spedding, M., Antoni, F.A., 2017. Behavioural pharmacology of the α5-GABA$_A$ receptor antagonist S44819: enhancement and remediation of cognitive performance in preclinical models. Neuropharmacology 125, 30–38.

Garay, R., Zarate, C.A., Cavero, I., Kim, Y.K., Charpeaud, T., Skolnick, P., 2018. The development of glutamate-based antidepressants is taking longer than expected. Drug Discov. Today 23, 1689–1692.

Golden, S.A., Covington III, H.E., Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice. Nat. Protoc. 6, 1183–1191.

Hashimoto, K., 2014. The R-stereoisomer of ketamine as an alternative for ketamine for treatment-resistant major depression. Clin. Psychopharmacol. Neurosci. 12, 72–73.

Hashimoto, K., 2015. Inflammatory biomarkers as differential predictors of antidepressant response. Int. J. Mol. Sci. 16, 7796–7801.

Hashimoto, K., 2016a. Letter to the Editor: R-ketamine: a rapid-onset and sustained antidepressant without risk of brain toxicity. Psychol. Med. 46, 2449–2451.

Hashimoto, K., 2016b. Ketamine's antidepressant action: beyond NMDA receptor inhibition. Expert Opin. Ther. Targets 20, 1389–1392.

Hashimoto, K., 2016c. Detrimental side effects of repeated ketamine infusions in the brain. Am. J. Psychiatry 173, 1044–1045.

Hashimoto, K., Shirayama, Y., 2018. What are the causes for discrepanmcies of antidepressant actions of (2*R*,6*R*)-hydroxynorketamine? Biol. Psychiatry 84, e7–e8.

Hashimoto, K., Yoshida, T., Ishikawa, M., Fujita, Y., Niitsu, T., Nakazato, M., Watanabe, H., Sasaki, T., Shiina, A., Hashimoto, T., Kanahara, N., Hasegawa, T., Enohara, M., Kimura, A., Iyo, M., 2016. Increased serum levels of serine enantiomers in patients with depression. Acta Neuropsychiatr. 28, 173–178.

Hashimoto, K., Kakiuchi, T., Ohba, H., Nishiyama, S., Tsukada, H., 2017. Reduction of dopamine $D_{2/3}$ receptor binding in the striatum after a single administration of es-ketamine, but not R-ketamine: a PET study in conscious monkeys. Eur. Arch. Psychiatry Clin. Neurosci. 267, 173–176.

Huang, C.C., Wei, I.H., Huang, C.L., Chen, K.T., Tsai, M.H., Tsai, P., Tun, R., Huang, K.H., Chang, Y.C., Lane, H.Y., Tsai, G.E., 2013. Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression. Biol. Psychiatry 74, 734–741.

Kalueff, A.V., Nutt, D.J., 2007. Role of GABA in anxiety and depression. Depress. Anxiety 24, 495–517.

Kawaura, K., Koike, H., Kinoshita, K., Kambe, D., Kaku, A., Karasawa, J., Chaki, S., Hikichi, H., 2015. Effects of a glycine transporter-1 inhibitor and D-serine on MK-801-induced immobility in the forced swimming test in rats. Behav. Brain Res. 278, 186–192.

Krystal, J.H., Sanacora, G., Duman, R.S., 2013. Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. Biol. Psychiatry 73, 1133–1141.

Luscher, B., Shen, Q., Sahir, N., 2011. The GABAergic deficit hypothesis of major depressive disorder. Mol. Psychiatry 16, 383–406.

Ma, M., Ren, Q., Yang, C., Zhang, J.C., Yao, W., Dong, C., Ohgi, Y., Futamura, T., Hashimoto, K., 2016. Adjunctive treatment of brexpiprazole with fluoxetine shows a rapid antidepressant effect in social defeat stress model: role of BDNF-TrkB signaling. Sci. Rep. 6, 39209.

Monteggia, L.M., Zarate Jr., C., 2015. Antidepressant actions of ketamine: from molecular mechanisms to clinical practice. Curr. Opin. Neurobiol. 30, 139–143.

Murrough, J.W., Abdallah, C.G., Mathew, S.J., 2017. Targeting glutamate signalling in depression: progress and prospects. Nat. Rev. Drug Discov. 16, 472–486.

Nestler, E.J., Carlezon Jr., W.A., 2006. The mesolimbic dopamine reward circuit in depression. Biol. Psychiatry 59, 1151–1159.

Ren, Q., Ma, M., Yang, C., Zhang, J.C., Yao, W., Hashimoto, K., 2015. BDNF–TrkB signaling in the nucleus accumbens shell of mice has key role in methamphetamine withdrawal symptoms. Transl. Psychiatry 5, e666.

Ren, Q., Ma, M., Ishima, T., Morisseau, C., Yang, J., Wagner, K.M., Zhang, J.C., Yang, C., Yao, W., Dong, C., Han, M., Hammock, B.D., Hashimoto, K., 2016. Gene deficiency and pharmacological inhibition of soluble epoxide hydrolase confers resilience to repeated social defeat stress. Proc. Natl. Acad. Sci. U. S. A. 113, E1944–E1952.

Rudolph, U., Knoflach, F., 2011. Beyond classical benzodiazepines: novel therapeutic potential of GABA A receptor subtypes. Nat. Rev. Drug Discov. 10, 685–697.

Rudolph, U., Möhler, H., 2014. GABA$_A$ receptor subtypes: therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. Annu. Rev. Pharmacol. Toxicol. 54, 483–507.

Shirayama, Y., Yang, C., Zhang, J.C., Ren, Q., Yao, W., Hashimoto, K., 2015. Alterations in brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in the brain regions of a learned helplessness rat model and the antidepressant effects of a TrkB agonist and antagonist. Eur. Neuropsychopharmacol. 25, 2449–2458.

Tian, Z., Dong, C., Fujita, A., Fujita, Y., Hashimoto, K., 2018. Expression of heat shock protein HSP-70 in the retrosplenial cortex of rat brain after administration of (*R*,*S*)-ketamine and (*S*)-ketamine, but not (*R*)-ketamine. Pharmacol. Biochem. Behav. 172, 17–21.

Torrey, E.F., Webster, M., Knable, M., Johnston, N., Yolken, R.H., 2000. The Stanley Foundation Brain Collection and Neuropathology Consortium. Schizophr. Res. 44, 151–155.

Wei, I.H., Chen, K.T., Tsai, M.H., Wu, C.H., Lane, H.Y., Huang, C.C., 2017. Acute amino acid D-serine administration, similar to ketamine, produces antidepressant-like effects through identical mechanisms. J. Agric. Food Chem. 65, 10792–10803.

Witkin, J.M., Knutson, D.E., Rodriguez, G.J., Shi, S., 2018. Rapid-acting antidepressants. Curr. Pharm. Des. https://doi.org/10.2174/1381612824666180730104707.

Yang, C., Shirayama, Y., Zhang, J.C., Ren, Q., Hashimoto, K., 2015a. Regional differences in brain-derived neurotrophic factor levels and dendritic spine density confer resilience to inescapable stress. Int. J. Neuropsychopharmacol. 18, pyu121.

Yang, C., Shirayama, Y., Zhang, J.C., Ren, Q., Yao, W., Ma, M., Hashimoto, K., 2015b. *R*-ketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects. Transl. Psychiatry 5, e632.

Yang, C., Han, M., Zhang, J.C., Ren, Q., Hashimoto, K., 2016. Loss of parvalbumin-immunoreactivity in mouse brain regions after repeated intermittent administration of esketamine, but not *R*-ketamine. Psychiatry Res. 239, 281–283.

Yang, C., Qu, Y., Abe, M., Nozawa, D., Chaki, S., Hashimoto, K., 2017a. (*R*)-ketamine shows greater potency and longer lasting antidepressant effects than its metabolite (2*R*,6*R*)-hydroxynorketamine. Biol. Psychiatry 82, e43–e44.

Yang, C., Qu, Y., Fujita, Y., Ren, Q., Ma, M., Dong, C., Hashimoto, K., 2017b. Possible role of the gut microbiota-brain axis in the antidepressant effects of (*R*)-ketamine sin a social defeat stress model. Transl. Psychiatry 7, 1294.

Yang, C., Ren, Q., Qu, Y., Zhang, J.C., Ma, M., Dong, C., Hashimoto, K., 2018. Mechanistic target of rapamycin–independent antidepressant effects of (R)-ketamine in a social defeat stress model. Biol. Psychiatry 83, 18–28.

Zanos, P., Moaddel, R., Morris, P.J., Georgiou, P., Fischell, J., Elmer, G.I., Alkondon, M., Yuan, P., Pribut, H.J., Singh, N.S., Dossou, K.S., Fang, Y., Huang, X.P., Mayo, C.L., Wainer, I.W., Albuquerque, E.X., Thompson, S.M., Thomas, C.J., Zarate Jr., C.A., Gould, T.D., 2016. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533, 481–486.

Zanos, P., Nelson, M.E., Highland, J.N., Krimmel, S.R., Georgiou, P., Gould, T.D., Thompson, S.M., 2017. A negative allosteric modulator for alpha5 subunit-containing GABA receptors exerts a rapid and persistent antidepressant-like action without the side effects of the NMDA receptor antagonist ketamine in mice. Eneuro 4, e0285-16.

Zarate Jr., C.A., Brutsche, N.E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., Selter, J., Marquardt, C.A., Liberty, V., Luckenbaugh, D.A., 2012. Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol. Psychiatry 71, 939–946.

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006. A randomized trial of an *N*-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63,

856–864.
Zhang, J.C., Li, S.X., Hashimoto, K., 2014. R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. Pharmacol. Biochem. Behav. 116, 137–141.
Zhang, J.C., Wu, J., Fujita, Y., Yao, W., Ren, Q., Yang, C., Li, S.X., Shirayama, Y., Hashimoto, K., 2015a. Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. Int. J. Neuropsychopharmacol. 18, pyu077.
Zhang, J.C., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., Han, M., Hashimoto, K., 2015b. Comparison of ketamine, 7, 8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. Psychopharmacology 232, 4325–4335.
Zhang, J.C., Yao, W., Hashimoto, K., 2016. Brain-derived neurotrophic factor (BDNF)-TrkB signaling in inflammation-related depression and potential therapeutic targets. Curr. Neuropharmacol. 14, 721–731.