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Comparison of antidepressant and side effects in mice after intranasal administration of (*R*,*S*)-ketamine, (*R*)-ketamine, and (*S*)-ketamine

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ABSTRACT

The *N*-methyl-D-aspartate receptor (NMDAR) antagonist (*R*,*S*)-ketamine produces rapid and sustained antidepressant effects in treatment-resistant patients with depression although intranasal use of (*R*,*S*)-ketamine in ketamine abusers is popular. In March 5, 2019, nasal spray of (*S*)-ketamine for treatment-resistant depression was approved as a new antidepressant by the US Food Drug Administration. Clinical study of (*R*)-ketamine is underway. In a chronic social defeat stress (CSDS) model, we compared the antidepressant effects of (*R*,*S*)-ketamine, (*R*)-ketamine, and (*S*)-ketamine after a single intranasal administration. Furthermore, we also compared the side effects (i.e., locomotion, prepulse inhibition (PPI), abuse liability) of these three compounds in mice. The order of potency of antidepressant effects after a single intranasal administration was (*R*)-ketamine > (*R*,*S*)-ketamine > (*S*)-ketamine. In contrast, the order of locomotor activity and prepulse inhibition (PPI) deficits after a single intranasal administration was (*S*)-ketamine > (*R*,*S*)-ketamine > (*R*)-ketamine. In the conditioned place preference (CPP) test, both (*S*)-ketamine and (*R*,*S*)-ketamine increased CPP scores in mice after repeated intranasal administration, in a dose dependent manner. In contrast, (*R*)-ketamine did not increase CPP scores in mice. These findings suggest that intranasal administration of (*R*)-ketamine would be a safer antidepressant than (*R*,*S*)-ketamine and (*S*)-ketamine.

1. Introduction

In 2000, Berman et al. (2000) reported a first double-blind, placebo-controlled study of the *N*-methyl-D-aspartate receptor (NMDAR) antagonist (*R*,*S*)-ketamine, demonstrating that (*R*,*S*)-ketamine exhibits rapid antidepressant effects in treatment-resistant patients with major depressive disorder (MDD). Subsequently, a number of groups replicated robust antidepressant effects of (*R*,*S*)-ketamine in treatment-resistant patients with MDD (Murrough et al., 2013; Su et al., 2017; Zarate et al., 2006;). Interestingly, (*R*,*S*)-ketamine could produce anti-suicidal effects in treatment-resistant patients with MDD (Grunebaum et al., 2018; Larkin and Beautrais, 2011; Murrough et al., 2015; Price et al., 2009). Several meta-analyses showed that (*R*,*S*)-ketamine exhibits rapid antidepressant and anti-suicidal ideation effects in treatment-resistant patients with MDD or bipolar disorder (Kishimoto et al., 2016; Newport et al., 2015; Wilkinson et al., 2018; Xu et al., 2016). Off-label use of (*R*,*S*)-ketamine (i.e., intravenous and intranasal administration) for antidepressant effects is increasing in the United State of America (USA) although the common adverse effects (e.g., psychotomimetic effects and dissociative effects) of (*R*,*S*)-ketamine are not resolved (Singh et al., 2017; Wilkinson et al., 2017; Zhu et al., 2016). Thus, although (*R*,*S*)-ketamine is the most attractive antidepressant in the treatment of severe depression, the precise mechanisms underlying its antidepressant actions remain elusive (Abdallah et al., 2018; Chaki, 2017a, 2017b; Duman, 2018; Gould et al., 2019; Hashimoto, 2016a, 2016b; Krystal et al., 2019; Monteggia and Zarate Jr, 2015; Murrough et al., 2017; Zanos et al., 2018; Zhang and Hashimoto, 2019a).

(*R*,*S*)-ketamine (Ki = 0.53 μM for NMDAR) is a racemic mixture containing equal parts of (*R*)-ketamine (or arketamine) (Ki = 1.4 μM for NMDAR) and (*S*)-ketamine (or esketamine) (Ki = 0.30 μM for NMDAR) (Ebert et al., 1997). (*R*)-ketamine is reported to show greater potency and longer-lasting antidepressant effects than (*S*)-ketamine in several animal models of depression (Fukumoto et al., 2017; Yang et al., 2015, 2017a, 2017b, 2018a; Zanos et al., 2016; Zhang et al., 2014). Unlike (*S*)-ketamine, (*R*)-ketamine might not induce psychotomimetic side effects or exhibit abuse potential in rodents (Yang et al., 2015, 2016). In addition, unlike (*R*,*S*)-ketamine and (*S*)-ketamine, (*R*)-ketamine did not

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cause the expression of heat shock protein HSP-70 (a marker for neuronal injury) in the rat retrosplenial cortex after a single intraperitoneal (i.p.) administration (Tian et al., 2018). A positron emission tomography (PET) study showed a marked reduction of dopamine $D_{2/3}$ receptor binding in conscious monkey striatum after a single intravenous (i.v.) infusion of (*S*)-ketamine but not that of (*R*)-ketamine, suggesting that (*S*)-ketamine-induced dopamine release might be associated with acute psychotomimetic and dissociative side effects in humans (Hashimoto et al., 2017). Therefore, it seems that (*R*)-ketamine could be a safer antidepressant in humans than (*R*,*S*)-ketamine and (*S*)-ketamine (Hashimoto, 2014, 2016a, 2016b, 2016c).

Fukumoto et al. (2017) reported that (*R*,*S*)-ketamine (10 mg/kg) and (*R*)-ketamine (10 mg/kg), but not (*S*)-ketamine (3 and 10 mg/kg), significantly reversed the depressive-like behavior induced by repeated treatments with corticosterone in rats at 24 h after a single i.p. administration, indicating that (*S*)-ketamine's antidepressant effects are less potent than (*R*,*S*)-ketamine and (*R*)-ketamine. On March 5, 2019, the US Food Drug Administration (FDA) approved Janssen Pharmaceutical Inc.'s (*S*)-ketamine nasal spray for treatment-resistant depression (FDA 2019). It is well known that bioavailability (17–29%) of intranasal administration of (*R*,*S*)-ketamine in humans is markedly lower than i.v. (100%) and intramuscular (i.m.) administration (93%) (Li and Vlisides, 2016; Peltoniemi et al., 2016; Zhang and Hashimoto, 2019a), suggesting lower efficacy and higher individual difference of intranasal administration compared to i.v. and i.m. administration. However, there are no reports showing the direct comparison of intranasal administration of (*R*,*S*)-ketamine and its two enantiomers for antidepressant and side effects in rodents.

The purpose of this study is to compare the antidepressant and side effects of intranasal administration of (*R*,*S*)-ketamine and its two enantiomers (*R*)-ketamine and (*S*)-ketamine. First, we compared the antidepressant effects of a single intranasal administration of (*R*,*S*)-ketamine, (*R*)-ketamine and (*S*)-ketamine in susceptible mice after chronic social defeat stress (CSDS). Second, we compared the side effects [i.e., locomotion, prepulse inhibition (PPI), conditioned place preference (CPP)] of intranasal administration of (*R*,*S*)-ketamine, (*R*)-ketamine and (*S*)-ketamine in mice.

2. Methods and Materials

2.1. Animals

Male adult C57BL/6 mice ($n = 400$), aged 8 weeks (body weight 20–25 g, Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 (ICR) mice (n = 40), aged 13–15 weeks (body weight > 40 g, Japan SLC, Inc., Hamamatsu, Japan) were used. Animals were housed under controlled temperatures and 128/dark cycles (lights on between 07:00 and 19:00 h), with ad libitum food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee (Permission number: 29-420). 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, USA. Animals were deeply anaesthetized with isoflurane before being killed by cervical dislocation. All efforts were made to minimize suffering.

2.2. Materials

(*R*)-Ketamine hydrochloride and (*S*)-ketamine hydrochloride were prepared by recrystallization of (*R*,*S*)-ketamine (Ketalar®, ketamine hydrochloride, Daiichi Sankyo Pharmaceutical Ltd., Tokyo, Japan) and D-(−)-tartaric acid and L-(+)-tartaric acid, respectively (Zhang et al., 2014). The dose (10, 20 or 40 mg/kg as hydrochloride) of (*R*,*S*)-ketamine and its enantiomers dissolved in the physiological saline was used as previously reported (Chang et al., 2019; Yang et al., 2015, 2017a, 2017b, 2018a; Zhang et al., 2018). Other reagents were purchased commercially.

2.3. Chronic social defeat stress (CSDS) model

The procedure of CSDS was performed as previously reported (Chang et al., 2019; Dong et al., 2017; Golden et al., 2011; Yang et al., 2015, 2017a, 2017b, 2018a; Xiong et al., 2018a, 2018b; Zhang et al., 2018). The C57BL/6 mice were exposed to a different CD1 aggressor mouse for 10 min per day for consecutive 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 (SIT) 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 “resilient”. Approximately 70–80% of mice were susceptible after CSDS. Susceptible mice were randomly divided in the subsequent experiments. Control C57BL/6 mice without CSDS were housed in the cage before the behavioral tests.

2.4. Treatment and behavioral tests

The CSDS susceptible mice were divided to four groups. Subsequently, saline (0.5 ml/kg), (*R*,*S*)-ketamine (10 mg/kg), (*R*)-ketamine (10 mg/kg), or (*S*)-ketamine (10 mg/kg) was administered intranasally into CSDS susceptible mice (Fig. 1A). Mice were restrained by hand, and saline or ketamine was administered intranasally into awake mice using Eppendorf micropipette (Eppendorf Japan, Tokyo, Japan). Behavioral tests, including locomotion test (LMT), tail suspension test (TST), forced swimming test (FST) and 1% sucrose preference test (SPT), were performed as reported previously (Dong et al., 2017; Yang et al., 2015, 2017a, 2017b, 2018a; Xiong et al., 2018a, 2018b; Zhang et al., 2018). LMT and TST were performed 2 and 4 h after a single injection, respectively. FST was performed 1 day after injection. SPT was performed 2, and 7 days after a single injection (Fig. 1A).

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 locomotor activity counts were recorded for 60 min. Cages were cleaned between testing session.

2.4.2. 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. FST

The FST was conducted using an automated forced-swim apparatus (SCANET MV-40; MELQUEST Co., Ltd., Toyama, Japan). Mice were placed individually in a cylinder (diameter: 23 cm; height: 31 cm) containing 15 cm of water maintained at a temperature of

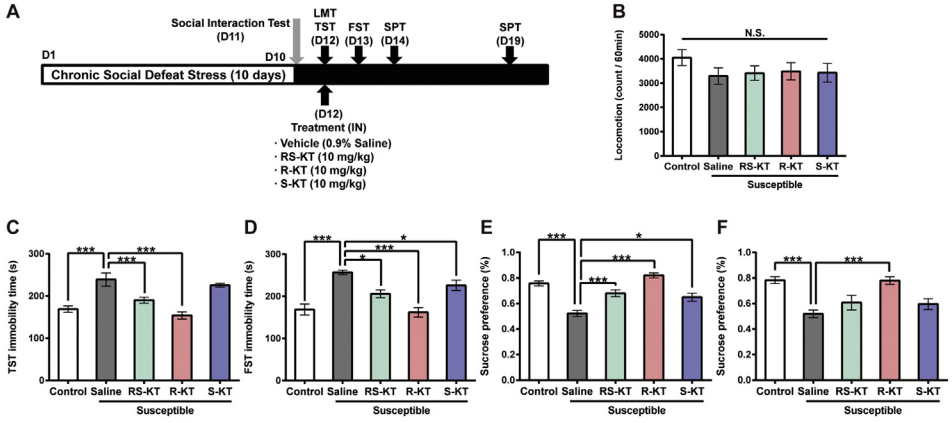

Fig. 1. Schedule of a CSDS model, treatment, and behavioral tests.
(A): CSDS was performed from day 1 to day 10, and the social interaction test (SIT) was performed on day 11. Saline (0.5 ml/kg), (*R*,*S*)-ketamine (10 mg/kg), (*R*)-ketamine (10 mg/kg), or (*S*)-ketamine (10 mg/kg) was administered intranasally into the susceptible mice on day 12. LMT and TST were performed 2 and 4 h after a single injection, respectively. SPT was performed 2, and 7 days after a single injection. (B): LMT. (day 12). (C): TST (day 12). (D): FST (day 13). (E): SPT (day 14). (F): SPT (day 19). The values represent the mean ± S.E.M. ($n = 15$ or 16). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with saline-treated susceptible mice. N.S.: not significant. LMT: locomotion test. TST: tail suspension test. FST: forced swimming test. SPT: 1% sucrose preference test. R-KT: (*R*)-ketamine. RS-KT: (*R*,*S*)-ketamine. S-KT: (*S*)-ketamine.

23 °C ± 1 °C. The immobility time was calculated using the activity time as (total) – (active) time by the apparatus analysis software. The immobility time of each mouse was recorded for a period of 6 min.

2.4.4. 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 (water and 1% sucrose solution). The bottles containing water and sucrose were weighed before and at the end of this period. The sucrose preference was calculated as a percentage of sucrose solution consumption to the total liquid consumption.

2.5. Side effects

2.5.1. Locomotion

After habituation (60 min) in the cage, saline (0.5 ml/kg), (*R*,*S*)-ketamine (10, 20 or 40 mg/kg), (*R*)-ketamine (10, 20 or 40 mg/kg), or (*S*)-ketamine (10, 20 or 40 mg/kg) was injected intranasally into male C57BL/6 mice. Locomotor activity was measured using an animal movement analysis system (SCANET MV-40, Melquest, Toyama, Japan). The system consisted of a rectangular enclosure (560 × 560 mm). The side walls (height, 60 mm) of the enclosure were equipped with 144 pairs of photosensors located at 6-mm intervals at a height of 30 mm from the bottom edge. An animal was placed in the observation cage 60 min from a single dose of saline or compounds. A pair of photosensors was scanned every 0.1 s to detect the animal's movements. The intersection of paired photosensors (10 mm apart) in the enclosure was counted as one unit of locomotor activity. Data collected for 60 min after a single injection were used in this study.

2.5.2. Prepulse inhibition (PPI) test

Male C57BL/6 mice were tested for their acoustic startle reactivity (ASR) in a startle chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA) using the standard methods described previously (Matsuura et al., 2015; Yang et al., 2015; Yang et al., 2018b). The test sessions were begun after an initial 10-min acclimation period in the chamber. The mice were subjected to one of six trials: (1) pulse alone, as a 40 ms broadband burst; a pulse (40 ms broadband burst) preceded by 100 ms with a 20 ms prepulse that was (2) 4 dB, (3) 8 dB, (4) 12 dB, or (5) 16 dB over background (65 dB); and (6) background only (no stimulus). The amount of PPI was expressed as the percentage decrease in the amplitude of the startle reactivity caused by presentation of the prepulse (% PPI). Saline (0.5 ml/kg), or (*R*,*S*)-ketamine (10, 20 or 40 mg/kg) [or (*R*)-ketamine (10, 20 or 40 mg/kg), (*S*)-ketamine (10, 20 or 40 mg/kg)] was administered intranasally 20 min (including the 10-min acclimation period) before the machine records. The PPI test lasted 20 min in total.

2.5.3. Conditioned place preference (CPP) test

The place conditioning paradigm (Brain Science Idea Inc., Osaka, Japan) was used for studying ketamine-induced rewarding effects, as reported previously (Yang et al., 2015; Yang et al., 2018b). Male C57BL/6 mouse was allowed to move freely between transparent and black boxes for a 15 min session once a day, for 3 days (days 1–3) as preconditioning. On day 3, the time spent in each box was measured. There was no significant difference between time spent in the black compartment with a smooth floor and the white compartment with a textured floor, indicating that there was no place preference before conditioning. On days 4, 6, and 8, saline (0.5 ml/kg), or (*R*,*S*)-ketamine (10, 20 or 40 mg/kg) [or (*R*)-ketamine (10, 20 or 40 mg/kg), (*S*)-ketamine (10, 20 or 40 mg/kg)] was intranasally administered, and then mice were confined to either the transparent or black box for 30 min. On days 5, 7, and 9, mice were given saline and placed in the opposite ketamine-conditioning box for 30 min. On day 10, the post-conditioning test was performed without drug treatment, and the time spent in each box was measured for 15 min. A counterbalanced protocol was used in order to nullify any initial preference by the mouse. The CPP score was designated as the time spent in the drug-conditioning sites, minus the time spent in the saline-conditioning sites.

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, Tokyo, Japan). The data were analyzed using the one-way analysis of variance (ANOVA), followed by *post-hoc* Fisher's Least Significant Difference (LSD) test. The PPI data were also analyzed using multivariate analysis of variance, followed by *post-hoc* Fisher's LSD test. The *P*-values of < 0.05 were considered statistically significant.

3. Results

3.1. Antidepressant effects of (R,S)-ketamine, (R)-ketamine and (S)-ketamine in CSDS susceptible mice

Locomotion showed no difference ($F_{4,72} = 0.735$, $P = 0.571$) among the five groups (Fig. 1B). One-way ANOVA of TST data showed a statistical significance ($F_{4,72} = 15.23$, $P < 0.001$) among the five groups (Fig. 1C). *Post-hoc* tests showed that (*R*,*S*)-ketamine (10 mg/kg) and (*R*)-ketamine (10 mg/kg) significantly attenuated the increased immobility times of TST in CSDS susceptible mice (Fig. 1C). However, (*S*)-ketamine (10 mg/kg) did not attenuate the increased immobility

time of TST in CSDS susceptible mice although (*S*)-ketamine slightly decreased the increased immobility time (Fig. 1C). One-way ANOVA of FST data showed a statistical significance ($F_{4,72} = 13.77$, $P < 0.001$) among the five groups (Fig. 1D). *Post-hoc* tests showed that three compounds (10 mg/kg) significantly attenuated the increased immobility times of FST in CSDS susceptible mice (Fig. 1D). One-way ANOVA of SPT data showed statistical significance (2 days after a single injection: $F_{4,72} = 20.78$, $P < 0.001$) among the five groups (Fig. 1E). *Post-hoc* tests showed that three compounds (10 mg/kg) significantly attenuated the decreased sucrose preference of SPT in CSDS susceptible mice (Fig. 1E). One-way ANOVA of SPT data showed statistical significance (7 days after a single injection: $F_{4,72} = 9.311$, $P < 0.001$,) among the five groups (Fig. 1F). *Post-hoc* tests showed that sucrose preference of (*R*)-ketamine-treated group was significantly higher from saline-treated group. However, sucrose preference of (*R*,*S*)-ketamine-treated group and (*S*)-ketamine -treated group was not different from saline-treated group (Fig. 1E and F). Collectively, the order of potency of antidepressant effects in a CSDS model was (*R*)-ketamine > (*R*,*S*)-ketamine > (*S*)-ketamine.

3.2. Effects of (R,S)-ketamine, (R)-ketamine, and (S)-ketamine on locomotion in mice after a single intranasal administration

Effects of three compounds on locomotion of male mice were examined after a single intranasal administration. One-way ANOVA of the data showed statistical significances ($F_{9,70} = 8.931$, $P < 0.001$) among the ten groups (Fig. 2A). *Post-hoc* tests showed that a single intranasal administration of (*R*,*S*)-ketamine (20 and 40 mg/kg) or (*S*)-ketamine (10, 20 and 40 mg/kg) significantly increased locomotion compared to saline-treated group (Fig. 2A). Furthermore, locomotion of (*S*)-ketamine (40 mg/kg) treated mice was significantly higher than that of (*R*,*S*)-ketamine (40 mg/kg) or (*R*)-ketamine (40 mg/kg) treated mice. In contrast, all doses (10, 20 or 40 mg/kg) of (*R*)-ketamine did not alter locomotion in mice (Fig. 2A).

3.3. Effects of (R,S)-ketamine, (R)-ketamine, and (S)-ketamine on PPI in mice after a single intranasal administration

PPI test was performed to examine the effects of three compounds in mice. There were no changes in the acoustic startle response among the four groups for (*R*,*S*)-ketamine [Wilks lambda = 0.717, $P = 0.589$], (*R*)-ketamine [Wilks lambda = 0.629, $P = 0.226$], and (*S*)-ketamine [Wilks lambda = 0.588, $P = 0.405$] (Fig. 3A-3C).

The MANOVA analysis of all PPI data of (*R*,*S*)-ketamine revealed that there was a significant effect [Wilks lambda = 0.497, $P = 0.042$]. Treatment with (*R*,*S*)-ketamine (10, 20 or 40 mg/kg) decreased PPI at all dB groups, in a dose dependent manner. Subsequent *post-hoc* tests indicated significant differences in PPI between the saline group and (*R*,*S*)-ketamine (40 mg/kg) group at all dB groups (Fig. 3A). In contrast, the MANOVA analysis of all PPI data of (*R*)-ketamine revealed that there was not a significant effect [Wilks lambda = 0.625, $P = 0.216$] (Fig. 3B). The MANOVA analysis of all PPI data of (*S*)-ketamine revealed that there was a significant effect [Wilks lambda = 0.299, $P < 0.001$]. Treatment with (*S*)-ketamine (10, 20 or 40 mg/kg) decreased PPI at all dB groups, in a dose dependent manner. Subsequent *post-hoc* tests indicated significant differences in PPI deficits between the saline group and (*S*)-ketamine (20 and 40 mg/kg) group at all dB groups. Furthermore, (*S*)-ketamine (10 mg/kg) significantly decreased PPI at 73 dB (Fig. 3C).

3.4. Effects of (R,S)-ketamine, (R)-ketamine, and (S)-ketamine on CPP scores in mice after repeated intranasal administration

In the conditioned place preference (CPP) test (Fig. 4A), both (*R*,*S*)-ketamine and (*S*)-ketamine, but not (*R*)-ketamine, increased CPP scores, in a dose dependent manner (Fig. 4). Repeated intranasal administration of (*R*,*S*)-ketamine (40 mg/kg), but not the low doses (10 and 20 mg/kg), significantly increased CPP scores ($F_{3,34} = 3.054$, $P = 0.042$) (Fig. 4B). In contrast, repeated intranasal administration of (*R*)-ketamine (10, 20 or 40 mg/kg) did not increase CPP scores ($F_{3,36} = 0.072$, $P = 0.974$) (Fig. 4C). Repeated intranasal administration of (*S*)-ketamine (20 and 40 mg/kg), but not the low dose (10 mg/kg), significantly increased CPP scores ($F_{3,36} = 14.0$, $P < 0.001$) (Fig. 4D).

Collectively, the order of potencies of side effects (i.e., psychosis, abuse liability) in mice after intranasal administration was (*S*)-ketamine > (*R*,*S*)-ketamine > (*R*)-ketamine.

4. Discussion

In the present study, we compared (*R*,*S*)-ketamine, and its two enantiomers, in CSDS susceptible mice (for antidepressant effects) and control mice (for side effects). The order of potency of antidepressant effects after a single intranasal administration to CSDS susceptible mice is (*R*)-ketamine > (*R*,*S*)-ketamine > (*S*)-ketamine. Furthermore, the order of potency of side effects (i.e., psychosis and abuse liability) after

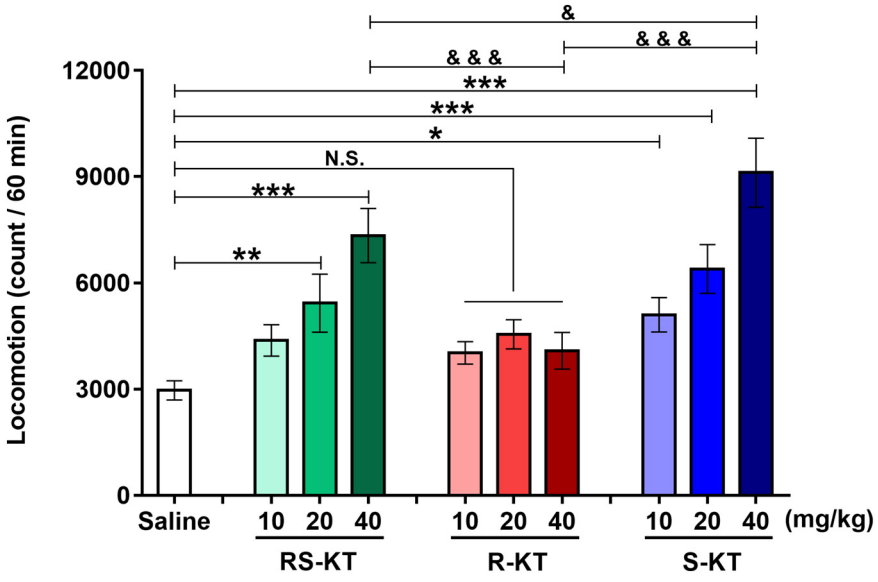

Fig. 2. Effects of (*R*,*S*)-ketamine, (*R*)-ketamine, and (*S*)-ketamine on locomotion after a single intranasal administration.
Saline (0.5 ml/kg), (*R*,*S*)-ketamine (10, 20, or 40 mg/kg), (*R*)-ketamine (10, 20, or 40 mg/kg), or (*S*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. Locomotor activity was measured 60 min after a single injection of the compounds. The values represent the mean ± S.E.M. ($n = 8$). *P < 0.05, **P < 0.01, ***P < 0.001 compared with saline-treated mice. $^{\&}$P < 0.05 compared to RS-KT (40 mg/kg). $^{\&\&\&}$P < 0.001 compared to R-KT (40 mg/kg). N.S.: not significant. R-KT: (*R*)-ketamine. RS-KT: (*R*,*S*)-ketamine. S-KT: (*S*)-ketamine.

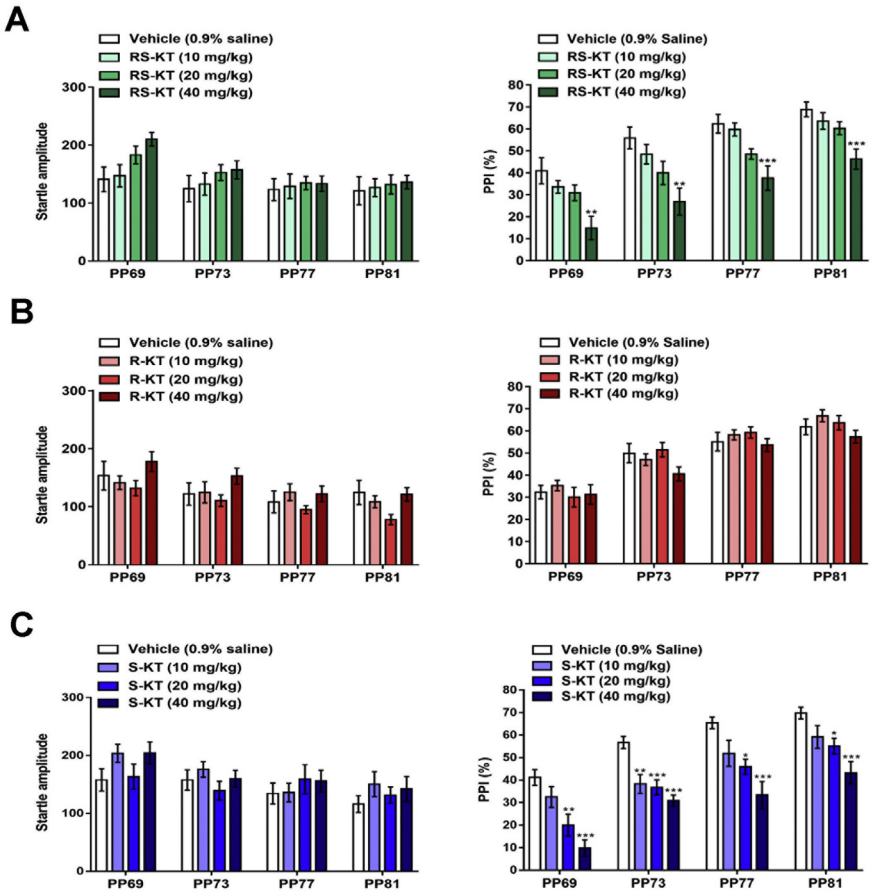

Fig. 3. Effects of (*R*,*S*)-ketamine, (*R*)-ketamine, and (*S*)-ketamine on PPI after a single intranasal administration. (A): Saline (0.5 ml/kg), or (*R*,*S*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. (B): Saline (0.5 ml/kg), or (*R*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. (C): Saline (0.5 ml/kg), or (*S*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. Startle response amplitude and PPI were measured as described in the Method Section. The values represent the mean ± S.E.M. ($n = 9$ or 10). *P < 0.05, **P < 0.01, ***P < 0.001 compared with saline-treated mice. N.S.: not significant. R-KT: (*R*)-ketamine. RS-KT: (*R*,*S*)-ketamine. S-KT: (*S*)-ketamine.

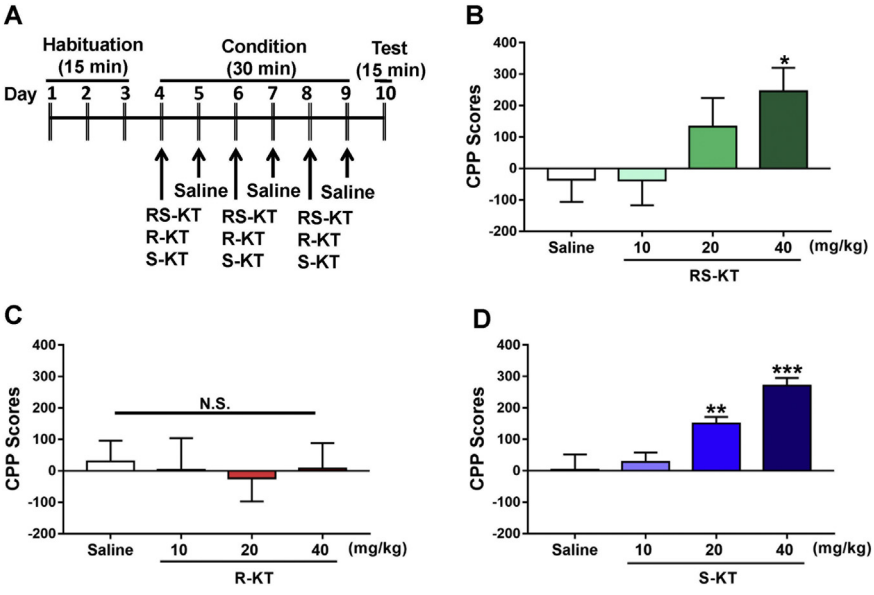

Fig. 4. Effects of (*R*,*S*)-ketamine, (*R*)-ketamine, and (*S*)-ketamine on CPP score after repeated intranasal administration.
(A): Schedule of habituation, treatment, and behavioral test. (B): Saline (0.5 ml/kg), or (*R*,*S*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. (C): Saline (0.5 ml/kg), or (*R*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. (D): Saline (0.5 ml/kg), or (*S*)-ketamine (10, 20, or 40 mg/kg) was administered intranasally into male mice. CPP score was measured as described in the Method Section. The values represent the mean ± S.E.M. (n = 8–10). *P < 0.05, **P < 0.01, ***P < 0.001 compared with saline-treated mice. N.S.: not significant. R-KT: (*R*)-ketamine. RS-KT: (*R*,*S*)-ketamine. S-KT: (*S*)-ketamine.

intranasal administration is (*S*)-ketamine > (*R*,*S*)-ketamine > (*R*)-ketamine. Collectively, it is likely that (*R*)-ketamine would be a rapid-acting and sustained antidepressant without side effects compared to (*R*,*S*)-ketamine and (*S*)-ketamine.

In this study, we found that antidepressant effects of (*R*,*S*)-ketamine and its two enantiomers in CSDS susceptible mice after a single intranasal administration may be less potent that those of a single i.p. administration (Dong et al., 2017; Yang et al., 2015, 2017a, 2017b, 2018a; Zhang et al., 2015; Zhang and Hashimoto, 2019b). Lower bioavailability of intranasal administration of (*R*,*S*)-ketamine and its two enantiomers may contribute to lower efficacy of intranasal administration compared to i.p. administration. Interestingly, the potency of antidepressant effects of (*R*,*S*)-ketamine and its two enantiomers was not correlated with the potencies of these compounds at the NMDAR (Ebert et al., 1997), suggesting that NMDAR inhibition may not play a key role in the antidepressant effects of (*R*,*S*)-ketamine and its enantiomers. Previously, Fukumoto et al. (2017) reported that (*R*,*S*)-ketamine and (*R*)-ketamine, but not (*S*)-ketamine, show antidepressant

effects in rats with repeated corticosterone treatments after a single i.p. administration, consistent with our current data.

Due to its serious side effects, clinical use of ketamine has remained limited (Domino, 2010; Sanacora et al., 2017; Singh et al., 2017; Zhu et al., 2016), although it has been used as an off-label antidepressant in the USA (Reardon, 2018; Wilkinson et al., 2017). In this study, we found that locomotion after a single intranasal administration of (*R*)-ketamine is lower than those of (*R*,*S*)-ketamine and (*S*)-ketamine, consistent with the previous reports (Ryder et al., 1978; Yang et al., 2015) of subcutaneous or i.p. administration. Furthermore, we found that a single intranasal administration of (*R*)-ketamine did not cause PPI deficits in mice compared to (*R*,*S*)-ketamine and (*S*)-ketamine, consistent with the previous reports of i.p. administration (Yang et al., 2015). Interestingly, it was reported that the ED_{50} of (*R*)-ketamine (6.33 mg/kg) for PPI deficits in rats was higher than that of (*S*)-ketamine (2.86 mg/kg), indicating that (*S*)-ketamine disrupts PPI with 2.5-fold higher potency than (*R*)-ketamine (Halberstadt et al., 2016). Finally, we found that repeated intranasal administration of (*R*)-ketamine did not increase CPP scores in mice although (*R*,*S*)-ketamine and (*S*)-ketamine increased CPP scores, in a dose dependent manner, consistent with the previous reports of i.p. administration (Yang et al., 2015, 2018b). A PET study showed that a single i.v. infusion of (*S*)-ketamine (0.5 mg/kg for 40-min), but not (*R*)-ketamine (0.5 mg/kg for 40-min), produced a marked reduction of dopamine $D_{2/3}$ receptor binding in conscious monkey striatum, suggesting that (*S*)-ketamine-induced dopamine release might be associated with acute psychotomimetic and dissociative side effects in humans (Hashimoto et al., 2017). Unlike (*R*,*S*)-ketamine and (*S*)-ketamine, it seems that intranasal infusion of (*R*)-ketamine does not appear to cause psychotomimetic effects or have abuse potential in humans, based on the lack of behavioral abnormalities (e.g., PPI deficits, CPP) observed in mice after single or repeated intranasal administration (Hashimoto, 2016a, 2016b, 2016c).

Mathisen et al. (1995) reported that the incidence of side effects (i.e., blurred vision, altered hearing, dizziness, proprioceptive disturbances, illusions) of (*S*)-ketamine (0.45 mg/kg, i.m.) treated group in patients with oral pain was higher than (*R*)-ketamine (1.8 mg/kg, i.m.) treated group, although the dose of (*S*)-ketamine (0.45 mg/kg) is lower than (*R*)-ketamine (1.8 mg/kg). Furthermore, it is also reported that experiencing illusion and alterations in hearing, vision, and proprioception is attributable to (*S*)-ketamine's actions (Oye et al., 1992; Vollenweider et al., 1997), whereas the feelings of relaxation are associated with (*R*)-ketamine's actions (Vollenweider et al., 1997; Zanos et al., 2018). Taken all together, it seems likely that (*S*)-ketamine contributes to the acute psychotomimetic and dissociative effects of (*R*,*S*)-ketamine, whereas (*R*)-ketamine may not be associated with these side effects (Zanos et al., 2018).

On March 5, 2019, the US FDA approved nasal spray of (*S*)-ketamine for treatment-resistant depression (FDA, 2019). Due to the risk of serious adverse outcomes from sedation and dissociation caused by administration of (*S*)-ketamine, as well as the potential for abuse and misuse of the drug, FDA said that the drug will only be available through a restricted distribution system, under a Risk Evaluation and Mitigation Strategy (REMS). Patients will self-administer (*S*)-ketamine under the supervision of a health care provider in a certified doctor's office or clinic; the nasal spray cannot be taken home (FDA, 2019). Given the lack of adverse side effects of (*R*)-ketamine, it is possible that patients may take (*R*)-ketamine to their home.

In conclusion, this study suggests that the order of potency for antidepressant effects in a CSDS model after a single intranasal administration is (*R*)-ketamine > (*R*,*S*)-ketamine > (*S*)-ketamine. In contrast, the order of potency for side effects in mice after intranasal administration is (*S*)-ketamine > (*R*,*S*)-ketamine > (*R*)-ketamine. Therefore, it is likely that (*R*)-ketamine could be a safer antidepressant without side effects than (*R*,*S*)-ketamine and (*S*)-ketamine.

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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.

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