J Ginseng Res 45 (2021) 254–263

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research Article

Korean Red Ginseng reduces chronic social defeat stress-induced mood disorders via N-methyl-D-aspartate receptor modulation in mice

Bo-Ram Lee ☆, Ju-Hyun Lee ☆, Yong-Hyun Ko, Jee-Yeon Seo, Kwang-Hyun Hur, Young-Jung Kim, Seon-Kyung Kim, Seong-Eon Kim, Seok-Yong Lee, Choon-Gon Jang*

Department of Pharmacology, School of Pharmacy, Sungkyunkwan University, Suwon, 16419, Republic of Korea

ARTICLE INFO

Article history:
Received 28 March 2019
Received in Revised form
11 September 2019
Accepted 1 November 2019
Available online 7 November 2019

Keywords:
Anxiety
Chronic social defeat stress
Korean Red ginseng
NMDA receptor
Social avoidance

ABSTRACT

Background: A chronic social defeat stress (CSDS) model has been proposed as relevant to stress-induced behavioral change in humans. In this study, we examined the effect of Korean Red Ginseng (KRG) on CSDS-induced mood disorders and protein expression in an animal model.
Methods: To evaluate the effect of KRG on social defeat stress, test mice were exposed in the resident aggressor's home cage compartment for 14 days beginning 1 h after KRG treatment (10, 20, and 40 mg/kg, per oral (p.o.)). After the exposure, behavioral tests to measure anxiety, social interaction, and depression-like behavior were performed. To investigate the underlying mechanism, N-methyl-D-aspartate receptor expression levels in CSDS-induced mice were evaluated using Western blot analysis.
Results: CSDS induced anxiety-like behaviors by decreasing central activity in the open-field test and open-arm approach in the elevated plus maze test and led to social avoidance behavior in the social interaction test. CSDS mice showed upregulated NR1, NR2A, and NR2B expression in the hippocampus. KRG 20 and 40 mg/kg ameliorated anxiety-like activities and KRG 20 mg/kg alleviated social avoidance by decreasing time in the corner zone. KRG treatment recovered CSDS-induced NR1, NR2A, and NR2B protein levels in the hippocampus.
Conclusion: These results indicate that KRG has a therapeutic effect on CSDS-induced mood disorder by alleviating N-methyl-D-aspartate receptor overexpression in the hippocampus.

1. Introduction

Continuous social stress, such as issues in interpersonal relationships and a role loss at work, can predispose people to mental illnesses, such as depression and anxiety, as well as fear of being excluded from social groups [1,2]. In addition, recent reports have indicated that children who have been subjected to school violence have a more dangerous level of post-traumatic stress disorder than those who were not victimized by peers [3]. Among laboratory stress models, the chronic social defeat stress (CSDS) model, which is associated with social conflict, is useful because of high predictive validity [4]. Many researchers have shown that social defeat is a crucial factor in causing various psychopathological changes similar to what people experience in their lives. Stefanski [5] had found a marked decrease in corticosteroid-binding globulin and testosterone after 7 days of social stress. After being defeated, rats, mice, and tree shrews have shown a variety of behavior changes, such as sleep pattern disturbances, anhedonia, decreased locomotor activity, and impaired memory performance [6–9]. From previous studies, CSDS is an appropriate tool for understanding stress-induced mood disorders. Therefore, we can use the CSDS model to study the treatments that relieve mental diseases due to social stress.

When suffering from stress-related illness, such as post-traumatic stress disorder and Cushing's disease, it has been shown that hippocampal volumes were significantly reduced [10], suggesting that there is a chronic imbalance in endogenous neurotransmitters, such as glutamate [11]. In addition, chronic restraint stress had

* Corresponding author.
E-mail address: jang@skku.edu (C.-G. Jang).
☆ These authors are equally contributed.

https://doi.org/10.1016/j.jgr.2019.11.003
p1226-8453 e2093-4947/$ – see front matter

induced a contraction of dendrites in CA3 of the hippocampus, which are involved in stress-induced hippocampal structural plasticity [12]. Many studies have evaluated that the hippocampus is involved in the stress-related pathological process. Therefore, the hippocampus is the best-studied brain region for evaluating the effects of CSDS on the brain.

Recent studies have confirmed that increased stress-induced modulation of the glutamate release and transmission is an important factor in inducing changes associated with depression [13–15]. For example, animals that have been through chronic mild stress showed increased N-methyl-D-aspartate receptor (NMDAR) subtype mRNA levels in the hippocampus [16]. Moreover, chronic restraint stress paradigms had induced increases in glutamate release from hippocampal synaptosomes, which led to dysregulation of glutamate secretion [17]. Furthermore, several glutamatergic modulating agents, such as N-methyl-D-aspartate antagonist ketamine, have been proven to show rapid antidepressant effects in mood disorders [18–20]. Within the glutamatergic system, NMDARs, which are composed of a subunit NR1 and a subunit NR2, play a key role in fast synaptic glutamate transmission [16]. NR2A and NR2B are activated when coexpressed with NR1, with each of the NR2 subunits having different roles in NMDAR function [21]. Specifically, a previous study recently showed that chronic mild stress increased NR1 and NR2B protein expression, as well as mRNA levels in the rat ventral hippocampus [16]. Furthermore, NR2B-knockout animals could not regulate stress-induced depression-like behaviors [22]. These results explained how the mechanism mediated by NR2B-containing receptors is important in modulating mood disorders. In addition, NMDARs at synaptic sites cluster through the C-terminal of NR2 subunits and are organized to the postsynaptic membrane through postsynaptic density-95 (PSD-95) [23,24]. PSD-95 acts as a scaffolding protein for the postsynaptic NMDAR, linking postsynaptic glutamate receptors together and connecting them to intracellular signaling pathways [25]. From these results, chronic stress affects mood disorders by modulating hippocampal NMDARs, in particular the NR2 subunits and PSD-95.

Because stress is the fundamental cause of mood disorders, antidepressants are thought to modulate stress-related responsiveness and susceptibility [26]. However, traditional antidepressants exhibit various side effects [27]. Thus, there is a need to develop natural products that have fewer side effects. Korean Red Ginseng (KRG) is the commonly used herbal medicine in Asia for enhancing energy and immunity. In general, KRG has a variety of biological activities that benefit human health, such as antiinflammatory activity in lipopolysaccharide (LPS)-stimulated *in vivo* and *in vitro* studies and antioxidant effects in stressed mice and in cellular stress [28–30]. In addition, KRG has positive effects on humans exposed to high stress by stabilizing the sympathetic nervous system and improving cognitive function [31]. However, there have been no studies researching the effect of KRG on a CSDS model. Therefore, to understand the mechanism of KRG in social defeat stress, we investigated the effect of KRG on CSDS-induced mood disorders using various behavioral tests, including anxiety, locomotor activity, social avoidance, and depression-like behavior. Furthermore, we examined molecular changes in NMDAR subunits, including PSD-95, in the hippocampus of CSDS mouse models.

2. Materials and methods

2.1. Animals

Seven-week-old male C57BL/6J mice (Dae Han Bio Link Co., Ltd, Eumseong, Korea) weighing 19-21 g were used. After arrival, the animals were kept 10 per cage (27 × 42 × 18 cm) and habituated for 1 week before using in experimental procedures.

For aggressors, we used male CD-1 mice (4 weeks old, 38-42 g) purchased from Koatech Co., Ltd (Pyongtaek, Korea). After arrival, the animals were singly housed (21 × 24 × 12 cm) and acclimatized for 6 weeks before using in experimental procedures. When male CD-1 mice were housed singly for an extended time, they become aggressive and territorial with unfamiliar males. In addition, at 10 weeks of age, the CD-1 mice weighed more than 40 g, which made them more threatening to C57BL/6J mice (the intruder).

The animals had approach to water and food ad libitum. The mice were sustained in a climate and humidity-controlled room under a 12-h light/dark cycle (lights on 07:00 to 19:00). All tests were carried out according the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the approval of the Institutional Animal Care and Use Committee of Sungkyunkwan University (SKKUIACUC2018-12-01-1).

2.2. KRG administration

KRG extract was obtained from the Korea Ginseng Corporation (Buyeo, Chungnam, Korea). In brief, ginseng was steamed (90-100°C, 3 h), dried in a chamber (50-80°C), extracted with circulating hot water (85-90°C), and then filtered. The filtrates were concentrated under reduced pressure and lyophilized. KRG contained the following ginsenosides by HPLC analysis: Rb1 7.98 mg/g; Rg3 3.23 mg/g; Rc 3.11 mg/g; Rb2 2.89 mg/g; Rg2s 2.20 mg/g; Re 1.86 mg/g; Rg1 1.63 mg/g; Rf 1.6 mg/g; Rh1 1.17 mg/g; Rd 1.03 mg/g; and other minor ginsenosides. The stressed animals were segregated at random into four groups: CSDS mice treated with a deionized water injection (CSDS group) and CSDS mice treated with 10 mg/kg, 20 mg/kg, or 40 mg/kg of KRG (CSDS–KRG groups). All mice were given oral injections of KRG or deionized water daily 1 h before social defeat stress.

2.3. Chronic social defeat stress

Nonexperimental C57BL/6J mice were used as screeners during the screening process. A screener was introduced into the CD-1 home cage for 180 s per session. We performed three consecutive sessions, once daily. On each subsequent day, we used different screeners for each CD-1 mice. An aggressor CD-1 mice was used in social defeat experiments according to the following criteria: (1) CD-1 mouse expressed aggression, for instance pulling a C57BL/6J mouse down and biting it for at least 5 s; (2) the initial aggression time had to be within 60 s of the start of the session; and (3) the CD-1 mouse attacked a C57BL/6J mouse in at least two consecutive sessions.

The CSDS procedure was performed as described [32,33] with minor modifications. Briefly, an aggressor mouse was put on one part of the divided home cage during the night before initiating the first social defeat stress. On the first day, a C57BL/6J mouse was introduced into the compartment of resident aggressor's home cage. The defeats lasted 10 min or less. At the end of time, the intruder was transferred to the opposite compartment divided by an acrylic divider with holes and maintained sensory contact until the next day. Intruder was exposed to social defeat stress for 14 days. For each subsequent daily defeat, they were moved daily to a novel resident's home cage. Control mice were housed in the compartment of equivalent cages and rotated to a new cage daily without physical contact with their cage members. The experiment was conducted between 9 and 12 a.m. Starting on the 11th day of chronic social defeat, behavioral experiments were performed in the afternoon (3-6 p.m.).

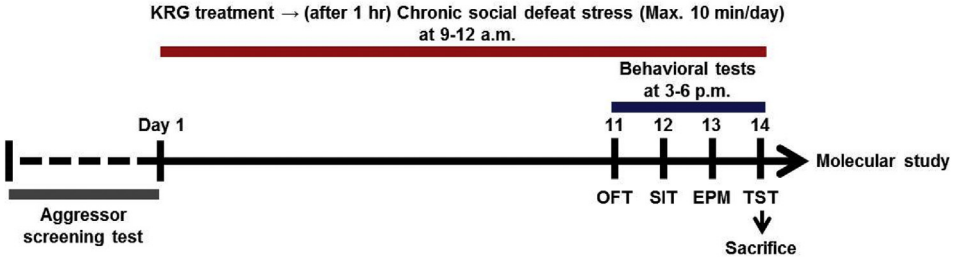

Fig. 1. Experimental schedule. The CSDS protocol lasted 14 days. Before the CSDS procedure, baseline body weight was measured. The KRG–CSDS group was treated with KRG 1 h before defeat stress. The CSDS group and the nonstress group received only DW. After 14 days of CSDS, behavioral tests and molecular analysis were carried out. KRG, Korean Red Ginseng; CSDS, chronic social defeat stress; DW, deionized water.

2.4. Open-field test

The animals explored an open-field chamber (30 × 30 × 30 cm) for 10 min under dim light. Using a tracking system (NeuroVision, Pusan, Korea), the distance traveled was recorded as a locomotor activity. The center time was used as an anxiolytic index.

2.5. Social interaction test

The social interaction test (SIT) was carried out depending on the method of Golden et al [32] and Tsankova et al [33] with slight modification. The SIT was assessed in the same condition as that of open-field test (OFT). A wire cage (13 × 11 × 25 cm) was put in the middle of the edge of the chamber. The test consisted of two 150-s phases, parted by a 30 s break. During the first session ("no target"), there was an empty wire cage. In the second session ("target"), an aggressor was put into the wire cage. In the testing, the aggressor is novel to the test mouse (the defeated C57BL/6J mouse). Movement of test mouse was recorded using an automated tracking system (EthoVision 3.0, Noldus, Wageningen, Netherlands). Cumulative times and movement in the "interaction zone" (the 5 cm width around the wire cage) and the "corner zone" (two corners of a 7 × 7 cm shaped square across the interaction zone) were calculated. The "interaction zone time" was the difference in time spent in the interaction zone between when the target was present and the target was not present. As an alternative method, SIT could also be expressed as a social interaction (SI) ratio. The SI ratio was assessed by dividing the time spent in the interaction zone when the target was in the wire cage by the time spent in the interaction zone when there was no target.

2.6. Elevated plus maze test

The elevated plus maze (EPM) test was conducted as described [34] with slight modification. The EPM test composed of two open arms (30 × 5 cm), two closed arms (30 × 5 cm), and a central platform (2.5 × 2.5 cm) with a height of 50 cm from the ground. At the beginning of the 5-min session, the animals were put in the central zone toward the closed arm. The percentage of open arm entries, time and movement, and total distance moved were measured using a tracking system.

2.7. Tail suspension test

We used a specifically manufactured box (40 × 120 × 25 cm depth) for the tail suspension test (TST). To avoid each animal interacting with or observing others, the box was divided into three-walled rectangular compartments. The animal tails were affixed to the middle top of the compartment using tape. Once all the tape was applied, recording was initiated and the session was identified before the mice were suspended. During the 6-min test session, the last 4-min was analyzed as the immobility time using a tracking system. Immobility time (percent change in body movement below a 10% threshold) and mobility time (above 20%) were captured.

2.8. Western blot method

Western blot method was carried out as previously provided [35]. Briefly, the hippocampus was dissected after behavioral experiments. Isolated hippocampus was homogenized and incubated on ice for 30 min. After centrifugation, the supernatant was extracted by lysis buffer. Eight percent sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to subject the protein samples. Anti-NR1 (1:1000), anti-NR2A (1:1000), anti-NR2B (1:1000), and anti-PSD-95 (1:1000) antibodies were purchased from Abcam (Cambridge, UK). Densitometric analysis was then performed using data obtained from at least three independent experiments. To determine band density, the enhanced chemiluminescence approach was used by immersing the probed membrane for 5 min in a 1:1 mixture of enhanced chemiluminescence reagents A and B (DonginLS, Seoul, Korea). Membranes were then exposed to a photographic film for a few minutes. Protein bands were quantified by densitometric analysis using ImageJ program from NIH (Bethesda, MD, USA).

2.9. Statistical analysis

All results are expressed as mean ± standard error of mean with Prism 6.0 software (GraphPad Software, Inc., San Diego, CA, USA). Changes in body weight and immobility time measured every minute were analyzed by two-way analysis of variance (ANOVA). Other behavioral tests were analyzed by one-way ANOVA. These behavioral data were analyzed with Fisher least significant difference (LSD) test. Western blot data were analyzed by one-way ANOVA followed by Bonferroni testing. Statistical significance was set at $p < 0.05$ in all statistical analysis (Fig. 1).

3. Results

3.1. KRG recovers weight loss caused by CSDS

Firstly, we observed changes in body weight every morning before social defeat to assess the CSDS-induced physiological changes. Beginning on day 5 of defeat stress, the CSDS group dramatically decreased body weight compared with the control group (Fig. 2, $F(36, 430) = 0.9860$, $p < 0.05$) and did not recover until day 10 of defeat stress ($p < 0.05$ and $p < 0.01$). However, the CSDS–KRG 20 mg/kg group effectively reversed this reduction observed in the CSDS group from day 5 and on day 10 ($p < 0.05$,

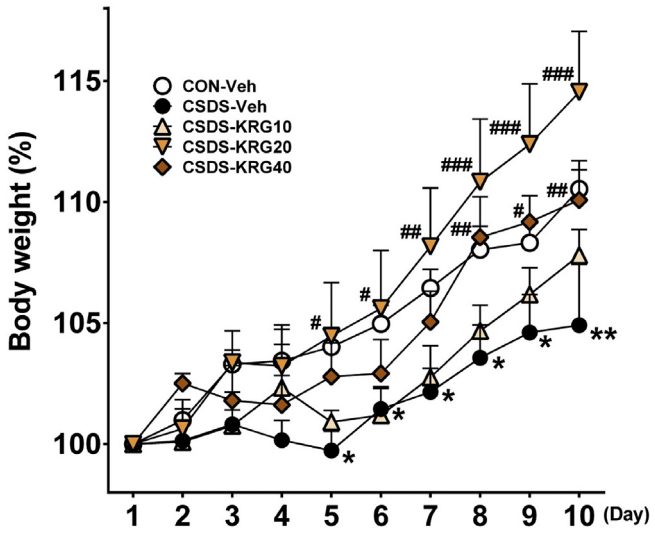

Fig. 2. Effect of KRG on body weight changes in CSDS-induced mice (n = 7-12/group). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ compared with the CSDS group. KRG, Korean Red Ginseng; CSDS, chronic social defeat stress.

$p < 0.01$, and $p < 0.001$). The CSDS–KRG 40 mg/kg group also effectively recovered body weight from day 8 and on day 10 ($p < 0.05$ and $p < 0.01$).

3.2. KRG shows anxiolytic-like behavior induced by CSDS in the EPM test

To evaluate anxiety-like behaviors, EPM test was conducted. When compared with nonstressed controls, the CSDS group showed a significant decrease in open arm entries (Fig. 3A, $F\ (4, 44) = 7.700$, $p < 0.01$), time spent (Fig. 3B, $F\ (4, 44) = 4.139$, $p < 0.01$), and movement (Fig. 3C, $F\ (4, 44) = 4.896$, $p < 0.001$). However, the CSDS–KRG 20 mg/kg group markedly recovered open arm entries, time spent, and movement as compared with the CSDS group ($p < 0.05$ and $p < 0.01$). In addition, when orally treated with KRG 40 mg/kg, the outcomes were also clearly increased as compared with the CSDS group ($p < 0.01$ and $p < 0.001$). In the KRG 10 mg/kg group, no significant differences were observed. There were no differences of total movement during the 5-min test in all groups (Fig. 3D, $F\ (4, 44) = 0.6595$).

3.3. KRG restores anxiety-like behaviors induced by CSDS in the OFT

The OFT was conducted for 10 min without adaptation to the open field. For the CSDS group, the center area count (Fig. 4A) and the time spent in the central zone (Fig. 4B) were significantly reduced when compared with the control group ($F\ (4, 38) = 7.674$, $p < 0.001$; $F\ (4, 38) = 8.100$, $p < 0.001$). Moreover, there was a significant decrease of locomotor activity in the CSDS group (Fig. 4C, $F\ (4, 38) = 5.741$, $p < 0.001$). However, for the CSDS 40 mg/kg group, the center area count, time in the center area, and locomotor activity were all recovered when compared with the CSDS group ($p < 0.05$, $p < 0.01$ and $p < 0.001$). There were no reversal in groups of KRG 10 mg/kg and KRG 20 mg/kg.

3.4. KRG changes social avoidance induced by CSDS

The day after the OFT, SIT was conducted to evaluate social behavior without adaptation to the open field. The CSDS group exhibited a substantial reduction in interaction zone time, in the SI ratio, and in total movement in interaction zone as compared with the control group (Fig. 5A, $F\ (4,37) = 6.389$, $p < 0.001$; Fig. 5C, $F\ (4,37) = 11.71$, $p < 0.001$; Fig. 5E, $F\ (4,37) = 6.919$, $p < 0.001$). Decreased movement in interaction zone was significantly recovered by treatment with 20 mg/kg of KRG ($p < 0.05$). However, there were no differences in interaction zone time and in the SI ratio. With time spent and distance moved in the corner zones, the CSDS group showed a notable increase (Fig. 5B, $F\ (4.37) = 5.087$, $p < 0.001$; Fig. 5D, $F\ (4,37) = 3.339$, $p < 0.01$; Fig. 5F, $F\ (4,37) = 3.876$, $p < 0.01$). These increases were significantly ameliorated by treatment with 20 mg/kg of KRG ($p < 0.05$).

3.5. KRG relieves depression-like behavior in the TST

The TST was performed on the last day of the behavioral experiment. There were no changes in immobility time, strong mobility time, and velocity between the control group and CSDS groups (Fig. 6A, $F\ (9,140) = 0.2334$; Fig. 6B, $F\ (3, 35) = 3.453$; Fig. 6C, $F\ (3, 35) = 2.753$; Fig. 6D, $F\ (3, 35) = 2.807$). However, the KRG 40 mg/kg group showed changes of immobility time, strong mobility time, and velocity when compared with the CSDS group ($p < 0.05$, $p < 0.01$, and $p < 0.001$).

3.6. KRG modulates NMDAR levels in CSDS-induced mice

The CSDS group showed significantly increased NMDAR subunit levels, including NR1 (Fig. 7A, $F\ (3, 8) = 71.81$, $p < 0.001$), NR2A (Fig. 7B, $F\ (3, 8) = 119.9$, $p < 0.001$), and NR2B (Fig. 7C, $F\ (3, 12) = 18.69$, $p < 0.01$), in the hippocampus as compared with the control group. As shown in Fig. 7D though, CSDS did not induce changes in PSD-95 in the hippocampus ($F\ (3, 8) = 8.366$). In KRG 40 mg/kg group, the increases of NMDAR and PSD-95 levels in the hippocampus were significantly reduced as compared with the CSDS group ($p < 0.01$ and $p < 0.001$).

4. Discussion

Social defeat associated with social conflict is not just a factor of physical stress. After short physical exposure, intruders are put into an opposite section of the resident cage for the rest of the test (until the next physical attack). This allows for psychological damage to the resident without physical exposure [36]. Social defeat stress induces a number of physiological changes. Specifically, body weight reduction is a general phenomenon in stress-related disorders, which results from appetite loss, and suggests a major indicator of stress [37,38]. In our study, as the defeat stress progressed, a marked decrease of body weight was observed in the CSDS group when compared with the control group. This result agrees with previous studies showing chronic stress effects, such as chronic mild stress, chronic restraint stress, and CSDS, on body weight [39–41]. In addition, a previous report had also demonstrated that rats consistently exposed to restraint stress lost body weight quickly and did not recover even if they were not stressed [42]. In this study, when mice were treated with 20 and 40 mg/kg of KRG 1 h before attack, the body weight loss recovered. Thus, this result suggests that KRG has an effect of restoring weight loss caused by CSDS in a short period of time. However, the body weight trajectory of treated animals was different among groups. A previous study showed that a ginseng extract–treated group did not seem to gain body weight compared with a control group [43]. In contrast, several studies have reported that KRG reduced body weight gain and fat content [44,45]. In particular, they suggested that KRG contributes to weight loss through modulation of lipid metabolism and insulin signaling. These results support our finding that the higher was the dose of KRG, the greater was the body

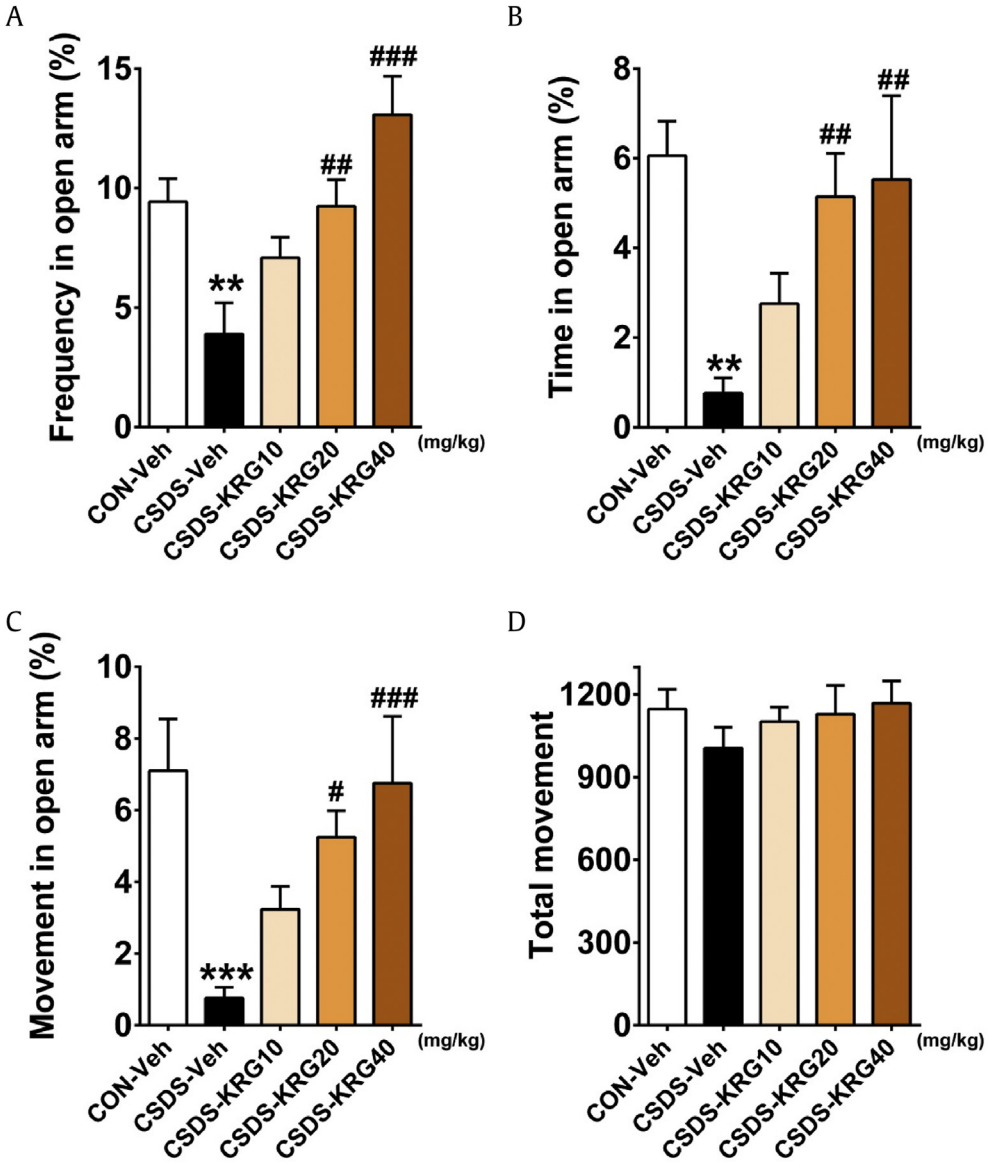

Fig. 3. Effects of KRG on anxiety-like behavior of CSDS-induced mice in the EPM test (n = 9-10/group). (A) The percentage of open arm entries, (B) The time spent in the open arm, (C) Movement in the open arm, and (D) Total movement were determined. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control group. $\#p < 0.05$, $\#\#p < 0.01$, and $\#\#\#p < 0.001$ compared with the CSDS group. EPM, elevated plus maze; KRG, Korean Red Ginseng; CSDS, chronic social defeat stress.

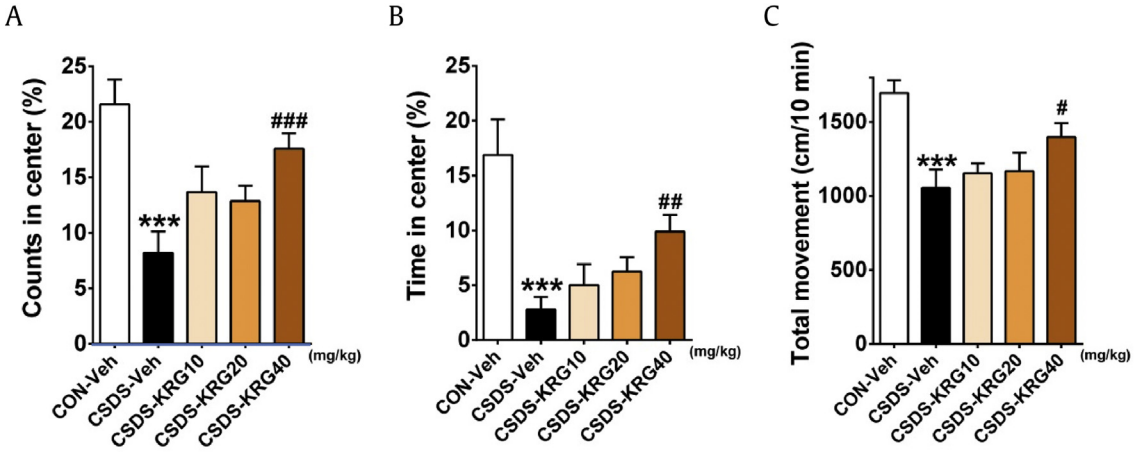

Fig. 4. Effects of KRG on anxiety-like behavior of CSDS-induced mice in the OFT (n = 7-10/group). (A) Counts in the center, (B) The time spent in the center, and (C) Total movement were determined. $^{***}p < 0.001$ compared with the control group. $\#p < 0.05$, $\#\#p < 0.01$, and $\#\#\#p < 0.001$ compared with the CSDS group. KRG, Korean Red Ginseng; CSDS, chronic social defeat stress; OFT, open-field test.

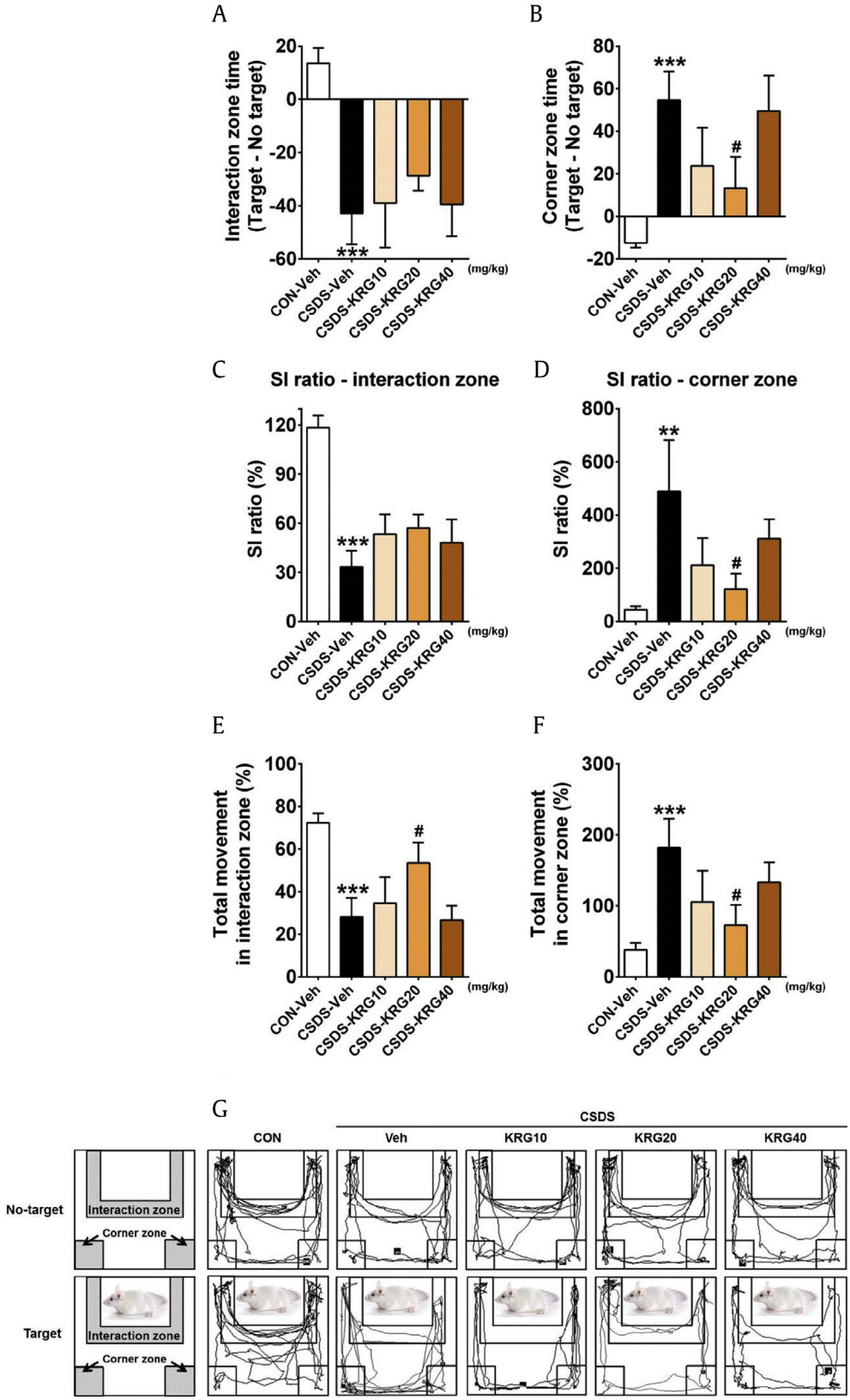

Fig. 5. Effects of KRG on social avoidance behavior of CSDS-induced mice in the SIT (n = 7-10/group). Each mouse was put into a chamber and allowed to interact with an unfamiliar target in the interaction zone. (A) Results are expressed as interaction zone time, (B) Corner zone time, (C) The SI ratio in the interaction zone, (D) The SI ratio in the corner zone, (E) Total movement in interaction zone, and (F) Total movement in corner zone. (G) Representative track image of total movement. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control group. $\#p < 0.05$ compared with the CSDS group. KRG, Korean Red Ginseng; CSDS, chronic social defeat stress; SIT, social interaction test; SI, social interaction.

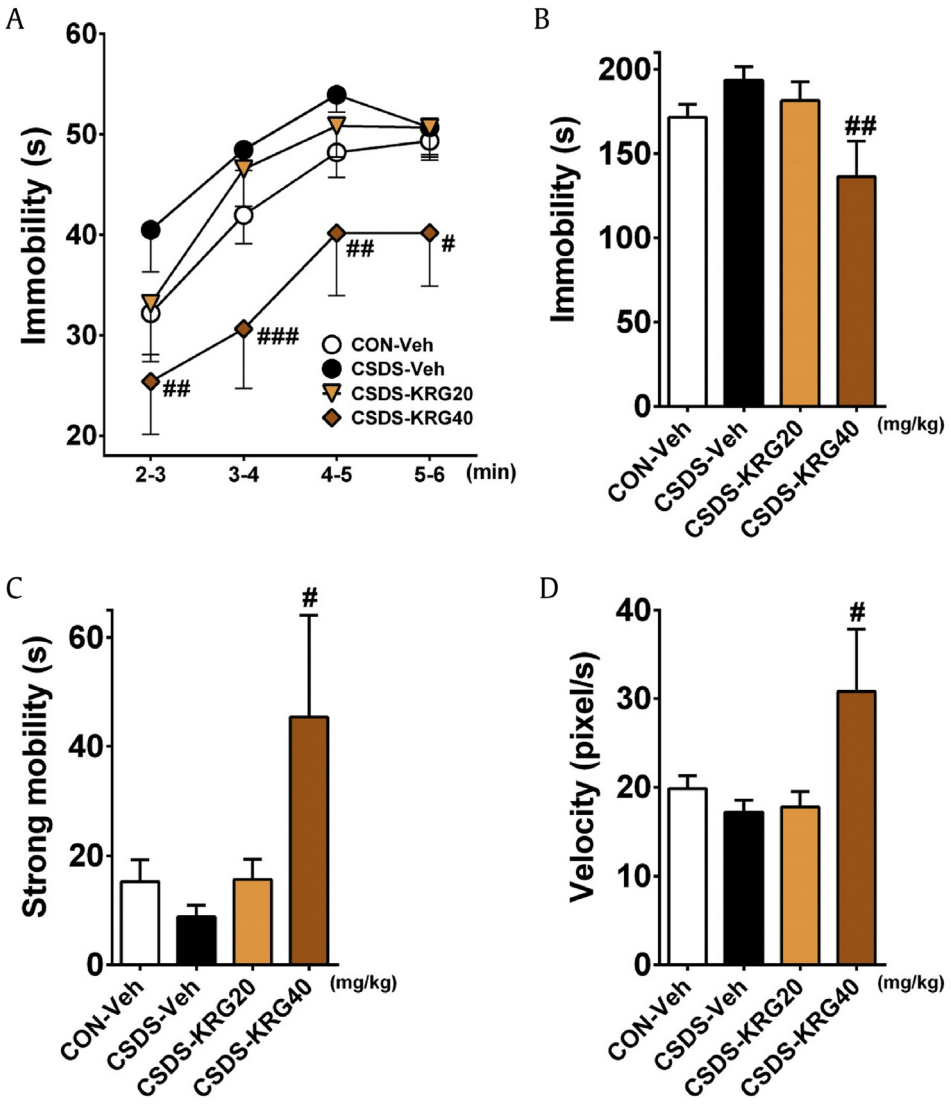

Fig. 6. Effects of KRG on depression-like behavior of CSDS-induced mice in the TST (n = 9-10/group). All data were analyzed in the final 4 min of total 6-min. (A) Immobility time measured at 1 min intervals, (B) Immobility time, (C) Strong mobility time, and (D) Velocity were determined. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ compared with the CSDS group. KRG, Korean Red Ginseng; CSDS, chronic social defeat stress; TST, tail suspension test.

weight loss effect, although this did not increase body weight gain compared with the 20-mg/kg KRG group. Thus, we cautiously conclude that animals treated with 20 and 40 mg/kg of KRG may show differences in body weight gain because of the antiobesity effect of KRG.

Anxiety is a frequent comorbid feature with other psychiatric disorders. Notably, symptoms of depression occur in up to 90% of patients with anxiety [46]. In accordance with the 10th revision of the International Statistical Classification of Disease and Related Health Problems, the “mixed anxiety and depressive disorder” classification has been included [47]. In this study, CSDS induced anxiety in both the EPM test and OFT. The CSDS group showed decreased open arm frequency, time spent in open arm, and open arm movement in the EPM test, as well as reduced center counts and center time in the OFT. A decrease in these factors would be suggestion of classical characteristics to induce anxiety [48]. These results correspond to previous findings that have observed the appearance of an “anxious profile” in stress-induced models [40,49,50]. However, when treated with KRG repeatedly, the anxiogenic features were significantly decreased, and the time in the open arm was restored to control group numbers in the EPM test. When the mice were presented a novel environment in OFT, exploratory behavior was decreased in the CSDS group. Similarly, Saul et al [51] had reported that a 3-day stress exposure during adolescence resulted in a reduction of locomotor activity in the OFT. These data proposed that the mice being exposed to a repeated stress developed anhedonia that was accompanied by anxiety-related behavior, including decreased exploration of novel environments [52]. As a result, it was confirmed that locomotor activity decreased because of anhedonia caused by CSDS, but was restored by continuous KRG administration.

Many studies using SIT have shown that mice exposed to social defeat become persistently aversive to social stimuli [53]. In the present study, defeated mice also displayed significantly reduced time in the interaction zone and showed increased time in the corner zone. This response is long-lasting [49] and changed by chronic administration of antidepressants [54]. Here, the results we presented did not show significant recovery of the difference of interaction zone time within the KRG group. In contrast, the corner zone time within the KRG group was effectively decreased. One explanation for this outcome is a mild effect of KRG on social avoidance induced by CSDS. The KRG dose we used in this study did

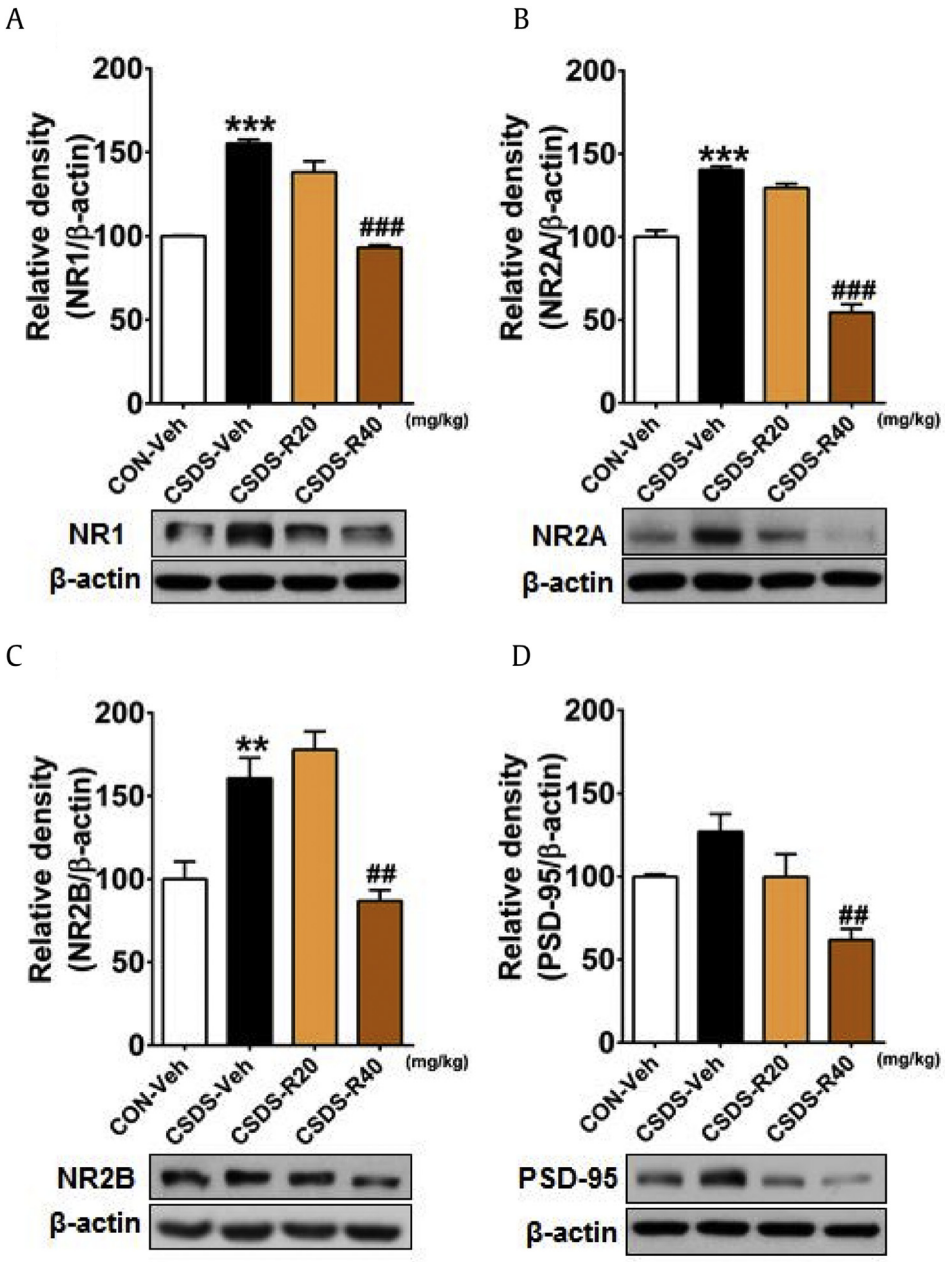

Fig. 7. Effects of KRG on expression levels of NMDARs and PSD-95 in the hippocampus of CSDS-induced mice (n = 3-4/group). (A) The expression levels of NR1, NR2A (B), NR2B (C), and PSD-95 (D) were examined by Western blot analysis. Mice were decapitated 60 min after TST. The hippocampus was dissected for Western blot analysis. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control group. $\#\#p < 0.01$ and $\#\#\#p < 0.001$ compared with the CSDS group. KRG, Korean Red Ginseng; CSDS, chronic social defeat stress; TST, tail suspension test; NMDARs, N-methyl-D-aspartate receptors; PSD-95, postsynaptic density-95.

not directly affect social interaction, such as notably recovering the interaction zone time reduced by CSDS, but indirectly affected it by reducing the difference of the corner zone. Another potential explanation is the effect of KRG on recovery of movement in the interaction zone. When we observed that the time in the corner zone was significantly reduced within the KRG 20 mg/kg group, it was thought that the decreased locomotor activity due to stress-induced anhedonia was restored. Therefore, we calculated the movement ratio according to presence or absence of target (Fig. 5E–5F). As a result, it was confirmed that the movement ratio in the interaction zone was significantly increased in KRG 20 mg/kg group. Thus, in the stressed mice, the movement ratio in the interaction zone was reduced by the effect of freezing, one of the fear-related behaviors of the target. However, when KRG was treated, the movement ratio in the interaction zone was increased. This change was confirmed in the movement ratio in the corner zone as well. However, we did not observe a dose-dependent effect, and only the KRG 20 mg/kg group showed a significant effect on these parameters. This implies that KRG influences social avoidance and other behaviors differently, perhaps because of dissimilar behavioral features. The SIT evaluates interactions with other animals as a measure of sociability. Therefore, the effective dose of KRG would be different because the SIT is distinct from other tests, such as the EPM test and OFT that evaluate behavioral characteristics of test mice in isolation.

Furthermore, we found that CSDS-induced mice trended toward increased immobility time and decreased strong mobility time and velocity. As shown in Fig. 6A, the CSDS group showed

increased immobility over time, but the difference was not significant. Depressive symptoms as evaluated by the TST were not entirely evident. Many researches have shown that depression-like behaviors induced by stress differ from each other depending on various stress factors (e.g., defeat stress, unpredictable stress, mild stress, etc.), stress duration, and the experimental animal strain [38,40,50,52]. Furthermore, C57BL/6 mice strongly tend to climb up their tail, which would have negated the results of the TST [55]. In our study, when the stressed group was treated with 40 mg/kg of KRG, the immobility time was decreased as compared with the CSDS group. In addition, as shown in Fig. 6C–6D, strong mobility time and velocity were increased. This result suggests that KRG can possibly act as an effective antidepressant for relieving stress-related responsiveness.

Here, we evaluated the effect of KRG on NMDARs and PSD-95 expression in the hippocampus. Alterations of NMDARs have been studied in brain regions involved in mood disorders, such as the hippocampus [56]. A previous study reported that rats underwent chronic mild stress significantly increased the mRNA expression of NR1, NR2A, and NR2B in the ventral hippocampus, whereas rats chronically administered with duloxetine, one of the antidepressants, reduced the mRNA level of NR1, NR2A, and NR2B [16]. In this study, significant increases in NR1, NR2A, and NR2B protein expression were observed in the hippocampus of the CSDS group. PSD-95 levels were also increased, although the increase was not significant. Therefore, the present study suggests that the CSDS-induced increase of NMDARs may lead to abnormal hippocampus function in stress-related mood disorders. However, in the CSDS–KRG 40 mg/kg group, expression of NMDARs and PSD-95 was significantly reversed. Collectively, these data suggest that KRG may optimize glutamate function by regulating NMDARs, which interact with PSD-95.

As described in the Introduction, we suggest that not only the simple expression level but also the ratio of NR2A and NR2B levels is important in modulation of NMDAR function. In a previous study, chronic mild stress increased NR2B mRNA level but not NR2A mRNA in the rat dorsal hippocampus [16]. However, in our study, there was no significant difference in ratio of NR2A to NR2B among groups (data not shown). We reject the assumption that the function of NMDARs related to mood disorders is dependent on change in NR2B. Instead, we conclude that expression of all hippocampal NMDARs is important in modulation of NMDAR function in our chosen CSDS model.

In previous reports, some of the ginsenosides, especially Rb1 and Rg3, were studied for their effects on stress-related changes [57–59]. When Rb1 was administered orally, immobilization-stressed animals showed recovery from decreased brain-derived neurotrophic factor (BDNF) levels in the hippocampus and from increased levels of polyamine, which is a known stress stimuli marker in the brain [57,58]. In addition, Rg3 displayed an anxiolytic effect on chronic unpredictable stress by normalizing the serotonergic system [59]. Consistent with the previous studies, the KRG powder in the present study had high levels of Rb1 (7.89 mg/g) and Rg3 (3.23 mg/g). This suggests that Rb1- and Rg3-enriched KRG plays an important role in inhibiting CSDS-induced mood disorders by mitigating NMDAR upregulation.

In conclusion, our two primary findings were that KRG significantly ameliorated CSDS-induced mood disorders, specifically social avoidance, anxiety, and locomotor activity, and KRG recovered upregulated expression of NMDARs and PSD-95 related to stress-induced affective disorders in the hippocampus. These findings provide valuable insights into mood disorders that are caused by social stress and the roles of NMDARs in a CSDS model. Overall, KRG has therapeutic potential as a treatment for stress-related mood disorders.

Conflicts of Interest

The authors have no conflicting interests to disclose.

Acknowledgments

This work was supported by the 2016 grant from the Korean Society of Ginseng with funding from the Korea Ginseng Corporation and by the Basic Science Research Program (No. 2012R1A5A2A28671860) through the National Research Foundation of Korea (NRF).

References

[1] Hammen C. Stress and depression. Annu Rev Clin Psychol 2005;1:293–319.
[2] Tennant C. Life events, stress and depression: a review of recent findings. Aust N Z J Psychiatry 2002;36(2):173–82.
[3] Litman L, Costantino G, Waxman R, Sanabria-Velez C, Rodriguez-Guzman VM, Lampon-Velez A, Brown R, Cruz T. Relationship between peer victimization and posttraumatic stress among primary school children. J Trauma Stress 2015;28(4):348–54.
[4] Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature 2008;455(7215):894–902.
[5] Stefanski V. Social stress in laboratory rats: hormonal responses and immune cell distribution. Psychoneuroendocrinology 2000;25(4):389–406.
[6] Fuchs E, Flugge G. Social stress in tree shrews: effects on physiology, brain function, and behavior of subordinate individuals. Pharmacol Biochem Behav 2002;73(1):247–58.
[7] Rygula R, Abumaria N, Flugge G, Fuchs E, Ruther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 2005;162(1):127–34.
[8] Rygula R, Abumaria N, Domenici E, Hiemke C, Fuchs E. Effects of fluoxetine on behavioral deficits evoked by chronic social stress in rats. Behav Brain Res 2006;174(1):188–92.
[9] Huang GB, Zhao T, Muna SS, Bagalkot TR, Jin HM, Chae HJ, Chung YC. Effects of chronic social defeat stress on behaviour, endoplasmic reticulum proteins and choline acetyltransferase in adolescent mice. Int J Neuropsychopharmacol 2013;16(7):1635–47.
[10] Sheline YI. Neuroimaging studies of mood disorder effects on the brain. Biol Psychiatry 2003;54(3):338–52.
[11] McEwen BS. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism 2005;54(5 Suppl 1):20–3.
[12] McEwen BS. Stress and hippocampal plasticity. Annu Rev Neurosci 1999;22:105–22.
[13] Lowy MT, Gault L, Yamamoto BK. Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the hippocampus. J Neurochem 1993;61(5):1957–60.
[14] Reznikov LR, Grillo CA, Piroli GG, Pasumarthi RK, Reagan LP, Fadel J. Acute stress-mediated increases in extracellular glutamate levels in the rat amygdala: differential effects of antidepressant treatment. Eur J Neurosci 2007;25(10):3109–14.
[15] Bagley J, Moghaddam B. Temporal dynamics of glutamate efflux in the prefrontal cortex and in the hippocampus following repeated stress: effects of pretreatment with saline or diazepam. Neuroscience 1997;77(1):65–73.
[16] Calabrese F, Guidotti G, Molteni R, Racagni G, Mancini M, Riva MA. Stress-induced changes of hippocampal NMDA receptors: modulation by duloxetine treatment. PLoS One 2012;7(5):e37916.
[17] Fontella FU, Vendite DA, Tabajara AS, Porciuncula LO, da Silva Torres IL, Jardim FM, Martini L, Souza DO, Netto CA, Dalmaz C. Repeated restraint stress alters hippocampal glutamate uptake and release in the rat. Neurochem Res 2004;29(9):1703–9.
[18] Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 2011;475(7354):91–5.
[19] Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, Li XY, Aghajanian G, Duman RS. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol Psychiatry 2011;69(8):754–61.
[20] Yang Y, Ju W, Zhang H, Sun L. Effect of ketamine on LTP and NMDAR EPSC in Hippocampus of the chronic social defeat stress mice model of depression. Front Behav Neurosci 2018;12:229.
[21] Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, et al. Molecular diversity of the NMDA receptor channel. Nature 1992;358(6381):36–41.
[22] Miller OH, Yang L, Wang CC, Hargroder EA, Zhang Y, Delpire E, Hall BJ. GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. Elife 2014;3:e03581.

[23] Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science 1995;269(5231):1737–40.
[24] Cousins SL, Papadakis M, Rutter AR, Stephenson FA. Differential interaction of NMDA receptor subtypes with the post-synaptic density-95 family of membrane associated guanylate kinase proteins. J Neurochem 2008;104(4):903–13.
[25] Sheng M. The postsynaptic NMDA-receptor–PSD-95 signaling complex in excitatory synapses of the brain. J Cell Sci 2001;114(Pt 7):1251.
[26] Calabrese F, Molteni R, Racagni G, Riva MA. Neuronal plasticity: a link between stress and mood disorders. Psychoneuroendocrinology 2009;34(Suppl 1):S208–16.
[27] McGrath PJ, Stewart JW, Fava M, Trivedi MH, Wisniewski SR, Nierenberg AA, Thase ME, Davis L, Biggs MM, Shores-Wilson K, et al. Tranylcypromine versus venlafaxine plus mirtazapine following three failed antidepressant medication trials for depression: a STAR*D report. Am J Psychiatry 2006;163(9):1531–41. quiz 666.
[28] Lee YY, Park JS, Jung JS, Kim DH, Kim HS. Anti-inflammatory effect of ginsenoside Rg5 in lipopolysaccharide-stimulated BV2 microglial cells. Int J Mol Sci 2013;14(5):9820–33.
[29] Kim EH, Kim IH, Lee MJ, Thach Nguyen C, Ha JA, Lee SC, Choi S, Choi KT, Pyo S, Rhee DK. Anti-oxidative stress effect of red ginseng in the brain is mediated by peptidyl arginine deiminase type IV (PADI4) repression via estrogen receptor (ER) beta up-regulation. J Ethnopharmacol 2013;148(2):474–85.
[30] Kang A, Xie T, Zhu D, Shan J, Di L, Zheng X. Suppressive effect of ginsenoside Rg3 against lipopolysaccharide-induced depression-like behavior and neuroinflammation in mice. J Agric Food Chem 2017;65(32):6861–9.
[31] Baek JH, Heo JY, Fava M, Mischoulon D, Choi KW, Na EJ, Cho H, Jeon HJ. Effect of Korean red ginseng in individuals exposed to high stress levels: a 6-week, double-blind, randomized, placebo-controlled trial. J Ginseng Res 2018.
[32] Golden SA, Covington 3rd HE, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. Nat Protoc 2011;6(8):1183–91.
[33] Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 2006;9(4):519–25.
[34] Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 1987;92(2):180–5.
[35] Ko YH, Kwon SH, Hwang JY, Kim KI, Seo JY, Nguyen TL, Lee SY, Kim HC, Jang CG. The memory-enhancing effects of liquiritigenin by activation of NMDA receptors and the CREB signaling pathway in mice. Biomol Ther (Seoul) 2018;26(2):109–14.
[36] Hollis F, Kabbaj M. Social defeat as an animal model for depression. ILAR Journal 2014;55(2):221–32.
[37] Iio W, Takagi H, Ogawa Y, Tsukahara T, Chohnan S, Toyoda A. Effects of chronic social defeat stress on peripheral leptin and its hypothalamic actions. BMC Neurosci 2014;15:72.
[38] Iniguez SD, Riggs LM, Nieto SJ, Dayrit G, Zamora NN, Shawhan KL, Cruz B, Warren BL. Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. Stress 2014;17(3):247–55.
[39] Jeong JY, Lee DH, Kang SS. Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. Endocrinol Metab (Seoul) 2013;28(4):288–96.
[40] Jung YH, Hong SI, Ma SX, Hwang JY, Kim JS, Lee JH, Seo JY, Lee SY, Jang CG. Strain differences in the chronic mild stress animal model of depression and anxiety in mice. Biomolecules and Therapeutics 2014;22(5):453–9.
[41] Pothion S, Bizot JC, Trovero F, Belzung C. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav Brain Res 2004;155(1):135–46.
[42] Harris RB, Zhou J, Youngblood BD, Rybkin II, Smagin GN, Ryan DH. Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. Am J Physiol 1998;275(6 Pt 2):R1928–38.
[43] Karu N, Reifen R, Kerem Z. Weight gain reduction in mice fed Panax ginseng saponin, a pancreatic lipase inhibitor. J Agric Food Chem 2007;55(8):2824–8.
[44] Song YB, An YR, Kim SJ, Park HW, Jung JW, Kyung JS, Hwang SY, Kim YS. Lipid metabolic effect of Korean red ginseng extract in mice fed on a high-fat diet. J Sci Food Agric 2012;92(2):388–96.
[45] Lee SH, Lee HJ, Lee YH, Lee BW, Cha BS, Kang ES, Ahn CW, Park JS, Kim HJ, Lee EY, et al. Korean red ginseng (Panax ginseng) improves insulin sensitivity in high fat fed Sprague-Dawley rats. Phytother Res 2012;26(1):142–7.
[46] Gorman JM. Comorbid depression and anxiety spectrum disorders. Depress Anxiety 1996;4(4):160–8.
[47] Organization WH. International statistical classification of diseases and related health problems, tenth revision (ICD-10)1992.
[48] Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463(1–3):3–33.
[49] Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 2007;131(2):391–404.
[50] Kinsey SG, Bailey MT, Sheridan JF, Padgett DA, Avitsur R. Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. Brain Behav Immun 2007;21(4):458–66.
[51] Saul ML, Tylee D, Becoats KT, Guerrero BG, Sweeney P, Helmreich DL, Fudge JL. Long-term behavioral consequences of stress exposure in adolescent versus young adult rats. Behav Brain Res 2012;229(1):226–34.
[52] Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharmacology 2004;29(11):2007–17.
[53] Venzala E, Garcia-Garcia AL, Elizalde N, Delagrange P, Tordera RM. Chronic social defeat stress model: behavioral features, antidepressant action, and interaction with biological risk factors. Psychopharmacology (Berl) 2012a;224(2):313–25.
[54] Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 2006;311(5762):864–8.
[55] Mayorga AJ, Lucki I. Limitations on the use of the C57BL/6 mouse in the tail suspension test. Psychopharmacology (Berl) 2001;155(1):110–2.
[56] Miczek KA, Yap JJ, Covington HE. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. Pharmacol Ther 2008;120(2):102–28.
[57] Kim M, Kim SO, Lee M, Park Y, Kim D, Cho KH, Kim SY, Lee EH. Effects of ginsenoside Rb1 on the stress-induced changes of BDNF and HSP70 expression in rat hippocampus. Environ Toxicol Pharmacol 2014;38(1):257–62.
[58] Lee SH, Jung BH, Kim SY, Lee EH, Chung BC. The antistress effect of ginseng total saponin and ginsenoside Rg3 and Rb1 evaluated by brain polyamine level under immobilization stress. Pharmacol Res 2006;54(1):46–9.
[59] Xu JN, Chen LF, Su J, Liu ZL, Chen J, Lin QF, Mao WD, Shen D. The anxiolytic-like effects of ginsenoside Rg3 on chronic unpredictable stress in rats. Sci Rep 2018;8(1):7741.