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Research Article

REGULAR RESEARCH ARTICLE

Serotonin Signaling Trough Prelimbic 5-HT1A Receptors Modulates CSDS-Induced Behavioral Changes in Adult Female Voles

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Abstract

Background: Most previous studies have focused on the effects of social defeat in male juvenile individuals. Whether chronic social defeat stress in adulthood affects female emotion and the underlying mechanisms remains unclear.
Methods: Using highly aggressive adult female mandarin voles (*Microtus mandarinus*), the present study aimed to determine the effects of chronic social defeat stress on anxiety- and depression-like behaviors in adult female rodents and investigate the neurobiological mechanisms underlying these effects.
Results: Exposure of adult female voles to social defeat stress for 14 days reduced the time spent in the central area of the open field test and in the open arms of the elevated plus maze and lengthened the immobility time in the tail suspension and forced swimming tests, indicating increased anxiety- and depression-like behaviors. Meanwhile, defeated voles exhibited increased neural activity in the prelimbic cortex of the medial prefrontal cortex. Furthermore, chronic social defeat stress reduced serotonin projections and levels of serotonin 1A receptors in the medial prefrontal cortex-prelimbic cortex. Intra-prelimbic cortex microinjections of the serotonin 1A receptor agonist 8-OH-DPAT reversed the alterations in emotional behaviors, whereas injections of the serotonin 1A receptor antagonist WAY-100635 into the prelimbic cortex of control voles increased the levels of anxiety- and depression-like behaviors.
Conclusions: Taken together, our results demonstrated that chronic social defeat stress increased anxiety- and depression-like behaviors in adult female voles, and these effects were mediated by the action of serotonin on the serotonin 1A receptors in the prelimbic cortex. The serotonin system may be a promising target to treat emotional disorders induced by chronic social defeat stress.

Keywords: 5-HT1A receptor, mPFC, CSDS, emotional disorders, mandarin voles

Introduction

Repeated exposure to stressful social environments strongly correlates with the development of stress-related psychological disorders, such as anxiety-like behaviors (Smith and Wang, 2014; Takahashi et al., 2017) and depression-like behaviors (Yu et al., 2011; Huang et al., 2013; Hollis and Kabbaj, 2014). Social defeat stress has proven to be a powerful method for exploring the mechanisms underlying stress susceptibility (Valmaggia et al., 2015; Solomon, 2017). Chronic social defeat stress (CSDS) is a predominant social stressor for many species, particularly species living in groups (Valmaggia et al., 2015; Solomon, 2017).

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Significance Statement

Studies indicate that women may have higher susceptibility to violence and higher rates of stress-associated disorders compared to men. However, most previous studies have focused on social defeat in male juvenile individuals. Whether CSDS at adulthood affects female emotion and its underlying mechanism remains unclear. Recent evidence has indicated that serotonergic transmission and the 5-HT_{1A}R in the mPFC has an important inhibitory effect in anxiety and depression. The present study demonstrated that the CSDS increased anxiety- and depressive-like behaviors in adult female voles, and these effects were mediated by the action of 5-HT on the 5-HT_{1A}R in the mPFC. These findings show that the serotonin system may be a promising target to treat emotional disorders in women induced by CSDS.

In humans, social defeat stress in the form of bullying is correlated with a greater incidence of stress-related psychiatric and addictive disorders (Bjorkqvist, 2001; Krishnan et al., 2007). Based on accumulating evidence, exposure to CSDS increases the levels of anxiety- and depression-like behaviors in rodents (Becker et al., 2008; Venzala et al., 2013; Jianhua et al., 2017). In addition, the consequences of chronic social stress may depend on the sex and age of the animals (McCormick et al., 2008).

Researchers are increasingly recognizing that women may have a higher susceptibility to violence and higher rates of stress-associated disorders than men (Laredo et al., 2015; Jianhua et al., 2017; Steinman and Trainor, 2017). More importantly, CSDS exposure increases the risk of many psychological disorders not only during development but also throughout adulthood (Kovalenko et al., 2014; McCormick et al., 2015). However, almost all behavioral and neuroscience studies examining social defeat stress have focused on adolescent male rodents, and the failure to include females in these studies is a serious limitation. According to multiple lines of evidence, exposure to social defeat stress in male adolescent rodents induces anxiety-like behaviors (Watt et al., 2009; Huang et al., 2013) and depression-like behaviors (Sanchez et al., 2001; Paus et al., 2008). Until now, few studies have investigated the alterations in emotional behaviors in female rodents after exposure to social defeat stress (Greenberg et al., 2015; Takahashi et al., 2017; Harris et al., 2018), and the neurobiological mechanisms by which CSDS induces anxiety and depression in adult female rodents remain poorly understood.

In mammals, serotonergic neurons are mainly located in the dorsal raphe of the brainstem (DRN) (Barnes and Sharp, 1999). Projections from these neurons release serotonin (5-hydroxytryptamine, 5-HT) to the entire central nervous system, including the medial prefrontal cortex (mPFC) and medial amygdala (MeA) in the limbic system (Bockaert et al., 2006). In these brain areas, 5-HT plays an important role in regulating numerous emotional disorders, including depression- and anxiety-related behaviors, in both humans and rodents (Canli and Lesch, 2007; Jans et al., 2007). For example, tryptophan (TPH, an amino acid necessary for serotonin synthesis) depletion followed by a decrease in 5-HT levels in the brain increases the risk of depression (Moreno et al., 1999; Neumeister et al., 2004). In addition, CSDS downregulated the expression of serotonergic genes (such as TPH2, SERT, Maoa, and Htr1a) in male mice (Boyarskikh et al., 2013) and decreased the level of 5-HT in the mPFC to induced depression and anxiety (Lowry et al., 2008; Venzala et al., 2013). In addition, 5-HT levels are reduced in the PFC of patients with major depression (Lowry et al., 2008; Michelsen et al., 2008). Researchers have not clearly determined whether CSDS induces emotional disorders by altering 5-HT projections to the PFC.

At least 14 different serotonin receptor subtypes have been identified in the brain, of which serotonin 1A receptors (5-HT_{1A}R) are among the best characterized (Polter and Li, 2010). More importantly, signalling through 5-HT_{1A}R has been shown to moderate mood-related behavior in animals (Garcia-Garcia et al., 2014). In the mammalian brain, 5-HT_{1A}R is divided into autoreceptors and heteroreceptors. The 5-HT1A autoreceptors located in the 5-HT neurons mediate negative feedback, and 5-HT1A heteroreceptors are expressed in brain areas that receive serotonergic projections and are expressed at particularly high levels in brain regions implicated in the regulation of emotion, such as the mPFC and MeA (Garcia-Garcia et al., 2014).

Among the brain areas modulating emotional responses to stress, the mPFC is considered to be an important region (Rosenkranz et al., 2003; Morrison et al., 2013). In rodents, the mPFC consists of the cingulate (Cg), prelimbic (PrL), and infralimbic (IL) cortices, these regions have different functions that can generate distinct patterns of behaviors (Heidbreder and Groenewegen, 2003). The PrL is involved in regulating emotional responses (Petty et al., 1997; Broersen et al., 2000). In addition, stimulation of postsynaptic 5-HT1A heteroreceptors in the mPFC is involved in the response to antidepressants (Fukumoto et al., 2018), and suppressing the 5-HT1A heteroreceptors in the mPFC is reported to result in a depression-like phenotype (Garcia-Garcia et al., 2017). More importantly, sustained antidepressant effects are mimicked by intra-mPFC injection of a 5-HT_{1A}R agonist and attenuated by a 5-HT_{1A}R antagonist (Fukumoto et al., 2018). Nevertheless, 5-HT1A autoreceptors have consistently been shown to impact anxiety-like behavior (De Vry, 1995; Albert and Lemonde, 2004; Garcia-Garcia et al., 2014). More importantly, the PFC-DRN pathway of 5-HT is reported to be a key neural circuit controlling the effect of stress (Amat et al., 2005). However, researchers have not yet determined whether 5-HT1A heteroreceptors in the mPFC are involved in the anxiety- and depression-like behaviors induced by CSDS in adult female rodents.

One limitation to incorporating females into studies of social defeat stress and its neural mechanism may be that most female rodents do not show spontaneous aggression in a resident-intruder situation (Steinman and Trainor, 2017). Although recent studies have focused on the female social defeat model in rodents, the establishment of those models is relatively complex (Lukas and Neumann, 2014; Takahashi et al., 2017; Finnell et al., 2018). Valid animal models are needed to investigate the effects of social defeat stress on female adults. The mandarin vole (*Microtus mandarinus*) is a socially monogamous species, all family members live in one burrow system, and both males and females defend territories. More importantly, the adult female mandarin vole displays stark spontaneous aggression (Tai and Wang, 2001). In particular, this species represents a valuable animal model for examining the effects of social defeat stress on females (Wang et al., 2018).

Thus, using this animal model, the goal of the present study was to determine the potential impact of CSDS on anxiety- and

depression-like behaviors in adult female voles and to investigate the underlying neurobiological mechanisms in the serotonin system. We predict that CSDS may increase levels of anxiety- and depression-like behavior in female mandarin voles, possibly via reducing the number of 5-HT projections and levels of 5-HT_{1A}R in the mPFC.

Materials and Methods

Animals

Mandarin voles were derived from a wild population in Henan province, China. The voles used in this experiment were adult virgin females obtained from the laboratory-reared F3 generation. Remarkably, socially monogamous female prairie voles (*Microtus ochrogaster*) do not display spontaneous ovarian activity or ovulation before encountering a male (Sawrey and Dewsbury, 1985), and behavioral estrus occurs after the female is introduced to a novel male (Morgan et al., 1997). Unpublished data from our laboratory also show a similar pattern that female mandarin voles are always in diestrus before encountering a male. Therefore, estrus cycles had no effects on the results reported in this article. Voles were housed with a female cagemate in polycarbonate cages (44 × 22 × 16 cm) with unlimited access to carrots and were maintained on a 12-hour-light/-dark photoperiod at a temperature of 23°C ± 1°C. All procedures were approved by the Animal Care and Use Committee of Shaanxi Normal University and in accordance with the Guide for the Care and Use of Laboratory Animals of China.

Chronic Social Defeat Stress

The resident-intruder paradigm of social defeat stress was conducted as previously reported (Wang et al., 2018). Based on the results of the preliminary experiment, female-female social defeat interactions were more intense than male-male social defeat interactions in mandarin voles. During the screening test, we randomly selected older (80–120 days) and heavier female mandarin voles to perform the social interaction test. The vole with an attack latency of less than 30 seconds in 3 consecutive tests was selected as aggressive resident vole. Six in 10 old females showed aggressive behaviors and were used as resident voles. These aggressive resident females were housed separately before the encounters. Young adult virgin female mandarin voles (70 days old, 23–27 g), which were designated as intruders, were assigned to the defeated group or the control group. Each experimental group included voles from different litters, and voles in each litter were divided across the experimental groups to avoid effects of genetic diversity. During the resident-intruder paradigm, almost every female intruder from the defeated group received attacks and exhibited at least 5 times submissive defeat posture (supine position). After a 10-minute confrontation, the animals were separated by a perforated Plexiglas panel (they could see, hear, and smell each other, but could not make physical contact) for 24 hours. This defeat process was repeated daily at 9:00 AM for 14 consecutive days. During the defeat process, if defeated females were injured, they were not involved in the next test. Approximately 30% of the voles were excluded because of injury. Control voles, which had similar backgrounds as animals in the defeated group, were also exposed to a female resident without aggression during a 10-minute period and housed in a manner similar to the defeated voles. On the second day after the last defeat stress, behavioral tests were performed in the defeated and control groups to assess anxiety- and depression-like behaviors using the open field test, elevated plus maze test, tail suspension test, and forced swimming test at 1-day intervals (Figure 1).

Open Field Test

The open field test (OFT) has been used to assess activity and levels of anxiety in previous studies (Choleris et al., 2001). The OFT was performed 1 day after the CSDS (day 86). The defeated and control voles (n = 10 animals per group) were placed individually in the center of a black-painted Plexiglas box (50 × 50 × 25 cm) for the OFT (the light intensity was approximately 20 lx). The box was divided into 16 quadrants: 4 inner sections (central area) and 12 outer sections. For the test, each vole was placed into the central area and allowed to explore for 5 minutes. The time spent in the central area and the total distance travelled in the entire area during this period was recorded with the digital video tracking system and quantified afterwards using OBSERVER (V5.0; Noldus, NL) software. At the end of each experiment, the box was thoroughly cleaned with 30% ethanol to remove odor cues (Kovalenko et al., 2014). All tests were conducted at the same time each day.

Elevated Plus Maze Test

The elevated plus maze test (EPM) was used to assess anxiety behavior (Lister, 1987). The EPM was performed 1 day after the CSDS (day 86). The elevated plus maze consisted of 2 open arms (25 × 5 cm) and 2 closed arms (25 × 5 × 5 cm), and the entire apparatus was elevated 50 cm above the ground (the light intensity was approximately 20 lx). The defeated and control voles (n = 12 animals per group) were placed in the center of the EPM facing an open arm and was allowed to explore for 5 minutes. During this period, the time spent in the open and closed arms was recorded and quantified.

Tail Suspension Test

The tail suspension test (TST) (Cryan and Slattery, 2007) is the most widely used test for evaluating depression in rodents. The TST was performed 2 days after the CSDS (day 87). Each vole from the defeated and control groups (n = 12 animals per group) was individually suspended approximately 50-cm above the floor with surgical tape for 6 minutes. The time the vole spent remaining completely motionless was defined as the immobility time. The duration of immobility was recorded during the final 4 minutes of the test by the tail suspension monitor (TSE Systems, V 2.2, Germany).

Forced Swimming Test

The forced swimming test (FST) is popularly used to assess levels of depression in rodents (Porsolt et al., 1977). The FST was performed 2 days after the CSDS (day 87). Each vole from the defeated and control groups (n = 12 animals per group) was placed individually in a glass cylinder (height = 24 cm, diameter = 14 cm) containing 15-cm of water (22°C–25°C) for 6 minutes. The duration of immobility during the last 4 minutes of the 6-minute testing period was monitored with a video camera located above the cylinder and analyzed using J Watcher software (http://www.jwatcher.ucla.edu/) by a trained and blinded observer.

Immunofluorescence Staining

CSDS-induced alterations in neuronal activity in different brain regions and in 5-HT projections were assessed in this experiment.

Six defeated and control voles were used for 5-HT and TPH2 immunofluorescence staining. To assess Fos expression in the brain, additional voles were killed 1-hour after EPM testing, which was 1 day after the last defeat in the defeated group (n=6 animals per group) and after the corresponding phase in the control group (n=6 animals per group). Voles were anesthetized with 2% sodium pentobarbital (3 mL/kg) and transcardially perfused with PBS buffer (0.1 M, pH 7.2–7.5) followed by 4% paraformaldehyde. The brains were collected and immersed in 4% paraformaldehyde and then dehydrated in sucrose solution. Serial transverse slices of the brain were cut in 30-μm intervals. Sections were incubated with 0.3% H_2O_2. After 3 rinses with PBS, sections were immersed in 0.2% Triton X-100 and then blocked with 5% BSA.

For Fos immunofluorescence staining, sections were incubated with the rabbit anti-Fos (1:500, Santa Cruz, CA) primary antibody overnight at 4°C. On the second day, sections were incubated with the goat anti-rabbit (TRITC, 1:400) secondary antibody in the dark for 2 hours. For serotonin and TPH2 immunofluorescence staining, sections were incubated with the goat anti-serotonin (1:400, Abcam) or rabbit anti-TPH2 (1:100, Santa Cruz) primary antibody and then incubated with the donkey anti-goat (DyLight 488, 1:200, YEASEN) or goat anti-rabbit (DyLight 488, 1:200, Boster) secondary antibody. Ultimately, all sections were photographed using a laser confocal microscope (FV-1000, Olympus). The number of 5-HT-immunoreactive (5-HT-ir) neurons and TPH2-ir neurons in the DRN, the density of 5-HT-ir fibers in the mPFC and MeA, and the number of Fos-ir neurons in the mPFC, MeA, and DRN were measured bilaterally using Image-Pro Plus software (V 6.0, Media Cybernetics).

Brain Tissue Preparation and Western Blot

Western blotting was used to measure the changes in 5-HT_{1A}R levels induced by CSDS. Voles from the defeated and control groups (n=6 animals per group) were anesthetized with 2% sodium pentobarbital (3 mL/kg) and decapitated. Brains were immediately extracted and frozen on dry ice. Coronal sections (200-μm) were cut on a cryostat and frost mounted onto microscope slides. Bilateral tissue punches with a 1-mm diameter were removed from the entire mPFC (Cg, PrL, IL), MeA, and DRN under a stereomicroscope with reference to brain atlas (Paxinos and Franklin, 2001) and stored at -80°C until processing. Based on the weight of the brain tissue, RIPA buffer (1:10000) and the protease inhibitor PMSF (1:100) were added to the tubes for sonication. Samples were centrifuged and the supernatant was collected. Total protein concentrations were quantified using the BCA Protein Assay kit (Tiangen). Protein samples were separated on SDS-PAGE gels and transferred to a PVDF membrane. Membranes were blocked and then incubated with rabbit anti-5-HT_{1A}R (1:2000, Abcam) or mouse anti-β-actin (1:3000, Abcam) primary antibody. Then, the membranes were incubated with the goat anti-rabbit or goat anti-mouse secondary antibody (1:10000, Zhongshan Goldenbridge). All protein bands were visualized using a fully automatic chemiluminescence image analysis system (Tanon) and analyzed using ImageJ software. 5-HT_{1A}R- and β-actin immunoreactive bands were visualized at molecular weights of 62 kDa and 43 kDa, respectively.

Pharmacological Studies

This experiment was used to test whether the microinjection of a 5-HT_{1A}R agonist and antagonist into the PrL of mPFC altered anxiety- and depression-like behaviors. Another cohort of defeated voles (n=32 animals per group) and control voles (n=16 animals per group) were anesthetized with a mixture of isoflurane and oxygen and then received stereotaxic cannulation surgery under sterile conditions. Next, 26-gauge stainless steel guide cannulae (RWD) were implanted bilaterally, aimed at the PrL (AL 2.2 mm, ML±0.5 mm, DV 2.1 mm). Finally, cannulae were affixed to the skull with dental cement. After 3 days of recovery, each vole with normal activity received microinjections of either saline/200 nL, 0.03-μg 8-OH-DPAT/200 nL, 0.3-μg 8-OH-DPAT/200 nL, 3-μg 8-OH-DPAT/200 nL, and 0.4-μg WAY-100635/200 nL (n=8 animals per group). The 5-HT_{1A}R agonist 8-OH-DPAT (Sigma-Aldrich) (0.03 μg, 0.3 μg, or 3 μg in 200 nL) and the 5-HT_{1A}R antagonist WAY-100635 (Sigma-Aldrich) (0.4 μg in 200 nL) were dissolved in saline, and doses were chosen based on effective doses used in previous studies (Cooper et al., 2008; Fukumoto et al., 2018) with little modification, based on their effects on mandarin voles in the preliminary experiment. The speed of injection was 0.1 μL/min for 1-minute per side. Fifteen minutes after the microinjection of the drug, anxiety- and depression-like behavior were assessed using the methods described above.

Statistical Analysis

All data were assessed for normality using one-sample Kolmogorov-Smirnov test. The time spent in the "central area"

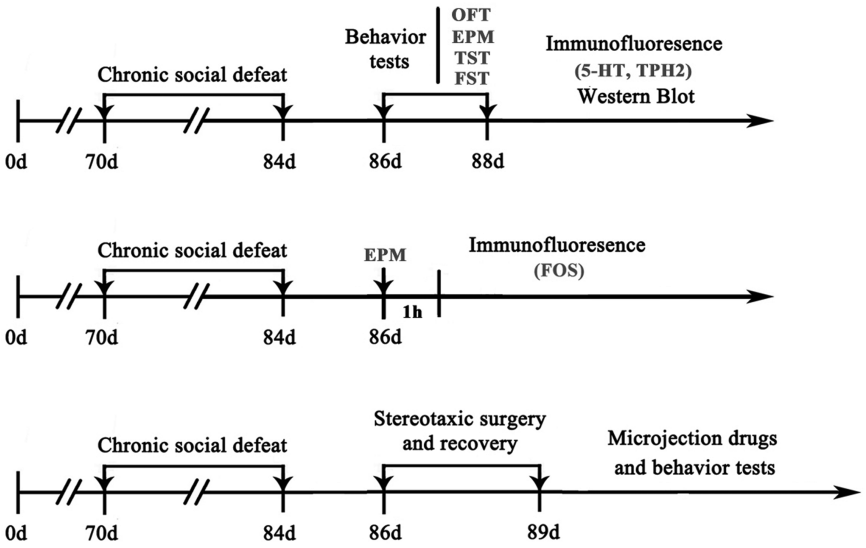

Figure 1. The timeline of the experimental procedures performed in this study.

and total distance travelled in the OFT, the time spent in the open or closed arms of the EPM, the immobility time during the TST and FST, the number of Fos-ir and 5-HT-ir cells, TPH2-ir and 5-HT-ir fibres densities, and 5-HT_{1A}R relative densities were compared using independent sample *t* tests. The effects of the treatment (saline, 8-OH-DPAT and WAY-100635) on the results of the behavior tests were compared using one-way ANOVA. Posthoc tests were performed using Tukey's test. All statistical analyses were performed using SPSS V 20.0 (SPSS Inc.) and presented as means ± SEM. The level of significance for all tests was .05.

Results

CSDS-Induced Anxiety-Like Behaviors in Adult Female Voles

In the OFT, defeated voles spent significantly less time in the central area than control voles (t (18) = 3.996, P < .01) (Figure 2A). The total distance travelled in the OFT did not differ between the 2 groups during the 5-minute test (t (18) = 1.006, P = .328) (Figure 2B). In the EPM, the defeated voles spent significantly more time in the closed arms than the controls (t (22) = -3.150, P < .01) (Figure 2C) and significantly less time in the open arms than the controls (t (22) = 3.301, P < .01) (Figure 2D). Based on the data from these experiments, CSDS results in anxiety-like behaviors in adult female voles.

CSDS Induced Depression-Like Behaviors in Adult Female Voles

CSDS significantly increased the immobility time of female voles in the TST (t (22) = -6.89, P < .01) (Figure 3A). In the FST, defeated voles also exhibited a significant increase in immobility time compared with control voles (t (22) = -2.543, P < .05) (Figure 3B). Thus, CSDS results in depression-like behaviors in adult female voles.

Brain Neural Activation Induced by CSDS

Fos is an endogenous marker of neuronal activity. Stress causes a rapid and transient increase in Fos expression (Martinez et al., 2002; Yu et al., 2011). Thus, we assessed Fos expression in specific brain areas using immunofluorescence staining 1 hour after the 14-day defeat phase. In the defeated voles, Fos expression was significantly increased in the mPFC-Cg (t (10) = -3.015, P < .05) (Figure 4A–C), mPFC-PrL (t (10) = -6.294, P < .01) (Figure 4D–F), and mPFC-IL (t (10) = -3.126, P < .05) (Figure 4G–I) compared with control voles, among which the increased in PrL area was the most significant. In addition, the density of Fos-ir cells in the MeA was higher in defeated voles than in the control voles (t (10) = -8.004, P < .01) (Figure 4J–L). Social defeat stress also induced Fos expression in the DRN (t (10) = -8.595, P < .01) (Figure 4M–O).

CSDS Reduced 5-HT Projections in PrL

CSDS changed the 5-HT projections in the brain in a region-specific manner. The control group exhibited higher densities of 5-HT-ir fibers in the mPFC-PrL than the defeated group had (t (10) = 2.679, P < .05) (Figure 5D–F). CSDS did not affect the densities of 5-HT-ir fibers in the mPFC-Cg (t (10) = 1.105, P = .295) (Figure 5A–C), mPFC-IL (t (10) = 0.214, P = .835) (Figure 5G–I), or MeA (t (10) = 1.053, P = .317) (Figure 5J–L), or the numbers of 5-HT-ir neurons (t (10) = -1.661, P = .128) (Figure 5M–O) and TPH2-ir neurons (t (10) = -1.189, P = .262) (Figure 5P–R) in the DRN.

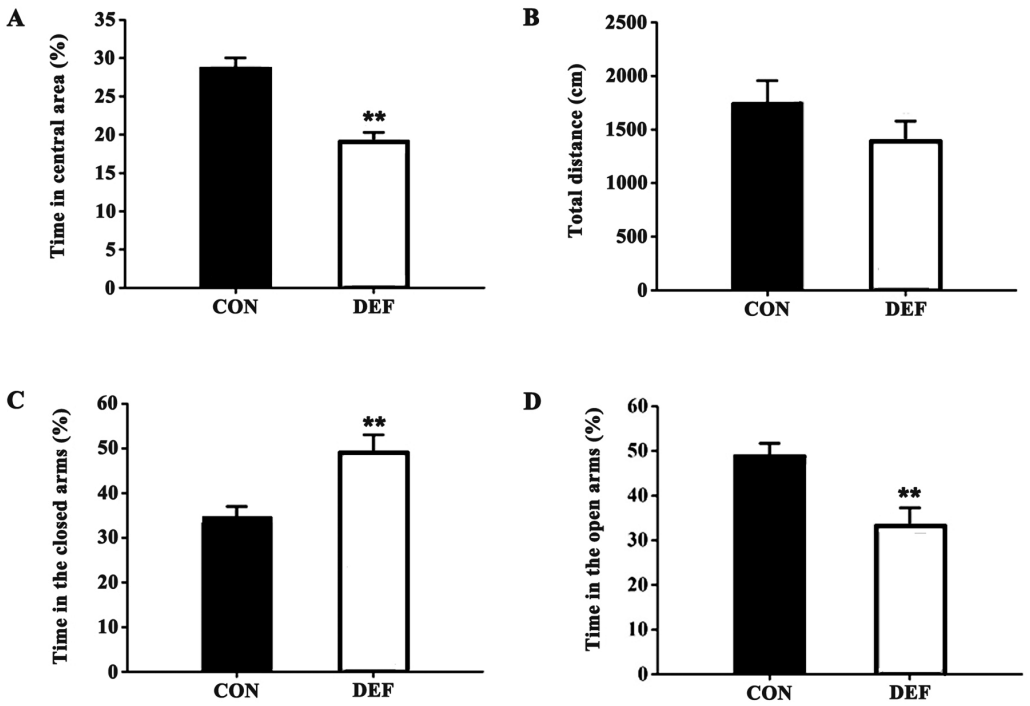

Figure 2. Chronic social defeat stress (CSDS) induced anxiety-like behaviors in adult female voles. (A) The time control and defeated voles spent in the central area in the open field test (OFT). (B) The total distance travelled by control and defeated voles in the OFT. (C) The time control and defeated voles spent in the closed arms in the elevated plus maze test (EPM). (D) The time of control and defeated voles spent in the open arms in the EPM. CON, control group; DEF, defeated group. Data are presented as the means ± SEM. *$P \leq .05$ and **$P \leq .01$, CON vs DEF.

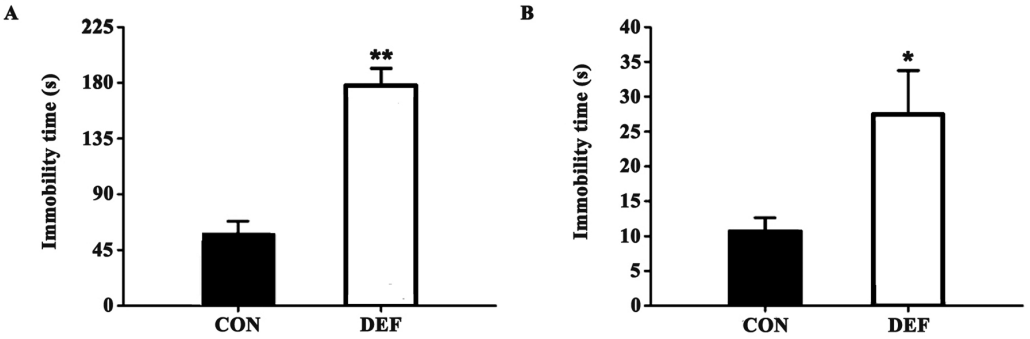

Figure 3. Chronic social defeat stress (CSDS) induced depression-like behaviors in adult female voles. (A) The immobility time of control and defeated voles in the tail suspension test (TST). (B) The immobility time of control and defeated voles in the forced swimming test (FST). CON, control group; DEF, defeated group. Data are presented as the means ± SEM. *P ≤ .05 and **P ≤ .01, CON vs DEF.

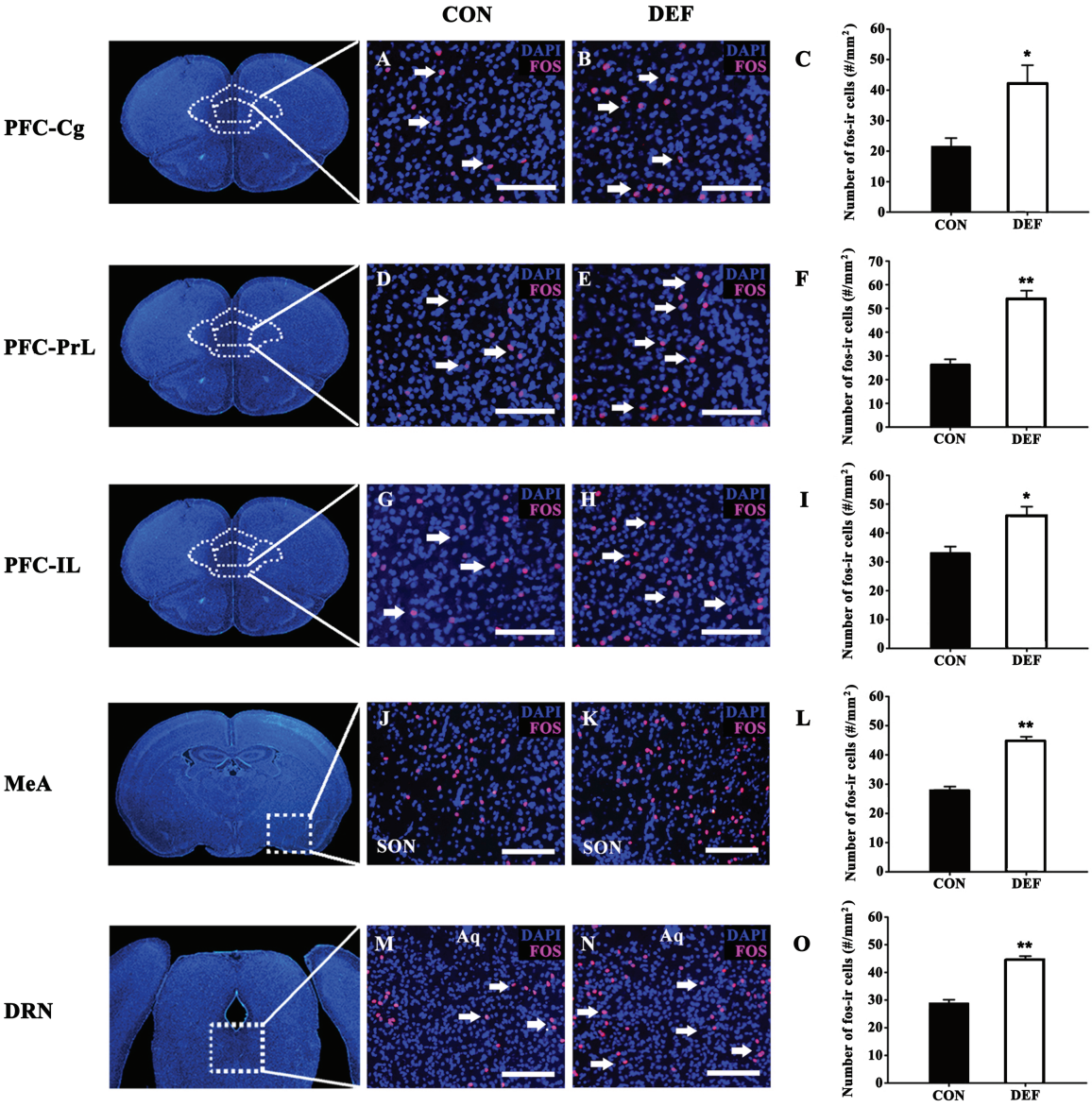

Figure 4. Effects of chronic social defeat stress (CSDS) on the number of Fos-ir cells in some brain areas. (A–C) Effects of CSDS on the number of Fos-ir cells in the medial prefrontal cortex- cingulate cortex (mPFC-Cg). (D–F) Effects of CSDS on the number of Fos-ir cells in the mPFC-prelimbic cortex (PrL). (G–I) Effects of CSDS on the number of Fos-ir cells in the mPFC- infralimbic cortex (IL). (J–L) Effects of CSDS on the number of Fos-ir cells in the medial amygdala (MeA). (M–O) Effects of CSDS on the number of Fos-ir cells in the dorsal raphe of the brainstem (DRN). Aq, aqueduct; CON, control group; DEF, defeat group; SON, supraoptic nuclei. Data are presented as the means ± SEM. *P ≤ .05 and **P ≤ .01, CON vs DEF. Bar = 200 μm.

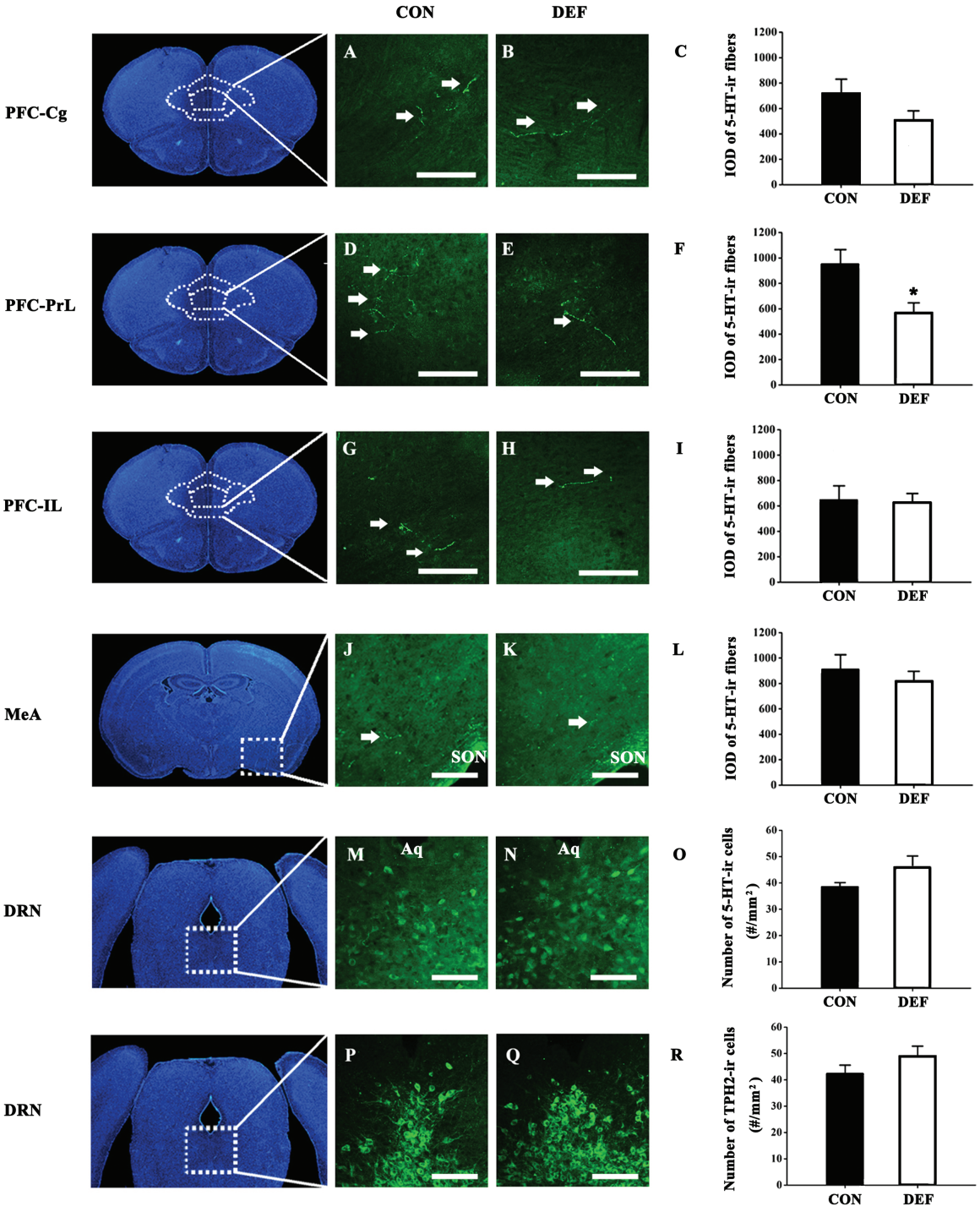

Figure 5. Effects of chronic social defeat stress (CSDS) on the density of serotonin-immunoreactive (5-HT-ir) fibers and the numbers of 5-HT-ir and TPH2-ir neurons in some brain areas. (A–C) Effects of CSDS on the density of 5-HT-ir fibers in the medial prefrontal cortex-cingulate cortex (mPFC-Cg). (D–F) Effects of CSDS on the density of 5-HT-ir fibers in the mPFC-prelimbic cortex (PrL). (G–I) Effects of CSDS on the density of 5-HT-ir fibers in the mPFC-infralimbic cortex (IL). (J–L) Effects of CSDS on the density of 5-HT-ir fibers in the medial amygdala (MeA). (M–O) Effects of CSDS on the number of 5-HT-ir neurons in the dorsal raphe of the brainstem (DRN). (P–R) Effects of CSDS on the number of TPH2-ir cells in the DRN. Aq, aqueduct; CON, control group; DEF, defeat group; SON, supraoptic nuclei. Data are presented as the means ± SEM. *$P \leq .05$, CON vs DEF. Bar = 200 μm.

CSDS Reduced the Relative 5-HT_{1A}R Density in the PrL

The schematic drawing illustrates tissue punch locations in the PFC (Cg, PrL, and IL), MeA, and DRN (Figure 6A). CSDS reduced the relative density of 5-HT_{1A}R in the PrL (t (10) = 4.025, $P < .01$). This effect on the relative density of 5-HT_{1A}R was not observed in other brain areas, including the Cg (t (10) = -0.091, $P = .929$), IL (t (10) = -0.300, $P = .771$), and MeA (t (10) = 0.221, $P = .829$), indicating that the effect was brain region specific. In addition, the relative 5-HT_{1A}R density in DRN was increased by CSDS compared with control conditions (t (10) = -2.496, $P < .05$) (Figure 6B).

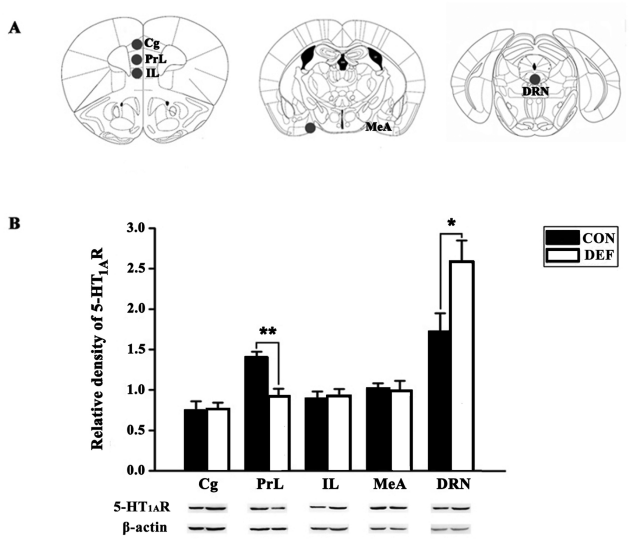

Figure 6. Effects of chronic social defeat stress (CSDS) on serotonin 1A receptors (5-HT_{1A}R) levels in the brain. (A) Schematic drawing illustrates tissue punch locations in the medial prefrontal cortex-cingulate cortex (mPFC-Cg), mPFC-prelimbic cortex (PrL), mPFC-infrlimbic cortex (IL), medial amygdala (MeA), and dorsal raphe of the brainstem (DRN). (B) Levels of 5-HT_{1A}R in the Cg, PrL, IL, MeA, and DRN of defeated and control female voles. CON, control group; DEF, defeated group. Data are presented as the means ± SEM. $^{*}P \leq .05$ and $^{**}P \leq .01$, CON vs DEF for 5-HT_{1A}R relative density.

An 8-OH-DPAT Infusion into the PrL Reduced the Anxiety-Like Behaviors in Adult Defeated Female Mandarin Voles

The 26-gauge stainless-steel guide cannulae were implanted in subjects aimed at the PrL (Figure 7A). A schematic drawing illustrating the location of microinjections in all subjects is presented in Figure 7B.

In the OFT, the 0.3-μg 8-OH-DPAT group spent more time in the central area than the other groups. In addition, 0.4-μg WAY-100635 treatments significantly reduced the time spent in the central area ($F_{(5, 42)} = 8.146$, $P < .01$) (Figure 7C). The total distance travelled in the OFT did not differ among the 6 groups of adult female voles ($F_{(5, 42)} = 1.686$, $P = .162$) (Figure 7D).

In the EPM, the 0.3-μg 8-OH-DPAT group spent less time in the closed arms, and treatment with 0.4-μg WAY-100635 significantly increased the time spent in the closed arms ($F_{(5, 42)} = 8.786$, $P < .01$) (Figure 7E). In addition, the 0.3-μg 8-OH-DPAT group spent more time in the open arms than the other groups, and the treatment with 0.4-μg WAY-100635 significantly reduced the time spent in the open arms ($F_{(5, 42)} = 11.748$, $P < .01$) (Figure 7F).

An 8-OH-DPAT Infusion into the PrL Reduced the Depressive-Like Behaviors in Adult Defeated Female Mandarin Voles

In the TST, the 0.3-μg 8-OH-DPAT group exhibited a significant increases in the immobility time compared with the other 3 groups of defeated female voles. In addition, the 0.4-μg WAY-100635 group displayed a lower immobility time than the saline group of control female voles ($F_{(5, 42)} = 7.160$, $P < .01$) (Figure 7G).

In the FST, the drug treatments significantly altered the immobility time. The 0.3-μg 8-OH-DPAT group displayed a longer immobility time than the other 3 groups of defeated female voles. In addition, 0.4-μg of WAY-100635 significantly reduced the immobility time compared with the saline treatment in control female voles ($F_{(5, 42)} = 7.101$, $P < .01$) (Figure 7H).

Discussion

In this paper, using highly aggressive adult female mandarin voles, we demonstrated that CSDS increased levels of anxiety- and depression-like behaviors in adult female voles. Moreover, CSDS reduced 5-HT projections and 5-HT_{1A}R levels in the PrL and reduced the levels of 5-HT_{1A}R in the DRN. We also found that microinjection of 8-OH-DPAT into the PrL effectively reversed the emotional disorders induced by CSDS, and an infusion of WAY-100635 into the PrL of control female voles increased anxiety- and depression-like behaviors. Based on these results, 5-HT acts on the 5-HT_{1A}R of the mPFC-PrL is involved in the anxiety- and depression-related behaviors induced by CSDS.

Effects of CSDS on Anxiety and Depression

According to our results from the OFT and EPM, CSDS increased anxiety-like behaviors in adult female mandarin voles. These findings are consistent with many previous studies that focused on adolescent males. For example, adolescent male mice or rats exposed to repeated social defeat stress show increased anxiety-like behaviors compared with controls (Watt et al., 2009; Huang et al., 2013). Likewise, after social defeat stress, subordinate male mice consistently show an increase in anxiety-like behavior (Huang et al., 2011; Boyarskikh et al., 2013). In addition, repeated exposure to social defeat stress enhances the anxiogenic effect on adult male rats (Jaisinghani and Rosenkranz, 2015). However, only a very limited number of studies have tested the effects of chronic social defeat stress on emotional behaviors in adult female rodents, and our result is consistent with the findings of these recent studies showing that CSDS increases anxiety-like behavior (Greenberg et al., 2015; Takahashi et al., 2017; Finnell et al., 2018; Harris et al., 2018).

Another interesting discovery from the TST and FST in the present study was that CSDS increased depression-like behaviors in adult female mandarin voles. Consistent with the results from our study, CSDS also increased depression-like behavior in adolescent male rodents (Becker et al., 2008; Hayashida et al., 2010; Huang et al., 2013), adult male C57BL/6J mice (Covington et al., 2009; Yu et al., 2011; Boyarskikh et al., 2013) and adult female C57BL/6J mice (Takahashi et al., 2017). However, a single exposure to defeat stress or 5 days of social defeat stress in adult male mice or rats did not affect sucrose preference (Von Frijtag et al., 2002; Croft et al., 2005; Razzoli et al., 2011). In male mice, social defeat stress did not produce significant effects on the immobility time during the TST (Kinsey et al., 2007; Krishnan et al., 2007). This inconsistency may be due to the different lengths of time of exposure to defeat stress, indicating that acute or short social defeat stress was too brief to induce depression-like behaviors (Lehmann et al., 2016) or that female mandarin voles with high levels of sociability are particularly sensitive to the CSDS.

Overall, our findings strikingly resemble those observed in CSDS-exposed male rodents (Krishnan et al, 2007) and some female rodents (Takahashi et al., 2017; Harris et al., 2018) and provide the foundation for comparing the underpinnings of emotional disorders in males and females. Future studies designed to compare the effects of CSDS on individuals of different sexes and ages would be interesting.

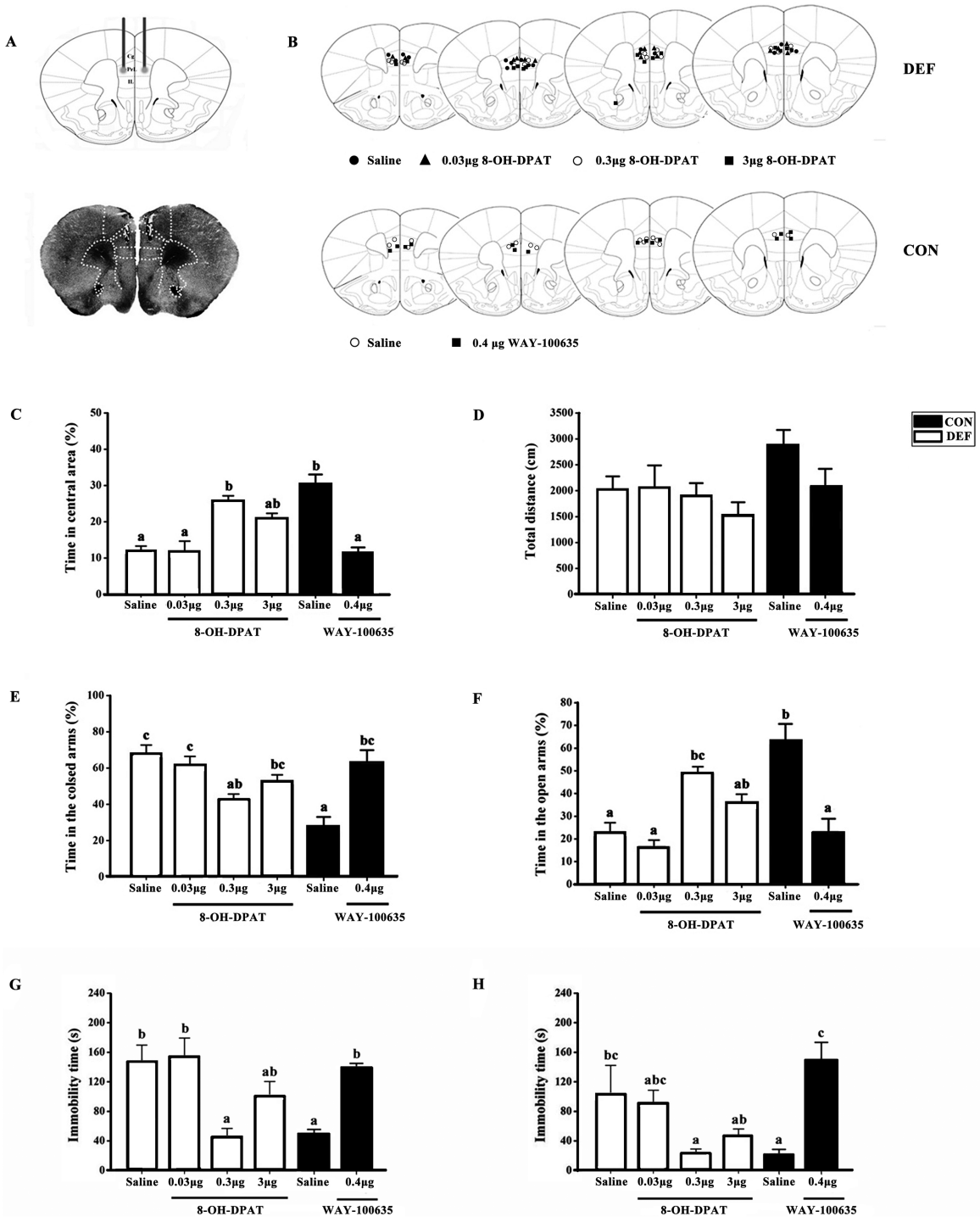

Figure 7. (A) The 26-gauge stainless steel guide cannulae were implanted in subjects aimed at the medial prefrontal cortex (mPFC)-prelimbic cortex (PrL). (B) Schematic drawing illustrating the location of microinjections for all subjects. (C and D) Effects of microinjection of saline, 8-OH-DPAT, or WAY-100635 in the PrL on the time in the central area or total distance travelled in the open field test (OFT). (E and F) Effects of microinjections of saline, 8-OH-DPAT, or WAY-100635 into the PrL on the time spent in the closed or open arms of the elevated plus maze test (EPM). (G and H) Effects of microinjections of saline, 8-OH-DPAT, or WAY-100635 into the PrL on immobility time in the tail suspension test (TST) and forced swimming test (FST). Groups not sharing the same letters are significantly different from each other ($P \leq .05$). Data are presented as the means ± SEM.

Social Defeat Stress and Serotonin System

In the present study, the defeated female voles showed significantly decreased 5-HT projections and 5-HT_{1A}R levels in the mPFC-PrL.

A previous study using the FST and foot shock test reported increases in the 5-HT content in DRN projection regions (Yoshioka et al., 1995). Exposure to stressors such as chronic restraint stress upregulated levels of the 5-HT1A mRNA in the PFC of male rats (Iyo et al., 2009). Furthermore, no differences

in the expression of the 5-HT1A mRNA are observed in the DRN and hippocampus of rats after maternal separation (Neumaier et al., 2002). Our current results using the CSDS paradigm contradict these previous studies. This finding is not entirely surprising because different stressors may induce different responses in different brain regions.

More recently, 4 days of social defeat stress were consistently shown to increase 5-HT levels in the hippocampus (Ahnaou and Drinkenburg, 2016). The inconsistency of 5-HT levels observed following social defeat stress may be due to observed different duration of social defeat stress. A short period of defeat stress promoted an exaggerated synthesis and release of 5-HT, and 4 days of threat stress may not be sufficient to deplete the 5-HT content. However, a greater number of 5-HT-ir fibers were observed within the hypothalamus and lateral septum of defeated male golden hamsters after exposure to 14 days of social defeat stress during development (Delville et al., 1998). Nevertheless, 4 weeks of CSDS reduced levels of 5-HT_{1A}R in the PFC of adult male Wistar rats (Kieran et al., 2010). Therefore, the discrepancy in the changes in the 5-HT level may also be due to the examination of different brain areas or rodents of different sexes and ages.

Our study focused on the mPFC, a region that has been implicated in psychological disorders and the modulation of emotional responses to stress (Kieran et al., 2010; Morrison et al., 2013), and our results seem to parallel the decreased 5-HT and 5-HT_{1A}R levels observed in the mPFC-PrL in brains of defeated adult female voles. Importantly, the discrepancy may be due to the analysis of different brain regions following exposure to the stressor for different duration or at different intensities. In addition, the inconsistency may be due to the different sexes and ages studied.

Serotonin System and Anxiety and Depression

In the present study, the 5-HT projections were decreased in the mPFC-PrL of voles that exhibited high levels of anxiety- and depression-like behaviors induced by CSDS. The mPFC is implicated in mediating anxiety and depression (Krishnan and Nestler, 2008; Morrison et al., 2013). The present results are consistent with recent reports showing that ketamine, an effective antidepressant, increases 5-HT release in the mPFC during treatment for depression (Nishitani et al., 2014; Pham et al., 2017). Furthermore, 5-HT levels are reduced in the PFC of patients with major depression (Lowry et al., 2008; Michelsen et al., 2008). Thus, the increased levels of anxiety- and depression-behavior induced by CSDS may be associated with fewer 5-HT projections to the mPFC.

CSDS significantly reduced 5-HT_{1A}R levels in the PFC-PrL of female adults that displayed higher levels of anxiety- and depression-related behaviors in the present study. This result is consistent with a previous report that levels of 5-HT_{1A}R protein were decreased in the PFC of patients with depression (Szewczyk et al., 2009). Stimulation of postsynaptic 5-HT_{1A}R in the mPFC and limbic system is involved in the response to antidepressants (Haddjeri et al., 1998), and suppressing the activity of 5-HT1A heteroreceptors in the mPFC has been reported to results in a depression-like phenotype (Garcia-Garcia et al., 2017). 5-HT1A knockout mice exhibit an anxiety-like phenotype in behavioral tests, indicating that a 5-HT_{1A}R deficit can elicit anxious behavior (Ramboz et al., 1998). 5-HT_{1A}R agonists seem to possess anxiolytic (Ramboz et al., 1998; Lacivita et al., 2008) and antidepressant effects (Robinson et al., 1990). Partial 5-HT_{1A}R agonists, such as buspirone and vilazodone, exert modest anxiolytic and antidepressant effects on animals (Detke et al., 1995; Bartoszyk et al., 1997). In addition, sustained antidepressant effects are mimicked by an intra-mPFC injection of 8-OH-DPAT, and the sustained antidepressant effects are attenuated by intra-mPFC injections of WAY-100635 (Fukumoto et al., 2018). The injection of 8-OH-DPAT into the DRN results in an anxiolytic action (Andrews et al., 1994; De Almeida et al., 1998). Furthermore, several preclinical studies have provided evidence that 5-HT1A autoreceptors impact anxiety-like behaviors, with 5-HT1A heteroreceptors being particularly important in the antidepressant response (De Vry, 1995; Garcia-Garcia et al., 2014). Nevertheless, whether 5-HT1A heteroreceptors in the mPFC also exert an anxiolytic effect on females remains unclear. Consistent with the previous reports described above, microinjection of the 5-HT_{1A}R agonist 8-OH-DPAT into the mPFC-PrL effectively reversed the emotional disorders induced by CSDS, and an infusion of the 5-HT_{1A}R antagonist WAY-100635 into the PrL of control adult female voles increased anxiety- and depression-like behaviors. Thus, we postulate that 5-HT acting via 5-HT1A heteroreceptors in the mPFC is involved in the CSDS-induced increase in anxiety- and depression-like behaviors in adult females.

Based on the findings from the present study, CSDS increased anxiety- and depression-like behaviors of adult female mandarin voles, and reduced 5-HT and 5-HT_{1A}R levels in the mPFC-PrL were possibly involved in these effects. This female social defeat model will allow us to expand previously published reports in males to an examination of anxiety- and depression-related biological pathways in females and provide a new target to develop an effective approach for treating violence-induced emotional disorders in women.

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Statement of Interest

We confirm that this material has not yet been published or submitted for publication elsewhere. We declare that we have no financial relationship with other people or organizations that can inappropriately influence our work.

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