

REGULAR RESEARCH ARTICLE

Dysfunction of Serotonergic and Dopaminergic Neuronal Systems in the Antidepressant-Resistant Impairment of Social Behaviors Induced by Social Defeat Stress Exposure as Juveniles

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Abstract

Background: Extensive studies have been performed on the role of monoaminergic neuronal systems in rodents exposed to social defeat stress as adults. In the present study, we investigated the role of monoaminergic neuronal systems in the impairment of social behaviors induced by social defeat stress exposure as juveniles.

Methods: Juvenile, male C57BL/6J mice were exposed to social defeat stress for 10 consecutive days. From 1 day after the last stress exposure, desipramine, sertraline, and aripiprazole were administered for 15 days. Social behaviors were assessed at 1 and 15 days after the last stress exposure. Monoamine turnover was determined in specific regions of the brain in the mice exposed to the stress.

Results: Stress exposure as juveniles induced the impairment of social behaviors in adolescent mice. In mice that showed impairment of social behaviors, turnover of serotonin and dopamine, but not noradrenaline, was decreased in specific brain regions. Acute and repeated administration of desipramine, sertraline, and aripiprazole failed to attenuate the impairment of social behaviors, whereas repeated administration of a combination of sertraline and aripiprazole showed additive attenuating effects.

Conclusions: These findings suggest that social defeat stress exposure as juveniles induces the treatment-resistant impairment of social behaviors in adolescents through dysfunction in the serotonergic and dopaminergic neuronal systems. The combination of sertraline and aripiprazole may be used as a new treatment strategy for treatment-resistant stress-related psychiatric disorders in adolescents with adverse juvenile experiences.

Keywords: social defeat stress, juvenile, adolescent, social behaviors, monoaminergic neuronal system

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Significance Statement

Monoaminergic neuronal systems have attracted the attention of researchers with regard to the impairment of social behaviors induced by social defeat stress exposure as adults. However, the importance of monoaminergic neuronal systems in the impairment of social behaviors induced by exposure to social defeat stress as juveniles remains poorly understood; thus, in the present study, we investigated the role of monoaminergic neuronal systems in the impairment of social behaviors induced by social defeat stress exposure as juveniles. Exposure to social defeat stress as juveniles leads to the treatment-resistant impairment of social behaviors in adolescents, because of dysfunction of the serotonergic and dopaminergic neuronal systems. Repeated administration of a combination of sertraline and aripiprazole showed additive effects in attenuating this impairment of social behaviors. This may be useful as a new treatment strategy for treatment-resistant stress-related psychiatric disorders in adolescents with adverse juvenile experiences.

Introduction

Adverse juvenile experiences, including physical or sexual violence and neglect, often induce adverse mental health outcomes later in life (Afifi, 2011; Annerback et al., 2012; McKenzie and Scott, 2012). Epidemiological studies have demonstrated that adverse juvenile experiences increase the risk for stress-related psychiatric disorders, particularly major depressive disorder (MDD), anxiety disorder, and posttraumatic stress disorder (Weber et al., 2008; Weich et al., 2009; McLaughlin et al., 2010). These psychiatric disorders induced by adverse juvenile experiences frequently involve marked dysfunction in social activity during adolescence and adulthood (Sandi and Haller, 2015).

The monoaminergic neuronal system has attracted increasing attention of researchers in the field of stress-related psychiatric disorders (Flugge, 2000; Pittenger and Duman, 2008; Krystal and Neumeister, 2009). Polymorphism of the serotonin (5-HT) transporter (5-HTTLPR genotype) has been reported to be a susceptibility factor for posttraumatic stress disorder in the interaction between adult traumatic events and childhood adversity (Steckler and Risbrough, 2012). Further, monoaminergic neuronal dysregulation may contribute vulnerability to stress as a factor in the development of anxiety disorders, because 5-HT reuptake inhibitors and dual 5-HT/noradrenaline (NA) reuptake inhibitors are effective in treating anxiety disorders (Morilak and Frazer, 2004). Low CSF levels of 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), and homovanilic acid (HVA) have been hypothesized to be involved in MDD, although the published literature is contradictory (Placidi et al., 2001). Extensive investigation on the role of monoaminergic neuronal systems has also been performed in rodents exposed to social defeat stress as adults (Krishnan et al., 2007; Cao et al., 2010; Chen et al., 2012; Boyarskikh et al., 2013).

Mice exposed to social defeat stress as adults display the impairment of social behaviors (Berton et al., 2006; Tsankova et al., 2006). Previous studies have shown that tryptophan hydroxylase 2 knockin mice, which show 60% to 80% reduction in brain 5-HT, have increased susceptibility to social defeat stress (Nakayama et al., 2003). The impairment of social behaviors induced by social defeat stress exposure is dependent on the mesolimbic dopamine (DA) circuit (Tanaka et al., 2012). In addition, NA transporter-knockout mice resist the impairment of social behaviors induced by social defeat stress exposure (Haenisch et al., 2009). These findings suggest that brain monoaminergic neuronal systems are involved in the impairment of social behaviors induced by social defeat stress exposure. We previously found that juvenile mice were more vulnerable to the impairment of social behaviors induced by social defeat stress exposure than adult mice (Mouri et al., 2018). However,

the role of monoaminergic neuronal systems in the impairment of social behaviors induced by social defeat stress exposure as juveniles remains unclear.

The present study was designed to investigate the role of monoaminergic neuronal systems in the impairment of social behaviors induced by social defeat stress exposure as juveniles. We determined the functional and neurochemical changes in the monoaminergic neuronal systems of mice exposed to social defeat stress as juveniles using biochemical techniques. We also investigated the effect of antidepressants and aripiprazole on the impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles.

Materials and Methods

Animals

Male C57BL/6J and ICR mice were obtained from Japan SLC, Inc. Juvenile, male C57BL/6J mice (3 weeks old) were used to exposure to social defeat stress. Aggressive, male ICR mice (over 7 weeks old) for social defeat stress were screened based on the duration of attacks on C57BL/6J mice, with more than 1/10 minutes as the inclusion criterion. Unfamiliar target male ICR mice (over 4–6 weeks old) were used for the social interaction test. They were housed in plastic cages in a regulated environment (23°C ± 1°C, 50 ± 5% humidity), with a 12-hour-light/-dark cycle (lights on at 9:00 AM). Food (CE2; Clea Japan Inc.) and tap water were available ad libitum. All experiments were conducted in accordance with the Guidelines for Animal Experiments of the Nagoya University Graduate School of Medicine. Procedures involving animals and their care were conformed to the international guidelines set out in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Drug Administration

Desipramine hydrochloride, sertraline hydrochloride, and aripiprazole were purchased from Sigma-Aldrich, Tokyo Chemical Industry Co, Ltd, and Wako Pure Chemical Industries, Ltd, respectively. Desipramine and sertraline were dissolved in distilled water. Aripiprazole was dissolved in 100% acetic acid and diluted with distilled water.

From 1 day after the last social defeat stress exposure, i.p. administration of 10 and 20 mg/kg desipramine, 5 and 10 mg/kg sertraline, or 0.003 and 0.01 mg/kg aripiprazole was commenced; this treatment was performed once a day for 15 days. The administration volume was 10 mL/kg per mouse.

Social Defeat Stress

Social defeat stress exposure was carried out according to the method outlined in previous reports (Berton et al., 2006; Krishnan et al., 2007) with minor modifications. Prior to social defeat stress, an aggressive ICR mouse was habituated to social defeat stress cages for 10 minutes. C57BL/6J mice (3 weeks old) were exposed to a different aggressive ICR mouse for 10 minutes each day for 10 consecutive days. The pairing of defeated and aggressive mice was randomized daily to minimize the effects of variability in aggression that the mice were exposed to.

Defeat was defined as the display of defensive behaviors by C57BL/6J mice, such as escape and submissive postures during physical attacks by an aggressive mouse. Submissive posture was defined as standing upright with the belly exposed to the aggressor. The duration of defensive behaviors was recorded according to our previous report (Mouri et al., 2018). After 10 minutes, an aggressive mouse was separated from the defeated mouse to avoid habituation to the defeated mouse and placed in its group-housed home cage for the next 24 hours.

We previously found that mice exposed to a nonaggressive ICR mouse (undefeated mice) as well as an empty cage (control mice) for 10 consecutive days showed significantly increased duration at the interaction zone in the second session compared with that in the first session (Mouri et al., 2018). Thus, it is unlikely that defeated mice were habituated to expose ICR mouse during social defeat stress and did not approach familiar target ICR mouse.

Control mice were exposed to an empty cage as described in our previous report (Mouri et al., 2018).

Social Interaction Test

The adolescent mice (4 or 6 weeks old) were subjected to the social interaction test at 1 and 15 days after the last stress exposure. The social interaction test was performed from 10:00 AM to 5:00 PM in a sound-attenuated room, as described in our previous report (Mouri et al., 2018). The apparatus consisted of an open gray nonreflecting acrylic box (W42 × D42 × H30 cm) and the transparent Plexiglas enclosure (W10 × D6.5 × H30 cm) with 30 holes (10 mm in diameter). A lightbulb (54 W), not directly seen by the mouse, was attached to the upper part of the apparatus and provided constant illumination of approximately 20 lux.

The social interaction test consisted of 2 sessions: in the first session, the mouse was allowed to freely explore and habituate to the test environment for 30 minutes in the absence of an unfamiliar target ICR mouse, in consistent with a previous report (Mouri et al., 2018). This was done to reduce the time spent in the interaction zone by exploring the apparatus itself during the second session. The second session commenced 1 minute after the first session, and the mouse was returned to the apparatus for 5 minutes in the presence of an unfamiliar target ICR mouse. During the test, the time spent in the interaction zone (6 cm width) was recorded for the last 5 minutes of the first (target absent) and for the 5 minutes of the second (target present) sessions, using a video-tracking system (EthoVision XT; Noldus Information Technology).

Preparation of Brain Samples

Each mouse was killed immediately before or soon after the social interaction test. Their brains were rapidly removed and the prefrontal cortex (PFC), nucleus accumbens (NAc), hippocampus (HIP), and amygdala (AMG) were dissected out on an

ice-cold plate according to the atlas (Paxinos and Franklin, 2004). Each tissue sample was quickly frozen on dry ice, weighed, and stored in a deep freezer at -80°C until assay.

Determination of Monoamines and Their Metabolites

The concentrations of monoamines and their metabolites were determined using a high-performance liquid chromatography system with an electrochemical detector (Eicom) as described in previous reports (Noda et al., 1997, 1998). Briefly, each frozen tissue sample was homogenized with an ultrasonic processor (475 W, Model XL2020, Heat Systems Inc.) in 350 μ L of 0.2 M perchloric acid containing isoproterenol (internal standard). The homogenate was placed in ice for 30 minutes and then centrifuged at 20000 × g for 15 minutes at 0°C. The supernatant was mixed with 1 M sodium acetate to adjust the pH to 3.0 and then injected into a liquid chromatography system equipped with a reversed-phase ODS-column (3 × 150 mm, diameter of stationary phase grains 5 μ m: Eicompak SC-50DS, Eicom) and an electrochemical detector (model ECD-700, Eicom). The column temperature was maintained at 25°C, and the detector potential was set at +750 mV. The mobile phase consists of 0.1 M citric acid and 0.1 M sodium acetate, pH 3.5, containing 15% methanol, 220 mg/L sodium-1-octanesulfonate, and 5 mg/L ethylenediaminetetraacetic acid; the flow rate was 0.5 mL/min. Data were collected and analyzed with the PowerChrom v2.6.4 software (eDAQ).

Statistical Analysis

All results are expressed as the mean ± SEM for each group. Statistical significance was determined using 1-way or 2-way ANOVA followed by Bonferroni's test. The Student's *t* test was used to compare 2 sets of data. *P* < .05 was taken to indicate significance. Data were analyzed with SPSS 24 software (IBM).

Results

The Impairment of Social Behaviors in Adolescent Mice Exposed to Social Defeat Stress as Juveniles

Adolescent, male C57BL/6J mice were exposed to social defeat stress as juveniles for 10 consecutive days. When juvenile mice body weight was measured immediately after the stress exposure of 10 consecutive days, the stress exposure did not affect body weight gain (data not shown), in consistent with a previous report (Mouri et al., 2018). One day after the last stress exposure, mice were subjected to the social interaction test. In the first session without an unfamiliar ICR mouse, there was no significant difference between groups in the time spent in the interaction zone and engaging in exploratory activity within the apparatus (Figure 1A). In the second session, control mice approached the unfamiliar target ICR mouse and showed significantly increased time spent in the interaction zone than in the first session (Figure 1A). However, in the second session, the mice exposed to the stress as juveniles for 10 consecutive days spent significantly less time in the interaction zone than the control group, although there were no difference in exploratory activity between control and defeated groups (Figure 1A).

When tested 15 days after the last stress exposure, mice exposed to the stress as juveniles still spent less time in the interaction zone in the second session, although there was no difference in exploratory activity between control and defeated

groups (Figure 1B). There was also no significant difference between groups in the time spent in the interaction zone and engaging in exploratory activity within the apparatus in the first session (Figure 1B). These results indicate that social defeat stress exposure as juveniles induces the persistent impairment of social behaviors in adolescents.

Changes in Monoamine Metabolisms in Brain Regions of Adolescent Mice Exposed to Social Defeat Stress as Juveniles

To examine the influence of social defeat stress exposure as juveniles on monoaminergic neuronal systems, we measured the concentrations of monoamines and their metabolites in the PFC, NAc, HIP, and AMG immediately before (nontested group) or after (tested group) the social interaction test (Table 1). The ratios of 5-HIAA/5-HT, [3, 4-dihydroxyphenyl acetic acid (DOPAC) + HVA]/DA, and MHPG/NA, which are used as indices of the 5-HT, DA, and NA turnover rates, were calculated in the PFC, NAc, HIP, and AMG of the nontested and tested groups (Figure 2).

A significant increase in the 5-HIAA/5-HT ratio (5-HT metabolism) was observed in the PFC and AMG of the tested control group compared with that of the nontested control group (Figure 2A). Such significant increase in the 5-HIAA/5-HT ratio was not observed in the PFC and AMG of the tested defeated

group (Figure 2A). In the PFC, NAc, and AMG, there was a significant decrease in the 5-HIAA/5-HT ratio in the tested defeated group compared with that in tested control group (Figure 2A). There were no significant changes in the 5-HIAA/5-HT ratio in the HIP when all groups were compared (Figure 2A). No changes in the (DOPAC + HVA)/DA ratio (DA metabolism) were observed in the tested control group (Figure 2B), whereas the ratio in the NAc, HIP, and AMG was significantly decreased in the both nontested and tested defeated groups compared with that in the corresponding control groups, respectively (Figure 2B). There were no significant changes in the MHPG/NA ratio (NA metabolism) in the all brain regions when all groups were compared (Figure 2C).

Significant changes in the concentrations of monoamines and their metabolites were observed as follows: the 5-HIAA concentration in the PFC, NAc, and AMG was significantly increased in the tested control group compared with that in the nontested control group. The increase in 5-HIAA concentration in the PFC, NAc, and AMG was significantly decreased in the tested defeated group compared with the tested control group. The 5-HT concentration in the PFC was significantly increased in the tested defeated group compared with that in the tested control group. The HVA concentration in the NAc was significantly increased in the tested control group compared with that in the nontested control group. The DA and DOPAC concentrations in the HIP and NAc were significantly increased and

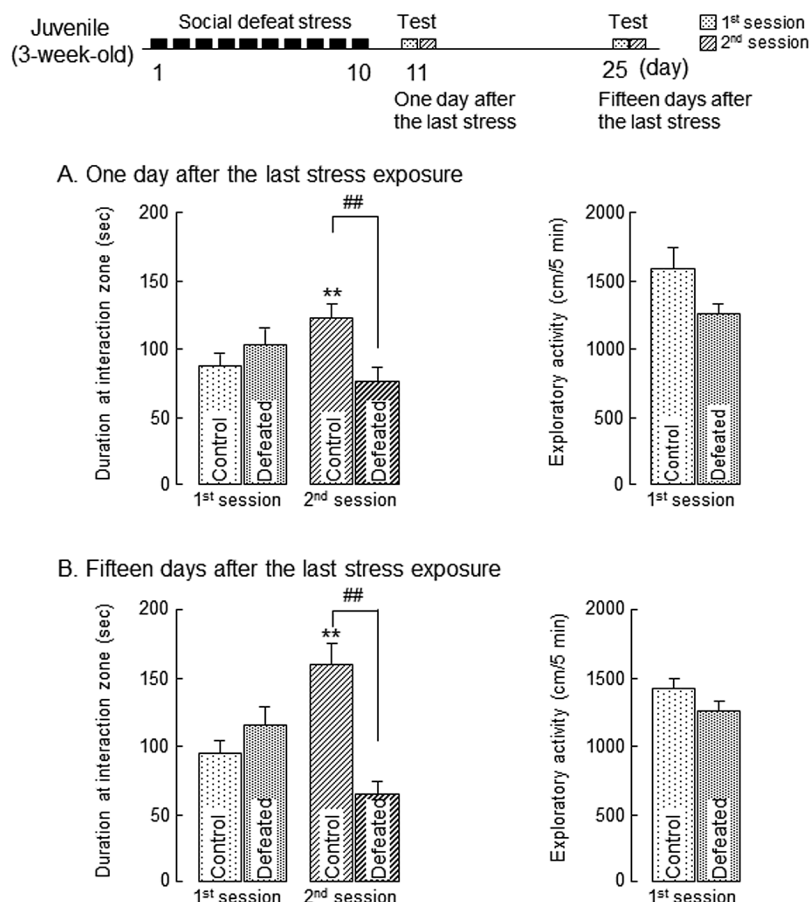


Figure 1. The impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. (A) Duration at interaction zone (left) and exploratory activity (right) 1 day after the last stress exposure. (B) Duration at interaction zone (left) and exploratory activity (right) 15 days after the last stress exposure. Each column represents the mean \pm SEM ($n = 12-15$). Two-way ANOVA: duration at interaction zone: (A) $F_{\text{defeat}}(1, 25) = 2.10, P = .16$; $F_{\text{session}}(1, 25) = 1.08, P = .31$; $F_{\text{defeat} \times \text{session}}(1, 25) = 13.7, P < .01$. (B) $F_{\text{defeat}}(1, 25) = 7.16, P = .013$; $F_{\text{session}}(1, 25) = 0.57, P = .46$; $F_{\text{defeat} \times \text{session}}(1, 25) = 22.3, P < .01$. ** $P < .01$ vs corresponding control in the first session (Student's *t* test). ## $P < .01$ vs control group (Student's *t* test). Control: mice exposed to an empty cage, defeated: mice exposed to an aggressive ICR mouse.

Table 1. Changes in Concentration of Monoamines and Their Metabolites in the Discrete Brain Regions of Adolescent Mice Exposed to Social Defeat Stress and Subjected to Social Interaction Test 1 Day after the Last Social Defeat Stress Exposure

Region	Group	5-HT	5-HIAA	Dopamine	DOPAC	HVA	Noradrenaline	MHPG
Prefrontal cortex (PFC)	Nontested							
	Control	202.1±7.2	208.6±5.9	96.9±27.9	87.4±14.8	73.5±6.6	246.6±2.6	50.0±1.6
	Defeated	193.1±19.1	229.8±9.2	114.7±27.1	76.4±11.0	85.0±6.8	275.6±8.2*	53.5±5.7
	Tested							
Nucleus accumbens (NAc)	Nontested							
	Control	599.3±51.4	348.2±11.3	5839.0±445.8	1279.5±97.9	531.2±40.8	174.6±25.9	71.0±2.4
	Defeated	549.7±55.2	336.5±18.4	6711.7±664.9	905.4±77.07	476.7±62.2	245.2±38.4	81.7±6.2
	Tested							
Hippocampus (HIP)	Nontested							
	Control	286.4±4.5	400.0±12.2	11.2±1.6	11.6±0.8	30.1±1.4	278.0±6.5	69.1±1.6
	Defeated	305.7±10.4	413.4±20.0	15.5±0.8	13.6±1.6	28.3±1.1	334.7±6.4*	69.7±6.3
	Tested							
Amygdala (AMG)	Nontested							
	Control	426.9±8.1	494.5±16.4	265.6±22.9	154.8±17.9	82.4±7.8	254.7±4.4	72.3±1.7
	Defeated	423.2±14.6	451.0±20.8	311.8±42.9	132.7±16.5	78.1±9.4	293.4±8.2	76.6±5.3
	Tested							
Nucleus accumbens (NAc)	Nontested							
	Control	794.2±147.2	550.7±70.0**	6845.1±708.8	1456.0±162.9	795.2±91.6*	305.0±54.2	108.1±14.7*
	Defeated	846.9±48.5	373.5±21.2#	7368.4±355.2	764.2±54.2##	647.7±28.8	342.5±47.1	114.4±8.5
	Tested							
Hippocampus (HIP)	Nontested							
	Control	271.9±26.2	455.7±36.0	14.5±1.6	15.1±2.6	37.4±3.5	263.1±17.0	73.0±5.1
	Defeated	303.4±15.4	411.1±19.4	21.3±2.2#	14.6±1.8	34.3±1.5	328.8±16.2##	80.8±1.6
	Tested							
Amygdala (AMG)	Nontested							
	Control	427.5±12.7	636.7±29.4**	279.1±18.5	153.9±12.3	96.9±5.3	270.1±6.4	88.5±2.2
	Defeated	447.4±27.1	460.3±31.4##	457.6±76.8	155.3±27.8	107.4±14.4	306.4±17.8	92.9±7.6
	Tested							

Values are expressed as ng/g wet weight and are the means ± SEM (n=8–9). The amounts of 5-HT, dopamine, noradrenaline, and their metabolites (5-HIAA, DOPAC, HVA, and MHPG) in the discrete brain regions were determined. Two-way ANOVA; 5-HT: $F_{\text{brain region}}(3, 120)=95.8, P<.01$; $F_{\text{group}}(3, 120)=4.18, P<.01$; $F_{\text{brain region} \times \text{group}}(9, 120)=2.63, P<.01$, 5-HIAA: $F_{\text{brain region}}(3, 120)=77.0, P<.01$; $F_{\text{group}}(3, 120)=22.8, P<.01$; $F_{\text{brain region} \times \text{group}}(9, 120)=2.09, P<.05$, dopamine: $F_{\text{brain region}}(3, 120)=535.7, P<.01$; $F_{\text{group}}(3, 120)=1.68, P=.18$; $F_{\text{brain region} \times \text{group}}(9, 120)=1.14, P=.34$, DOPAC: $F_{\text{brain region}}(3, 120)=363.8, P<.01$; $F_{\text{group}}(3, 120)=9.76, P<.01$; $F_{\text{brain region} \times \text{group}}(9, 120)=8.78, P<.01$, HVA: $F_{\text{brain region}}(3, 120)=312.4, P<.01$; $F_{\text{group}}(3, 120)=7.70, P<.01$; $F_{\text{brain region} \times \text{group}}(9, 120)=4.53, P<.01$, noradrenaline: $F_{\text{brain region}}(3, 120)=0.19, P=.90$; $F_{\text{group}}(3, 120)=8.47, P<.01$; $F_{\text{brain region} \times \text{group}}(9, 120)=1.17, P=.32$, MHPG: $F_{\text{brain region}}(3, 120)=21.0, P<.01$; $F_{\text{group}}(3, 120)=13.6, P<.01$; $F_{\text{brain region} \times \text{group}}(9, 120)=1.21, P=.29, P<.05$, * $P<.05$ vs nontested control group, # $P<.05$, ## $P<.01$ vs tested control group (Bonferroni's test). Nontested, mice sacrificed without performing the social interaction test; tested, mice sacrificed immediately after social interaction test; control, mice exposed to an empty cage; defeated, mice exposed to an aggressive ICR mouse.

decreased, respectively, in the tested defeated group compared with those in the tested control group. The NA concentration in the PFC or HIP and the MHPG concentration in the NAc were significantly increased in the nontested defeated and the tested control groups, respectively, compared with those of the nontested control group. Lastly, the NA concentration in the HIP was significantly increased in the tested defeated group compared with that in the tested control group (Table 1).

Effects of Antidepressants and Aripiprazole on the Impairment of Social Behaviors in Mice Exposed to Social Defeat Stress as Juveniles

To investigate the role of monoaminergic neuronal systems in the impairment of social behaviors induced by social defeat stress exposure as juveniles, desipramine (10 and 20 mg/kg i.p.), a NA reuptake inhibitor; sertraline (5 and 10 mg/kg i.p.), a selective 5-HT reuptake inhibitor; and aripiprazole (0.003 and 0.01 mg/kg i.p.), a DA receptor partial agonist, were administered once a day from 1 day after social defeat stress exposure for 15 days (Figure 3A). The doses used followed previous publications; aripiprazole at 20 mg/kg (p.o.) and sertraline at 10 mg/kg (i.p.) attenuated depressive-like behaviors in a forced swim test (Noda et al., 1997; Mouri et al., 2012). Aripiprazole at 0.03 mg/kg (p.o.) attenuated phencyclidine-induced the impairment of recognition memory (Nagai et al., 2009); however, it did not affect locomotor activity at 0.01 mg/kg (i.p.) (Bourin et al., 2009).

Acute administration of desipramine, sertraline, and aripiprazole did not affect the decrease in time spent in the interaction zone in the second session to expose an unfamiliar ICR mouse when tested 1 day after the last stress exposure (Figure 3A1–3). When tested 15 days after the last stress exposure, their repeated administration also did not affect body weight gain (data not shown) and the decrease in time spent in the interaction zone in the second session (Figure 3B1–3).

In the first session of acute administration, there was no significant difference among the groups in the time spent in the interaction zone (data not shown), whereas the repeated administration of 20 mg/kg desipramine and 0.003 and 0.01 mg/kg aripiprazole led to significantly increased time spent in the interaction zone in the first session compared with vehicle in the defeated group [2-way ANOVA; desipramine: $F_{\text{defeat}}(1, 70)=3.62, P=.06$; $F_{\text{drug}}(2, 70)=1.11, P=.34$; $F_{\text{defeat} \times \text{drug}}(1, 70)=8.53, P<.01$, sertraline: $F_{\text{defeat}}(1, 60)=2.00, P=.16$; $F_{\text{drug}}(2, 60)=0.28, P=.76$; $F_{\text{defeat} \times \text{drug}}(1, 60)=7.34, P<.01$, aripiprazole: $F_{\text{defeat}}(1, 96)=0.66, P=.42$; $F_{\text{drug}}(2, 96)=2.53, P=.09$; $F_{\text{defeat} \times \text{drug}}(1, 96)=4.07, P<.05$] (data not shown).

Additive Effect of Sertraline with Aripiprazole on the Impairment of Social Behaviors in Adolescent Mice Exposed to Social Defeat Stress as Juveniles

Both clinical and preclinical studies have demonstrated adjunctive use of aripiprazole as an augmentation strategy when combined with SSRIs (Kamei et al., 2008; Bourin et al., 2009). The present results shown in Figures 3A1–3 and 3B1–3 indicate

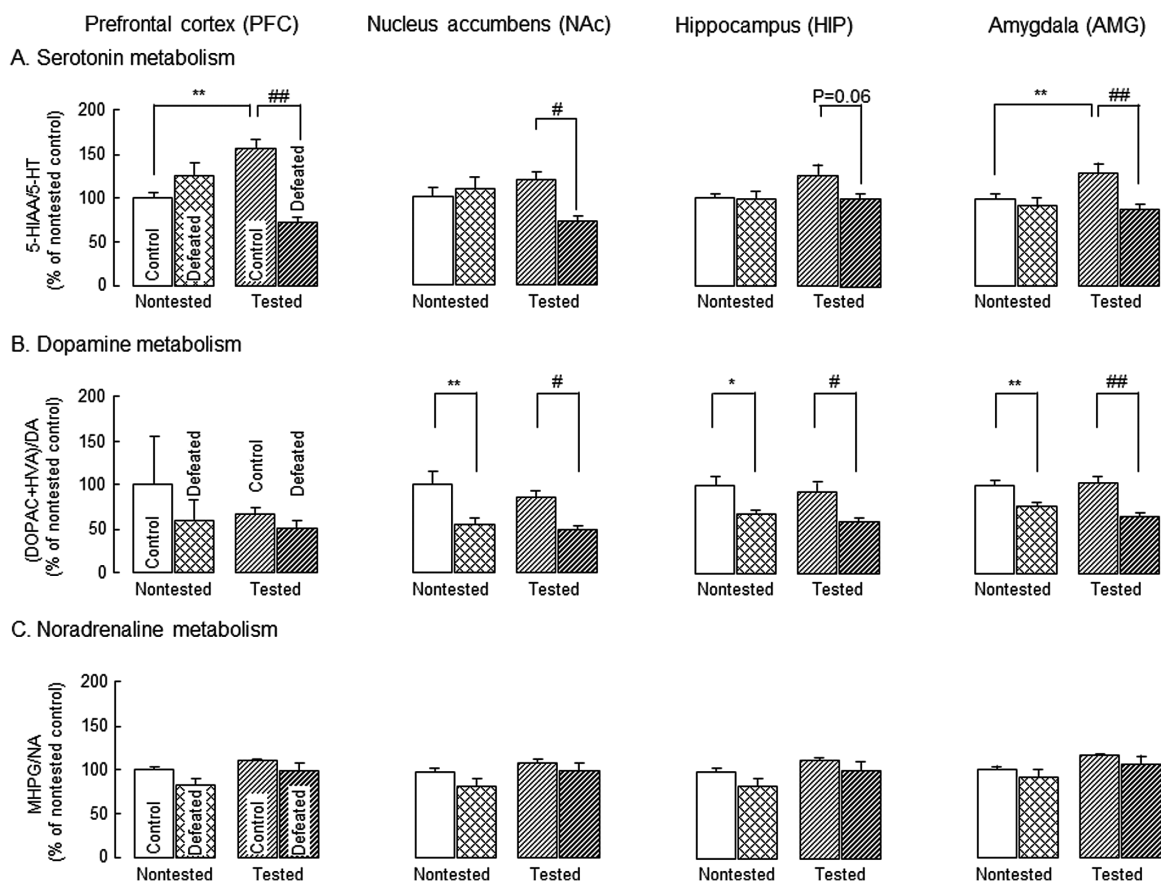


Figure 2. Changes in monoamine metabolites in discrete brain regions of adolescent mice exposed to social defeat stress as juveniles. Changes in serotonin (A), dopamine (B), and noradrenaline (C) utilizations in brain regions of adolescent mice exposed to social defeat stress as juveniles. Data are expressed as a percentage of nontested control group (set at 100%). Each column represents the mean \pm SEM ($n=8-9$). Other details are shown in Table 1. Two-way ANOVA: (A) $F_{\text{brain region}}$ (3, 120) = 2.21, $P = .09$; F_{group} (3, 120) = 19.9, $P < .01$; $F_{\text{brain region} \times \text{group}}$ (9, 120) = 1.90, $P = .60$. (B) $F_{\text{brain region}}$ (3, 120) = 1.02, $P = .39$; F_{group} (3, 120) = 7.00, $P < .01$; $F_{\text{brain region} \times \text{group}}$ (9, 120) = 0.28, $P = .98$. (C) $F_{\text{brain region}}$ (3, 120) = 6.06, $P < .01$; F_{group} (3, 120) = 2.94, $P = .36$; $F_{\text{brain region} \times \text{group}}$ (9, 120) = 1.20, $P = .30$. * $P < .05$, ** $P < .01$ vs nontested control group, # $P < .05$, ## $P < .01$ vs tested control group (Bonferroni's test). Nontested: mice sacrificed without the social interaction test, tested: mice sacrificed immediately after social interaction test, control: mice exposed to an empty cage, defeated: mice exposed to an aggressive ICR mouse.

exposure to social defeat stress as juveniles leads to the treatment-resistant impairment of social behaviors in adolescents, which is potentially caused by dysfunction of serotonergic and dopaminergic neuronal systems (Table 1; Figure 2). To investigate the effect of combined administration of sertraline and aripiprazole on the impairment of social behaviors, we used their ineffective doses, which were 10 mg/kg and 0.01 mg/kg, respectively (Figure 3A4 and B4).

In the second session, acute combined administration of sertraline with aripiprazole did not affect the impairment of social behaviors when tested 1 day after the last stress exposure, whereas repeated combined administration significantly attenuated it when tested 15 days after that (Figures 3A4 and B4). In the first session, however, there was no significant difference between groups in the time spent in the interaction zone when tested at 1 and 15 days after the last stress exposure, except for repeated combined administration in the defeated group at 15 days [2-way ANOVA; when tested 15 days: F_{Defeat} (1, 69) = 0.27, $P = .60$; F_{Drug} (1, 69) = 2.34, $P = .13$; $F_{\text{Defeat} \times \text{Drug}}$ (1, 69) = 0.16, $P = .68$, when tested 1 day: F_{Defeat} (1, 69) = 1.05, $P = .31$; F_{Drug} (1, 69) = 2.88, $P = .09$; $F_{\text{Defeat} \times \text{Drug}}$ (1, 69) = 3.86, $P = .05$] (data not shown).

Combined administration of sertraline and aripiprazole did not affect body weight gain (data not shown) and the exploratory activity (supplemental Figure S2).

Discussion

Adult rodents exposed to social defeat stress repeatedly display anxiety- and depressive-like behaviors (Kudryavtseva et al., 1991; Avustinovich et al., 2005) such as the impairment of social behaviors (Berton et al., 2006; Tsankova et al., 2006), resembling the clinical symptoms of stress-related psychiatric disorders. In the present study, mice exposed to social defeat stress as juveniles showed the persistent impairment of social behaviors in adolescents, which is consistent with our previous finding (Mouri et al., 2018). The impairment of social behaviors was not due to motor dysfunction, since the mice showed no difference in locomotor activity in the first session of social interaction test regardless of their exposure to social defeat stress. The impairment of social behaviors was also replicated even when the C57BL/6J (same strain) mouse was present 1 day after last stress exposure (Mouri et al., 2018). Thus, it is unlikely that the defeated mice did not approach the unfamiliar target ICR mouse only because of the social fear response to the aggressor. On the other hand, social defeat stress exposure as juveniles did not induce pronounced anxiety-like behaviors (Mouri et al., 2018). The immobility in the forced swimming test was enhanced between 1 day and 4 weeks after the last stress, suggesting that vulnerability against intense stress was generated by repeated exposure to juvenile stress (Mouri et al., 2018). Therefore,

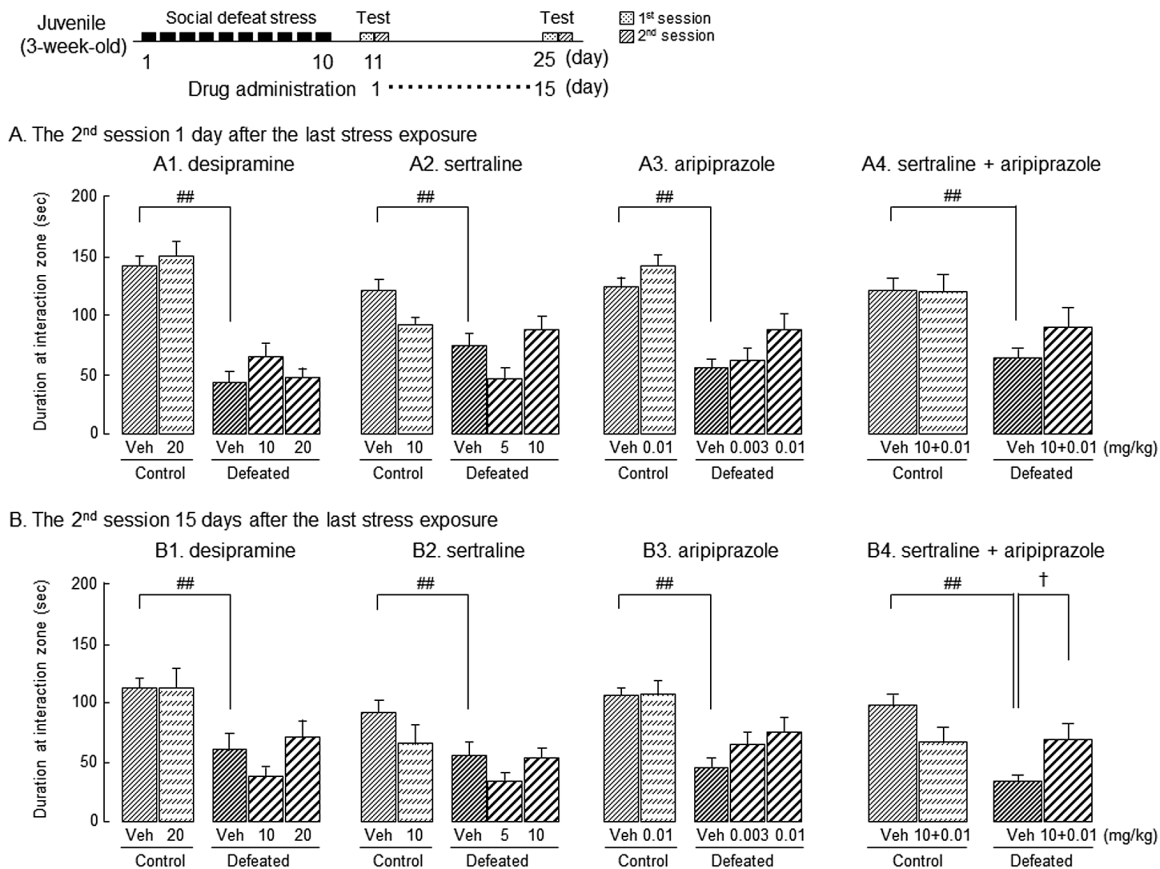


Figure 3. Effects of antidepressants and aripiprazole on the impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. From 1 day after the last stress exposure, the i.p. administration of vehicle (veh), desipramine 10 or 20 mg/kg (A1 and B1), sertraline 5 or 10 mg/kg (A2 and B2), aripiprazole 0.003 or 0.01 mg/kg (A3 and B3) or combined administration of sertraline 10 mg/kg with aripiprazole 0.01 mg/kg (A4 and B4) was started and continued once a day for 15 days. The social interaction test was performed 1 day and 15 days after the last stress exposure. Each column represents the mean \pm SEM ($n = 8-24$). Two-way ANOVA. (A1) $F_{\text{defeat}}(1, 70) = 94.7, P < .01$; $F_{\text{drug}}(2, 70) = 1.23, P = .30$; $F_{\text{defeat} \times \text{drug}}(1, 70) = 0.51, P = .82$. (A2) $F_{\text{defeat}}(1, 60) = 5.28, P < .05$; $F_{\text{drug}}(2, 60) = 3.30, P < .05$; $F_{\text{defeat} \times \text{drug}}(1, 60) = 3.77, P = .57$. (A3) $F_{\text{defeat}}(1, 96) = 33.3, P < .01$; $F_{\text{drug}}(2, 96) = 3.10, P < .05$; $F_{\text{defeat} \times \text{drug}}(1, 96) = 0.17, P = .68$. (A4) $F_{\text{defeat}}(1, 69) = 9.83, P < .01$; $F_{\text{drug}}(1, 69) = 0.95, P = .33$; $F_{\text{defeat} \times \text{drug}}(1, 69) = 0.57, P = .45$. (B1) $F_{\text{defeat}}(1, 70) = 13.1, P < .01$; $F_{\text{drug}}(2, 70) = 1.84, P = .17$; $F_{\text{defeat} \times \text{drug}}(1, 70) = 0.17, P = .68$. (B2) $F_{\text{defeat}}(1, 60) = 4.58, P < .05$; $F_{\text{drug}}(2, 60) = 1.87, P = .16$; $F_{\text{defeat} \times \text{drug}}(1, 60) = 1.48, P = .23$. (B3) $F_{\text{defeat}}(1, 96) = 18.6, P < .01$; $F_{\text{drug}}(2, 96) = 0.73, P = .49$; $F_{\text{defeat} \times \text{drug}}(1, 96) = 1.34, P = .25$. (B4) $F_{\text{defeat}}(1, 69) = 6.80, P < .05$; $F_{\text{drug}}(1, 69) = 0.14, P = .71$; $F_{\text{defeat} \times \text{drug}}(1, 69) = 10.1, P < .01$. ## $P < .01$ vs vehicle-administrated control group, † $P < .05$ vs vehicle-administrated defeated group (Bonferroni's test). control: mice exposed to an empty cage, defeated: mice exposed to an aggressive ICR mouse.

juvenile rodents exposed to social defeat stress repeatedly display depressive-like but not anxiety-like behaviors, and the impairment of social behaviors by social defeat stress might be caused by decrease of sociality rather than anxiety to the target mouse. In addition, the present stressed mouse model may be useful as a juvenile stress-based experimental model for the study of psychiatric disorders in adolescents.

The detailed mechanisms of social behavioral changes in this model have not been elucidated yet. However, it has been suggested that monoaminergic neuronal systems are important modulators of the responses to stress (Torres et al., 2002). Continued or chronic stress exposure is found to have a negative influence on the serotonergic neuronal system and may increase the 5-HT sensitivity or vulnerability as a compensatory response (Firk and Markus, 2007). In this model, the 5-HT metabolism in the PFC and AMG of the nontested control group were increased following the social interaction test, whereas there were no changes in NA and DA metabolisms. Interestingly, an increase in the utilization of 5-HT was not observed following the social interaction test in mice exposed to the stress. Thus, these findings suggest that the serotonergic neuronal dysfunction is involved in the expression of impaired social behaviors. Meanwhile, the utilization of not only 5-HT but also DA in the

NAC, HIP, and AMG was decreased in adolescent mice exposed to social defeat stress as juveniles following the social interaction test. Namely, no change in DA metabolism of the tested control group was observed, while the metabolism was significantly decreased on social defeat stress exposure in the tested mice (the tested defeated group) compared with that in the tested control group. Stress-induced inhibition of DA release in the NAC can explain the impaired defensive reactions under aversive conditions observed following stressful experiences (Cabib and Puglisi-Allegra, 1996). Taken together with these reports (Cabib and Puglisi-Allegra, 1996; Firk and Markus, 2007), our findings suggest that the not only serotonergic, but also dopaminergic neuronal systems in adolescent mice are impaired by social defeat stress exposure as juveniles. There are functional changes associated with the decreased the utilization of 5-HT and DA, which might be involved in the impairment of social behaviors. This could be a model of decreased interest as the symptom of depression.

The utilization of DA was increased in the PFC and Nac of adolescent mice exposed to social defeat stress (Tanaka et al., 2012). The reason for this discrepancy is unknown but may be because of difference in aging between samples exposed to social defeat stress at 3 weeks (juvenile) and 6 weeks (adolescent).

Prominent developmental transformations are seen in the PFC and limbic regions of the brain of adolescents across a variety of species; alterations include an apparent shift in the balance between the mesocortical and mesolimbic dopaminergic neuronal system (Spear, 2000). Different development transformations in mesocortical and mesolimbic dopaminergic neuronal systems between juvenile and adolescent mice exposed to social defeat stress may be involved.

Acute or repeated administration of traditional antidepressants have been shown to be effective in many animal models of MDD (Caldarone et al., 2015). Repeated administration of paroxetine, which is used widely in humans, attenuated impairment of social interaction in adult, defeated mice (Xu et al., 2018). In the present study, acute and repeated administration of desipramine and sertraline did not attenuate the persistent impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. Our findings suggest that exposure to social defeat stress as juveniles induces an antidepressant-resistant impairment of social behaviors in adolescents. Antidepressants are widely used in the treatment of depression in adolescents, although there are antidepressant-resistant in adolescents (Cipriani et al., 2016). Augmentation pharmacotherapy refers to the addition of drugs that are not standard antidepressants to enhance the effect of a classical antidepressant drug (Carvalho et al., 2007). Clinically, antidepressant activity of antipsychotics has been observed when administered either alone or in combination with an antidepressant (Kamei et al., 2008). DA-D₂ and D₃ receptor agonists have been tested as augmenting agents in antidepressant-resistant forms of MDD (Carvalho et al., 2007). Aripiprazole is an atypical antipsychotic with a novel pharmacological profile, acting as a partial DA-D₂ and D₃ receptor agonist (Bourin et al., 2009). In the present study, however, acute and repeated administration of aripiprazole itself did not attenuate the persistent impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles despite the DA hypo-function observed in the defeated mice. The functional changes in the utilization of 5-HT and DA were observed in adolescent mice exposed to social defeat stress as juveniles. These results indicate that multiple functional changes in monoaminergic neuronal systems are involved in the treatment-resistant impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. Treatment with a combination of atypical antipsychotic drugs and SSRIs had antidepressant effects in patients who did not respond to SSRI monotherapy (Kamei et al., 2008). Previous studies have indicated that aripiprazole combined with inactive doses of antidepressants attenuated depressive-like behaviors in a forced swim test (Bourin et al., 2009). At noneffective doses individually, repeated administration of a combination of sertraline and aripiprazole showed additive effects without affecting body weight gain and the exploratory activity on the impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles in the present study. Thus, the combined administration of sertraline with aripiprazole may be useful as a new treatment strategy for treatment-resistant of stress stress-related psychiatric disorders.

It is unclear why repeated but not acute administration of a combination of sertraline and aripiprazole attenuates the impairment of social behaviors induced by social defeat stress exposure as juveniles, whereas the serotonergic and dopaminergic neuronal systems were impaired 1 day after the last exposure to social defeat stress. There are only a few clinical or preclinical studies that have assessed the effects of chronic administration of aripiprazole in combination with antidepressants in adolescent depressed patients or in mice. We found

changes in the utilizations of the 5-HT ($P < .05$), and DA ($P = .08$), NA ($P = .06$) in the AMG (supplemental Figure S1) but not in other brain regions (PFC, NAs, and HIP: data not shown) 15 days after the last social defeat stress exposure, indicating monoaminergic neuronal dysfunction in the AMG. Multiple functional changes in the monoaminergic neuronal systems of the AMG occurred over the 15 days and led to persistent impairment of the social behaviors in the adolescent mice. Repeated administration of a combination of sertraline and aripiprazole prevented the development of multiple functional changes in monoaminergic neuronal systems, leading to reversal of social impairment. Emotionally related learning, including stress exposure events, is mediated through the interactions of the basolateral HIP and AMG formation (Benes, 2010). We reported that the stress exposure induced the persistent impairment of social behaviors associated with suppression of the hippocampal neurogenesis (Mouri et al., 2018). In the present study, we found the changes in the utilizations of the 5-HT and DA in the AMG 15 days after the last social defeat stress exposure (supplemental Figure S1). Thus, repeated administration of a combination of sertraline and aripiprazole may improve monoaminergic neuronal dysfunctions in the HIP and/or AMG 1 and/or 15 days after the stress exposure. Further studies are needed to investigate the effect of social defeat stress exposure in monoaminergic neuronal systems of the HIP-AMG pathways and morphological changes, such as spine densities and dendritic lengths, during development of monoaminergic neuronal systems (Lyttle et al., 2015; Suri et al., 2015).

In the present study, there were no significant changes in NA metabolism in the all brain regions of the mice exposed to social defeat stress when the all groups were compared. Thus, it is unlikely that the impairment of social behaviors is due to changes in the noradrenergic neuronal system relative to that of the controls, although the noradrenergic neuronal system has been explicitly implicated in pathophysiological conditions induced by the stress exposure (Glavin, 1985). Further, previous studies have shown that NA transporter-knockout mice are resistant to the impairment of social behaviors induced by social defeat stress exposure (Haenisch et al., 2009). It remains unclear whether noradrenergic neuronal systems are involved in the treatment-resistant impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. The mechanisms of additive effect also remain unclear and might be mediated by multiple receptor properties and other neuronal systems including the glutamatergic and cholinergic neuronal systems. Aripiprazole acts as not only a partial agonist at the DA-D₂ and D₃ receptors but also as a partial agonist at the serotonin 5-HT_{1A} receptors and an antagonist at the serotonin receptors (Bourin et al., 2009). Both monoaminergic and glutamatergic (Sanacora et al., 2012) as well as cholinergic (Mineur et al., 2013) neuronal systems have driven the research on the impairment of social behaviors induced by social defeat stress exposure. The additive effect may be mediated, at least partly, by the activation of the serotonergic and dopaminergic neuronal systems. Further studies are needed to investigate the complex mechanisms behind the functional changes in both the noradrenergic and serotonergic/dopaminergic neuronal systems, including receptor functions and other neuronal systems, leading to the development of treatment-resistant impairment of social behaviors.

Aripiprazole is a dopaminergic neuronal system stabilizer; namely, a drug that can enhance dopaminergic neuronal activity when it is diminished or suppress it when it is increased (Stahl, 2001a, 2001b). Bourin et al. (2009) referred that the combination of aripiprazole and selective serotonin reuptake inhibitors

(SSRIs) in outpatients with depression who did not experience significant clinical improvement after taking antidepressants alone was performed in clinical trials (Papakostas et al., 2004; Simon and Nemeroff, 2005; Marcus et al., 2008). These findings suggest that aripiprazole adjunction could be used as an augmentation strategy when combined with SSRIs (Bourin et al., 2009). Bourin et al (2009) demonstrated that combined administration of subactive doses of antidepressants (which activate the serotonergic system) with aripiprazole (which activates the dopaminergic system) induced an antidepressive-like effect in a mouse model of depression. Thus, we speculated that aripiprazole may work as an agonistic rather than antagonistic for dopamine-D₂ receptor in the present experiment.

In conclusion, exposure to social defeat stress as juveniles induces the treatment-resistant impairment of social behaviors in adolescents through the functional changes in the serotonergic and dopaminergic neuronal systems. Administration of a combination of sertraline and aripiprazole may be useful as a new treatment strategy in adolescents, who had been exposed to adverse juvenile experiences, with treatment-resistant stress-related psychiatric disorders.

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