博士論文

Roles of serotonergic system during the postnatal period in the development of brain and behavior

生後発達期のセロトニン神経系の脳と行動の発達における役割

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CONTENTS

ABSTRACT

It is well known that early-life experiences modulate brain development and affect the behavior in later life. However, the brain mechanisms underlying effects of environmental factors on the development of behaviors are less known. In the present study, I investigated the roles of 5-HT and the 5-HT_{1A} receptor during the postnatal period in the behaviors in adulthood. I orally administered the selective 5-HT reuptake inhibitor (SSRI) (fluoxetine) or 5-HT_{1A} receptor agonist (8-OH-DPAT) from postnatal day (P) 1 to P21. Treatment with fluoxetine and 8-OH-DPAT during the postnatal period decreased anxiety-like behavior in adulthood, suggesting that 5-HT has anxiolytic effect, which is at least mediated through 5-HT_{1A} receptor. In contrast, 8-OH-DPAT treatment during the postnatal period increased depression-like behavior in the forced swim test, but not in the sucrose preference test, whereas fluoxetine treatment had no effects in depression-like behavior. This suggests that the postnatal 5-HT_{1A} receptor has opposite effects on anxiety and depression in adulthood. Treatment with fluoxetine and 8-OH-DPAT during the postnatal period had no significant effects on the spatial learning and memory and pain sensitivity. Next, as an approach to elucidate the brain mechanisms underlying the effects of fluoxetine and 8-OH-DPAT on the anxiety and depression-like behaviors in adulthood, I measured the gene expressions that have been shown to be involved in anxiety and depression-like behaviors. Treatment with both fluoxetine and 8-OH-DPAT decreased the mRNA expressions of the $GABA_A$ receptor α3 subunit in the medial prefrontal cortex (mPFC) and ventral hippocampus at P71. On the basis of the correlative changes between behavior and the mRNA expression, it is possible that the $GABA_A$ receptor α3 subunit in the mPFC and ventral hippocampus may modulate

anxiety-like behavior. In contrast, 8-OH-DPAT treatment decreased the mRNA expressions of the 5-HT_{1A} receptor, brain-derived neurotrophic factor (BDNF) and the $GABA_A$ receptor α2 subunit in the mPFC and the $GABA_A$ receptor α2 subunit in the ventral hippocampus at P71, whereas fluoxetine had no effects on these gene expressions. On the basis of the correlative changes between behavior and the mRNA expression, it is suggested that the 5-HT_{1A} receptor, BDNF, and the $GABA_A$ receptor α2 subunit in the mPFC and the $GABA_A$ receptor α2 subunit in the ventral hippocampus may modulate depression-like behavior. In summary, activation of the 5-HT_{1A} receptor during the postnatal period may reduce anxiety levels, but increase depression levels during adulthood via different multiple molecules in the mPFC and ventral hippocampus.

INTRODUCTION

Effects of early-life experiences on the development of brain and behaviors

Early-life experiences are known to alter brain development and behaviors in later life. The quality of maternal care and mother-infant relationship is associated with various developmental outcomes, such as emotionality and cognition. In humans, a secure attachment with mothers, in which mothers respond to infant's signals accurately and consistently, provides the infants with better emotional regulation and better handling stressful situation (Baram et al., 2012). In contrast, poor maternal care or mother-infant relationship makes the infants vulnerable to stressful situations and at high risk of developing depression (Lupien et al., 2009; Baram et al., 2012). To study the roles of maternal care and mother-infant relationship in the development of infant, maternal separation is used as animal models. Prolonged maternal separation for 1-6 hours a day increases both anxiety and depression-like behaviors in the adulthood, and impairs the brain development including hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis and low level of neurotrophins (Nishi et al., 2014; Tractenberg et al., 2016). In contrast, brief maternal separation for 3-15 minutes a day during the postnatal period decreases anxiety-like behavior and improve spatial learning and memory in the adulthood, which is accompanied by lower HPA axis responsiveness (Vetulani et al., 2013; Nishi et al., 2014; Raineki et al., 2014). These positive effects may be due to increased maternal care, induced by brief maternal separation (Vetulani et al., 2013). Thus, human and animal studies have reported the crucial roles of early-life experiences in the development of the brain and behaviors. However, the brain mechanisms (molecules and

brain regions), which link early-life experiences to behavioral changes in later lifer, are not well known.

Serotonin in the development

Serotonin (5-hydroxytryptamine, 5-HT) is known as a neurotransmitter in the adult brain. In addition, 5-HT neurons appear early in the embryonic brain and 5-HT has neurotrophic activity. In mice, 5-HT neurons emerge at embryonic day (E) 10 to E12 in the raphe nuclei, and extend the axons to widespread brain regions like the prefrontal cortex, hippocampus, and amygdala between E14.5 and postnatal day (P) 0 (Gaspar et al., 2003; Kepser and Homberg, 2015). Both *in vivo* and *in vitro* studies have revealed various roles of 5-HT in the neural development. For example, *in vivo* studies show that selective 5-HT reuptake inhibitor (SSRI) fluoxetine treatment during the postnatal period decreases the complexity of dendritic arbors in the prefrontal cortex (Ko et al., 2014; Rebello et al., 2014), whereas increases the number and the length of dendritic spines in the hippocampus in adolescence and adult (Zheng et al., 2011; Guidi et al., 2013). Besides dendrite formation and synaptogenesis, postnatal 5-HT also affects adult behaviors. For example, fluoxetine treatment during the postnatal period increases adult anxiety level (Ansorge et al., 2004; Rebello et al., 2014; Sarkar et al., 2014) and depression level (Karpova et al., 2009; Ko et al., 2014; Rebello et al., 2014; Sarkar et al., 2014). In contrast, postnatal fluoxetine treatment recovers impaired spatial learning as well as 5-HT turnover and synapse formation in prenatally stressed mice (Ishiwata et al., 2005). Thus, 5-HT during the postnatal period may be one of important factors to modulate adult behaviors.

The 5-HT$_{1A}$ receptor in the development

Until today, at least 14 5-HT receptors with 7 classes were reported. Among these 5-HT receptors, the 5-HT$_{1A}$ receptor has been extensively studied. In the development, the 5-HT$_{1A}$ receptor begins to appear at E12 in rat (Hillion et al., 1993) and by E14.5 in mouse brains (Bonnin et al., 2006). Subsequently, the expression level of the 5-HT$_{1A}$ receptor reaches to the peak during the prenatal and postnatal periods, depending on the brain regions (Daval et al., 1987; Hillion et al., 1993; Bonnin et al., 2006). The 5-HT$_{1A}$ receptor is localized both presynaptically and postsynaptically. Both presynaptic and postsynaptic 5-HT$_{1A}$ receptors are coupled to G$\alpha_{i/o}$ proteins to increase intracellular calcium concentration through adenylate cyclase and protein kinase A (Polter and Li, 2010). Presynaptic 5-HT$_{1A}$ receptor (autoreceptor) is expressed in 5-HT neurons in the raphe nucleus, leading to a decrease in the firing of 5-HT neurons and 5-HT release. On the other hand, postsynaptic 5-HT$_{1A}$ receptor (heteroreceptor) is localized on non-5-HT neurons, such as glutamatergic, GABAergic and cholinergic neurons, in the cortex, amygdala, and hippocampus. Activation of the 5-HT$_{1A}$ heteroreceptor usually decreases neuronal excitability and firing.

Similar to the 5-HT actions, the 5-HT$_{1A}$ receptor modulates dendrite formation and synaptogenesis. The 5-HT$_{1A}$ receptor inhibits dendritic spine formation in the cortex (Yoshida et al., 2011) whereas it promotes dendritic spine formation and synaptogenesis in the hippocampus (Yan et al., 1997; Wilson et al., 1998; Mogha et al., 2012).

In addition, many studies have reported the relationships between postnatal 5-HT$_{1A}$ receptor and adult behaviors. Postnatal 5-HT$_{1A}$ receptor is particularly correlated with

anxiety-like behavior. By use of conditional knockout of the 5-HT_{1A} receptor and treatment with a 5-HT_{1A} receptor antagonist, it has been shown that blockade of the 5-HT_{1A} receptor during the postnatal period increases anxiety-like behavior in adulthood (Gross et al., 2002; Vinkers et al., 2010). In contrast to anxiety, the studies of roles of postnatal 5-HT_{1A} receptor on depression-like behavior and learning and memory are limited. Blockade of the 5-HT_{1A} receptor during the postnatal period normalizes depression-like behavior in adult 5-HT transporter knockout mice (Alexandre et al., 2006). The role of the 5-HT_{1A} receptor in the depression-like behavior is extensively studied, using 5-HT_{1A} receptor knockout mice, in which the 5-HT_{1A} receptor is blocked throughout the life. Many studies showed that 5-HT_{1A} receptor knockout mice reduce depression-like behavior in adulthood (Parks et al., 1998; Ramboz et al., 1998; Richardson-Jones et al., 2010; Bortozzi et al., 2012; Ferres-Coy et al., 2013; Piszszek et al., 2013). However, some studies also reported that the 5-HT_{1A} receptor-overexpressed mice reduce depression-like behavior (Gunther et al., 2011) and the 5-HT_{1A} receptor knockout in heteroreceptor (vs. autoreceptor) increases depression-like behavior (Richardson-Jones et al., 2011). Therefore, the relationship between the 5-HT_{1A} receptor and depression is somewhat controversial among studies. Regarding learning and memory, only a few studies have investigated using 5-HT_{1A} receptor knockout mice. They show that blockade of the 5-HT_{1A} receptor throughout the life impairs spatial learning and memory (Sarnyai et al., 2000; Wolff et al., 2004).

Taken together, in the present study I examined the roles of postnatal 5-HT and the 5-HT_{1A} receptor on the formation of adult behaviors using pharmacological treatment with SSRI and 5-HT_{1A} receptor agonist. Next, I examined the effects of postnatal SSRI and the 5-HT_{1A} receptor agonist on the expression of brain-derived neurotrophic factor (BDNF), the $GABA_A$

receptor and the AMPA receptor in order to elucidate the underlying brain mechanisms.

Brain-derived neurotrophic factor

BDNF is a member of the neurotrophin family and binds to TrkB receptor, leading to dimerization and phosphorylation of the intracellular tyrosine kinase domain (Yoshii and Constantine-Paton, 2010). Subsequently, it triggers the activation of intracellular signaling cascades, including mitogen-activated protein kinase (MAPK), phosphatidyl inositol-3 kinase (PI3K), and phospholipase C (PLC)-γ pathways. In consequence, BDNF contributes to various functions in neuronal survival, cell migration, dendritic growth, synaptogenesis and synaptic plasticity (Park and Poo, 2013).

It is suggested that 5-HT and BDNF interact and cooperate with each other (Homberg et al., 2014). For example, BDNF increases 5-HT synthesis in the 5-HT neuronal cell line RN46A and in the embryonic raphe culture *in vitro* (Eaton et al., 1995). BDNF also promotes the survival and sprouting of 5-HT axons *in vivo* (Mamounas et al., 1995). Conversely, enhancing 5-HT signaling, using monoamine oxidase (MAO) inhibitor, increases BDNF expression in the frontal cortex, while it decreases BDNF expression in the hippocampus (Zetterstrom et al., 1999). Hence, it is possible that changes of 5-HT system by treatment with SSRI and the 5-HT_{1A} receptor agonist during the postnatal period may affect BDNF expression.

It has been also reported that BDNF is involved in depression-like behavior and learning and memory. Expression of BDNF is reduced in the brains of depressed patients (Castren and Kojima, 2107). In animal studies, both acute and chronic stresses decrease the expression of

BDNF in the cortex and hippocampus, whereas antidepressants increase the expression of BDNF to recover the stress-induced reduction of BDNF (Nestler, 2002). The relationship between BDNF and learning and memory is also extensively studied. Genetic and pharmacological deprivation of BDNF impairs hippocampus-dependent learning and memory (Yamada and Nabeshima, 2003).

Taken together, it is possible that BDNF is a candidate molecule linking postnatal 5-HT system and adult behaviors such as depression and spatial learning and memory.

The $GABA_A$ receptor

The $GABA_A$ receptor is a target of benzodiazepines, anxiolytics. Benzodiazepines have acute effects in the treatment of generalized anxiety disorder, social anxiety disorder, and panic disorder (Griebel and Holmes, 2013), whereas SSRIs show their effect after several weeks of treatment (Vaswani et al., 2003). The $GABA_A$ receptor has 19 subunits, which are α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3 (Rudolph and Knoflach, 2011). Among these subunits, benzodiazepines bind to one of the α subunits (α1, α2, α3, or α5) and a γ subunit, and drugs which target on the α2 or α3 subunit have already been made to clinical trials (Griebel and Holmes, 2013). Studies using rodents have suggested that the α2 and α3 subunits modulate anxiety-like behavior. The diazepam-induced anxiolytic effect is absent in mice with the point mutation of the α2 subunit, suggesting the anxiolytic effect is displayed via the α2 subunit (Low et al., 2000). In addition, the selective agonist for the α3 subunit shows an anxiolytic effect (Dias et al., 2005) and the α3 subunit inverse agonist shows an anxiogenic effect in rats (Attack et al., 2005).

The AMPA receptor

In addition to the 5-HT system, glutamate receptor is known as a target of antidepressants. Recently, it was shown that ketamine has acute antidepressant activity (Murrough, 2012). Although SSRI requires a few weeks to show antidepressant effect, ketamine shows antidepressant effect in a few hours to several days. The glutamate receptor is comprised of the NMDA receptor, AMPA receptor, kainate receptor, and metabotropic glutamate receptor (mGluR). Ketamine potentiates the AMPA receptor in addition to the NMDA receptor antagonism, and the AMPA receptor has a crucial role in antidepressant effect (Koike and Chaki, 2014).

The AMPA receptor is a tetramer, which is composed of either GluR1, GluR2, GluR3, or GluR4 subunit, and most of the AMPA receptor is comprised of GluR1 and GluR2 (Derkach et al., 2007). The AMPA receptor usually permeates Na^+, K^+, and Ca^{2+}, but a receptor with GluR2 subunit is not permeable to Ca^{2+}. This characteristic is important in long-term potentiation (LTP) and long-term depression (LTD) (Huganir and Nicoll, 2013). LTP and LTD are controlled by the AMPA receptor trafficking between cell surface and cytoplasm. LTP is triggered by the transient increase of the AMPA receptors that has no GluR2 on the cell surface. This transient increase is caused by the phosphorylation of GluR1 and exocytosis of the AMPA receptors. On the other hand, LTD is triggered by the transient decrease of expression of the AMPA receptors on the cell surface. This transient decrease is caused by the phosphorylation of GluR2 and endocytosis of the AMPA receptors.

BALB/c mouse

A single nucleotide polymorphism of tryptophan hydroxylase 2 (Tph2), 5-HT synthesizing enzyme, has been identified in BALB/c mice, and accordingly mRNA and protein levels of Tph2 in the dorsal raphe and protein level of 5-hydroxytryptophan, precursor of 5-HT, are decreased (Zhang et al., 2004; Siesser et al., 2010; Bach et al., 2011). Concomitantly, 5-HT level in the cerebral cortex and midbrain are decreased (Zhang et al., 2004; Bach et al., 2011). In addition, the mRNA expression of the 5-HT_{1A} receptor is increased in the mPFC (unpublished observation) and the hypothalamus, although it is also reported that the protein level of 5-HT_{1A} receptor are unchanged (Popova et al., 2009). The 5-HT_{2C} receptor pre-mRNA editing, that is affected in depressed animals and patients (Gurevich et al., 2002; Iwamoto et al., 2005), is also altered in BALB/c mice (Englander et al., 2005; Hackler et al., 2006). Besides the 5-HT system, BALB/c mice show increased level of anxiety, impairment of learning and memory, vulnerability to chronic stress, and high sensitivity to antidepressants (Francis et al., 2003; Brodkin, 2007; Uchida et al., 2011).

I used BALB/c mice in the present study, because I hypothesized that I could see more clearly the effects of SSRI and 5-HT_{1A} receptor agonist on the behaviors.

Purpose

The roles of 5-HT and its receptors during the postnatal stages on the development of behavior are not well known. In this study, I examined the roles of 5-HT and the 5-HT_{1A} receptor during the postnatal 3 weeks on the adult behavior. For this purpose, I treated postnatal mice with SSRI or the 5-HT_{1A} receptor agonist. I first examined the adult behaviors,

such as anxiety, depression, spatial learning and memory, and pain sensitivity. Next, to elucidate the underlying brain mechanisms of anxiety and depression, I measured the mRNA expressions of the 5-HT_{1A} receptor, BDNF, the $GABA_A$ receptor α2 and α3 subunits, and the AMPA receptor GluR1, and GluR2 subunits in the medial prefrontal cortex (mPFC), amygdala, and dorsal and ventral hippocampi during the postnatal period and the adulthood. By comparing the changes of behaviors with those of the mRNA expressions induced by SSRI and the 5-HT_{1A} receptor agonist, it is possible to find some candidate genes, which may regulate adult behaviors.

MATERIALS AND METHODS

Animals

Pregnant female BALB/c mice (Japan SLC Inc., Shizuoka, Japan) were individually housed and maintained under a 12:12 h light/dark cycle (lights on 8:00AM) at 24^{o}C, with food and water available *ad libitum*. The date of the birth was considered as postnatal day 0 (P0). Only male offspring were used. All the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (USA) and were approved by the Animal Experimentation Committee of the University of Tsukuba.

Drug treatment

Offspring were randomly assigned to 3 groups; control group, group of treatment with the selective 5-HT reuptake inhibitor (SSRI), and group of treatment with the 5-HT$_{1A}$ receptor agonist group. Pups from each group received an oral administration of 5% sucrose (or saline) (control), 5 mg/kg fluoxetine hydrochloride (a SSRI, Sigma-Aldrich, St. Louis, MO, USA), or 5 mg/kg (R)-(+)-8-hydroxy-DPAT hydrobromide (8-OH-DPAT) (Sigma-Aldrich) (5-HT$_{1A}$ receptor agonist), using a pipettor (Pipetman, Gilson, Middleton, WI, USA) with a small tip at its end, from P1 to P21. The dose of fluoxetine was decided on the basis of a previous study (Ishiwata et al., 2005). The dose of 8-OH-DPAT was determined by the evaluation of the concentration of 0.5 and 5 mg/kg in the anxiety-like behavior (Fig. 1).

Body weight

Body weight of offspring were measured from P1 to P21 during the drugs were administered and at P71 when the brains were removed.

Elevated plus maze (EPM)

All behavioral tests were conducted from postnatal week 9 to 10. To investigate the effects on the anxiety-like behavior, the offspring of each experimental group were tested on P57 using the EPM (Ohara & Co., Ltd., Tokyo, Japan). The apparatus was elevated 60 cm from the floor and consisted of two opposing open arms (25 cm length x 5 cm width x 0.3 cm height) and two opposing closed arms (25 cm x 5 cm x 15 cm) that were connected by a central platform (6 cm x 6 cm). Each animal was placed in the central platform, facing a closed arm. All animals were tested once between 10:00 and 13:00, under regular room light (360 lux). The behavior was recorded for 5 min by use of an overhead color CCD camera. The time spent in the open arms and the number of entries into open arms were calculated as indices of anxiety-like behavior.

Morris water maze (MWM)

On P59-P65, the spatial learning and memory of the offspring of each experimental group was examined using the MWM (Ohara & Co., Ltd., Tokyo, Japan). The maze consisted of a circular pool (100 cm diameter and 30 cm depth) filled with water (22 ± 1 ^{o}C) colored by white poster color. A transparent escape platform (10 cm diameter) was situated 15 cm away

from the side wall and hidden 1 cm below the water surface. A series of tests was conducted between 9:30 and 13:00, under regular room light (450 lux). During the 5 day-course of the training, the platform was placed in a stable position that was centered in one of the four quadrants of the pool. Each daily session consisted of 3 trials in which animals were forced to swim from each of three random starting positions. They were allowed to search for the hidden platform for up to 90 sec, and remained on the platform for 30 sec after reaching it. Animals, which did not reach the platform, were moved onto the platform to rest for 30 sec. On day 6, mice were subjected to a probe test in which the platform was removed from the pool, and the mice were allowed to swim freely for 90 sec. The time spent in the quadrant where the platform had been located was measured. On day 7, a cued test was carried out during which the platform was changed to one that has a visible black box above the water surface and the time it took animals to reach it was measured.

Hot plate test

On P67, the pain sensitivity of the offspring of each experimental group was tested using the hot plate test (Ugo Basile, Monvalle VA, Italy). Each mouse was placed on the hot plate (54 °C) and the reaction time to lick or rub paws, jump, or shack the body was measured.

Forced swim test (FST)

Depression-like behavior was assessed as previously described (Porsolt et al., 1977) with slight modifications. The offspring of each experimental group were tested on P69 using the

FST. All animals were tested between 12:00 and 14:00, under regular room light (360 lux). Each animal was placed into the water (23 ± 1 ^{o}C) (7-cm height) in a 4L beaker (20-cm diameter). The behavior was analyzed during the last 4 min of the 6-min testing period. The first 2 min were considered as habituation. The time spent floating on the water surface without any movement except for minimal activity that the mouse kept them from drowning, was measured as an index of depression-like behavior. The time spent climbing was recorded when vigorous vertical movements with the forepaws against the wall of the beaker were displayed. Swimming time was calculated by subtraction of the floating and climbing times from the total testing period.

Sucrose preference test (SPT)

For the SPT, the offspring were acclimatized to two identical drinking bottles, one filled with 2% sucrose solution and the other with water, for 4 hr/day for 4 days. After overnight water deprivation, the SPT was conducted for 4 hr on the day 5. To avoid the side preference, the positions of the bottles were switched after 2 hr and interchanged daily. Sucrose and water consumption were determined by measuring the change in the volume of fluid consumed. Sucrose preference was defined as the ratio of the volume of sucrose versus total volume of sucrose and water consumed during the 4-hr test.

Brain Dissection

Under anesthesia by isoflurane, the mice were decapitated and their brains were quickly removed at P22 and P71. 2 mm thick coronal slices were cut on ice from the frontal pole of

the cerebral cortex using a Mouse Brain Matrix (Muromachi Kikai Co., Ltd., Tokyo, Japan), and the left hemisphere was used for the analysis of the expression of mRNA. The mPFC (Leuner and Shors, 2013) was punched out from the first slice and the amygdala was punched out from the third slice using a Harris Micro-Punch (GE healthcare, buckinghamshir, UK). The dorsal and ventral halves of the hippocampus were dissected out from the third and forth slices using a Noyes surgical scissor. We divided the hippocampus into dorsal and ventral halves because these regions have been shown to be involved in cognition and emotion, respectively (Bannerman et al., 2004).

Real-time reverse transcription-PCR

Each region was homogenized in RNAiso (Takara Bio, Shiga, Japan) and total RNA was isolated using RNAiso (Takara Bio), according to the manufacturer's instructions. The isolated 1 μg of total RNA was reverse-transcribed using the QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany). PCR was performed using the Thermal Cycler Dice Real Time TP800 System (Takara Bio) with SYBR Premix Ex Taq II (Takara Bio). The thermal cycling conditions comprised an initial step at 95°C for 10 sec, followed by 50 cycles at 95°C for 5 sec and at 60°C for 30 sec. Table 1 lists the primer sequences. The primers used in the present study are designed to capture all the splicing variants. The 5-HT_{1A} receptor, $GABA_A$ receptor α2 and α3 subunits, and 18S rRNA genes do not have any splicing variants. The sizes of the PCR products were verified using agarose gel electrophoresis. Quantitation was performed using the crossing point method. The data was normalized to 18S rRNA.

Statistical analysis

SPSS (SPSS Inc., Japan) was used for statistical analysis. Analyses of training of MWM and body weight from P1 to P21 were performed using repeated measures analysis of variance (ANOVA), with day as the within-subject factor and experiment group as between-subject factor, followed by student's t test (training of MWM) or one-way ANOVA (body weight). For evaluations of probe test and cued test of MWM and hot plate test, data were analyzed by student's t test. For evaluation of body weight at P71, EPM, FST, SPT, and mRNA expression, data were analyzed by one-way ANOVA, followed by post hoc analysis (Fisher's protected least significant difference test). For the evaluation of correlation between the mRNA expression of the 5-HT_{1A} receptor and BDNF at P71, Pearson's test was performed. $p < 0.05$ was considered as statistically significant. All data are expressed as mean ± S.E.M.

RESULTS

Postnatal 8-OH-DPAT treatment showed anxiolytic effects at the concentration of 5 mg/kg

The concentration of 8-OH-DPAT was determined by evaluating effects on the anxiety-like behavior using the EPM, since the previous studies reported that blockade of the 5-HT_{1A} receptor during postnatal period increased anxiety-like behavior in adulthood (Gross et al., 2002; Vinkers et al., 2010). ANOVA revealed marginally significant main effects on the time spent in open arms ($F(2,30) = 3.129$, $p = 0.058$) (Fig. 1A) and the ratios of entries into open arms ($F(2,30) = 2.917$, $p = 0.07$) (Fig. 1B), but not for the total number of entries into both open and closed arms ($F(2,30) = 0.722$, $p = 0.494$) (Fig. 1C). Post hoc test showed that mice treated with 5 mg/kg 8-OH-DPAT increased the time spent in open arms ($p < 0.05$) and the ratios of entries into open arms ($p < 0.05$). On the other hand, mice treated with 0.5 mg/kg 8-OH-DPAT showed no changes in the time spent in the open arms ($p = 0.827$) and the ratios of entries into open arms ($p = 0.311$). Thus, I treated with 8-OH-DPAT at the concentration of 5 mg/kg for the further analyses.

Postnatal treatment with fluoxetine and 8-OH-DPAT did not alter body weight

During the treatment with fluoxetine and 8-OH-DPAT from P1 to P21, main day effect on the body weight was significant ($F(20,1960) = 2624.008$, $p < 0.001$), but the interaction between day and group was not significant ($F(2,98) = 1.95$, $p = 0.148$) (Table 2). At P71, ANOVA revealed no significant main effects on the body weight ($F(2,38) = 0.175$, $p = 0.84$). Thus, the drug treatment had no effect on the body weight.

Postnatal treatment with fluoxetine and 8-OH-DPAT decreased anxiety-like behavior in adulthood

I examined effects of postnatal treatment with fluoxetine and 8-OH-DPAT on anxiety-like behavior in adulthood, using the EPM (Fig. 2). ANOVA revealed significant main effects on the time spent in open arms ($F(2,38) = 8.788$, $p < 0.001$) (Fig. 2A), the ratios of entries into open arms ($F(2,38) = 9.152$, $p < 0.001$) (Fig. 2B), and total number of entries into both open and closed arms ($F(2,38) = 4.104$, $p < 0.05$) (Fig. 2C). The post hoc test showed that postnatal treatment with both fluoxetine and 8-OH-DPAT increased the time spent in open arms (fluoxetine, $p < 0.01$; 8-OH-DPAT, $p < 0.001$) (Fig. 2A) and the entries into open arms (fluoxetine, $p < 0.001$; 8-OH-DPAT, $p < 0.01$) (Fig. 2B), as compared with the control mice. In addition, postnatal 8-OH-DPAT treatment decreased the total number of entries into both open and closed arms ($p < 0.01$), whereas fluoxetine treatment had no effect, as compared with the control mice ($p = 0.103$) (Fig. 2C).

Postnatal 8-OH-DPAT treatment increased depression-like behavior in adulthood

Next, I used the FST (Fig. 3) and the SPT (Fig. 4) to examine the effects of postnatal treatment with fluoxetine and 8-OH-DPAT on depression-like behavior in adulthood. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), major depressive disorder in humans is defined by depressed mood (behavioral despair) or a loss of interest or pleasure (anhedonia) in daily activities. Therefore, I used the FST to measure behavioral despair (Porsolt et al., 2001) and the SPT to measure anhedonia (Papp et al.,

1991).

In the FST, ANOVA revealed significant main effects on the floating ($F(2,37) = 5.069$, $p < 0.05$) (Fig. 3A), swimming ($F(2,37) = 6.841$, $p < 0.01$) (Fig. 3B), and the climbing ($F(2,37) = 4.851$, $p < 0.05$) (Fig. 2C) times. Postnatal 8-OH-DPAT treatment increased the floating time ($p < 0.01$) (Fig. 3A), whereas it decreased the swimming ($p < 0.01$) (Fig. 3B) and climbing ($p < 0.01$) (Fig. 3C) times. Postnatal fluoxetine treatment had no effects on any parameters: the floating ($p = 0.62$) (Fig. 3A), the swimming ($p = 0.714$) (Fig. 3B), and the climbing ($p = 0.489$) (Fig. 3C) times.

In the SPT, ANOVA revealed no significant main effect on the sucrose preference ($F(2,38) = 0.466$, $p = 0.631$) (Fig. 4).

Postnatal treatment with fluoxetine and 8-OH-DPAT had no effects on spatial learning and memory in adulthood

I examined the effects of postnatal fluoxetine and 8-OH-DPAT on adult spatial learning and memory in adulthood by the MWM (Figs. 5, 6). In the 5 days-training of MWM, the main day effects were significant (fluoxetine, $F(4,52) = 13.065$, $p < 0.001$; 8-OH-DPAT, $F(4,76) = 18.435$, $p < 0.001$), but the interactions between day and group were not significant (fluoxetine, $F(4,52) = 0.864$, $p = 0.49$; 8-OH-DPAT, $F(4,76) = 1.168$, $p = 0.323$) (Figs. 5A, 6A). The probe test showed no significant effects on the time spent in the platform quadrant (fluoxetine, $t(13) = -1.053$, $p = 0.312$; 8-OH-DPAT, $t(19) = -0.786$, $p = 0.441$) and the number of crossing of the platform (fluoxetine, $t(13) = -1.332$, $p = 0.206$; 8-OH-DPAT, $t(19) = -1.334$, $p = 0.198$) (Figs. 5B, 6B). The cued test also showed no significant effects (fluoxetine, $t(13) = 0.355$, $p = 0.728$; 8-OH-DPAT, $t(19) = -0.159$, $p = 0.876$) (Figs. 5C, 6C).

Postnatal treatment with fluoxetine and 8-OH-DPAT had no effect on pain sensitivity in adulthood

I examined the effects of postnatal fluoxetine and 8-OH-DPAT on adult pain sensitivity by the hot plate test. Neither postnatal fluoxetine nor 8-OH-DPAT affected the reaction time (fluoxetine, $t(16) = -1.179$, $p = 0.256$; 8-OH-DPAT, $t(29) = 0.829$, $p = 0.414$) (Fig. 7).

Postnatal 8-OH-DPAT treatment decreased the mRNA expression of the 5-HT_{1A} receptor in the mPFC and the reduction was maintained in adulthood

As described above, postnatal treatment with fluoxetine and 8-OH-DPAT induced significant changes in anxiety and/or depression-like behaviors in adulthood. To elucidate the brain mechanisms underlying the effects on behaviors, I examined gene expression focusing on the genes and brain regions, which are reported to relate with anxiety and depression.

In order to investigate the chronic effects of fluoxetine and 8-OH-DPAT, I measured the mRNA expression of the 5-HT_{1A} receptor at P22 and at P71 (Fig. 8). ANOVA revealed significant main effects on the mRNA expression of the 5-HT_{1A} receptor at P22 in the mPFC ($F(2,23) = 4.228$, $p < 0.05$), dorsal hippocampus ($F(2,22) = 6.061$, $p < 0.01$), and ventral hippocampus ($F(2,23) = 5.017$, $p < 0.05$), but not in the amygdala ($F(2,19) = 2.548$, $p = 0.105$) (Fig. 8A). The treatment with fluoxetine and 8-OH-DPAT between P1 and P21 decreased the mRNA expression of the 5-HT_{1A} receptor in the mPFC (fluoxetine, $p < 0.05$; 8-OH-DPAT, $p < 0.05$) and ventral hippocampus (fluoxetine, $p < 0.05$; 8-OH-DPAT, $p < 0.01$). 8-OH-DPAT treatment also decreased the mRNA expression of the 5-HT_{1A} receptor in the dorsal hippocampus ($p < 0.01$).

In contrast, ANOVA revealed a significant main effect on the mRNA expression of the 5-HT_{1A} receptor at P71 in the mPFC ($F(2,20) = 5.369$, $p < 0.05$), but not in the amygdala ($F(2,18) = 1.626$, $p = 0.224$), dorsal hippocampus ($F(2,18) = 2.057$, $p = 0.157$) or ventral hippocampus ($F(2,18) = 0.652$, $p = 0.533$) (Fig. 8B). 8-OH-DPAT treatment decreased the mRNA expression of the 5-HT_{1A} receptor in the mPFC at P71 ($p < 0.05$).

Postnatal 8-OH-DPAT treatment decreased the mRNA expression of BDNF in the adult mPFC

Next, I examined the mRNA expression of BDNF at P22 and P71 (Fig. 9). ANOVA revealed a marginally significant main effects on the mRNA expression of BDNF at P22 in the mPFC ($F(2,23) = 3.132$, $p = 0.063$), but not in the amygdala ($F(2,16) = 0.103$, $p = 0.903$), dorsal hippocampus ($F(2,20) = 2.05$, $p = 0.155$), or ventral hippocampus ($F(2,24) = 1.799$, $p = 0.187$) (Fig. 9A). The post-hoc test revealed that the treatments with fluoxetine and 8-OH-DPAT during the postnatal period decreased the mRNA expression of BDNF in the mPFC at P22 (fluoxetine, $p < 0.05$; 8-OH-DPAT, $p < 0.05$) (Fig. 9A).

In contrast, ANOVA revealed a significant main effect on the mRNA expression of BDNF at P71 in the mPFC ($F(2,23) = 11.411$, $p < 0.001$), but not in the amygdala ($F(2,18) = 1.769$, $p = 0.199$), dorsal hippocampus ($F(2,16) = 0.323$, $p = 0.728$), or ventral hippocampus ($F(2,16) = 2.658$, $p = 0.101$) (Fig. 9B). 8-OH-DPAT treatment during the postnatal period decreased the mRNA expression of BDNF in the mPFC at P71 ($p < 0.001$).

Postnatal 8-OH-DPAT treatment decreased the mRNA expression of the $GABA_A$ receptor α2 subunit in the adult mPFC and ventral hippocampus

I measured the mRNA expression of the $GABA_A$ receptor α2 subunit at P71 (Fig. 10A). ANOVA revealed significant main effects on the mRNA expression of $GABA_A$ receptor α2 subunit in the mPFC ($F(2,16) = 8.646$, $p < 0.01$), dorsal hippocampus ($F(2, 20) = 4.546$, $p < 0.05$) and ventral hippocampus ($F(2,17) = 9.782$, $p < 0.001$), but not in the amygdala ($F(2,18) = 0.028$, $p = 0.973$). 8-OH-DPAT treatment during the postnatal period decreased the mRNA expression of the $GABA_A$ receptor α2 subunit in the mPFC and the ventral hippocampus (mPFC, $p < 0.01$; ventral hippocamous, $p < 0.01$). In addition, 8-OH-DPAT treatment during the postnatal period decreased the mRNA expression of the $GABA_A$ receptor α2 subunit in the dorsal hippocampus, as compared with the treatment with fluoxetine ($p < 0.01$).

Postnatal treatment with fluoxetine and 8-OH-DPAT decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the adult mPFC and ventral hippocampus

I measured the mRNA expression of the $GABA_A$ receptor α3 subunit at P71 (Fig. 10B). ANOVA revealed significant main effects on the mRNA expression of $GABA_A$ receptor α3 subunit in the mPFC ($F(2,23) = 10.996$, $p < 0.001$), amygdala ($F(2,16) = 6.889$, $p < 0.01$) and ventral hippocampus ($F(2,19) = 5.882$, $p < 0.01$), but not in the dorsal hippocampus ($F(2,18) = 1.876$, $p = 0.182$). Both the treatment with fluoxetine and 8-OH-DPAT during the postnatal period decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the mPFC (fluoxetine, $p < 0.01$; 8-OH-DPAT, $p < 0.001$) and ventral hippocampus (fluoxetine, $p < 0.05$; 8-OH-DPAT, $p < 0.01$). In addition, only the treatment with fluoxetine decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the amygdala ($p < 0.01$).

Postnatal treatment with fluoxetine and 8-OH-DPAT did not affect the mRNA expression of GluR1 and GluR2 in adulthood

I measured the mRNA expression of GluR1 and GluR2 at P71 as presumptive mediators of depression-like behavior (Fig. 11). ANOVA revealed a significant main effect on the mRNA expression of GluR1 in the ventral hippocampus ($F(2,22) = 3.675$, $p < 0.05$) and a marginally significant main effect in the mPFC ($F(2,19) = 3.211$, $p = 0.063$), but not in the dorsal hippocampus ($F(2,17) = 0.12$, $p = 0.888$) (Fig. 11A). The treatment with fluoxetine increased the mRNA expression of GluR1 in the ventral hippocampus and decreased in the mPFC, as compared with the treatment with 8-OH-DPAT ($p < 0.05$). ANOVA revealed no significant main effects on the mRNA expression of GluR2 in the mPFC ($F(2,19) = 1.666$, $p = 0.215$) or the dorsal ($F(2,18) = 0.319$, $p = 0.731$) and ventral hippocampi ($F(2,21) = 0.206$, $p = 0.816$) (Fig. 11B).

DISCUSSION

Early-life experiences are known to alter brain structures and functions in later life. Dysfunction of 5-HT and the 5-HT_{1A} receptor during development is closely related to the alterations of behaviors in adulthood (Daubert and Condron, 2010). The present study showed that treatment with fluoxetine and 8-OH-DPAT between P1 and P21 decreased anxiety-like behavior in adulthood, while the same postnatal 8-OH-DPAT treatment increased depression-like behavior in terms of behavioral despair in adulthood (Table 3). There were no significant effects of fluoxetine and 8-OH-DPAT on the spatial learning and memory and pain sensitivity. To elucidate the brain mechanisms that regulate anxiety and depression, we examined the fluoxetine and 8-OH-DPAT-induced changes in the mRNA expression (Tables 4, 5). Both the treatment with fluoxetine and 8-OH-DPAT decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the mPFC and the ventral hippocampus (Table 5). In contrast, only the 8-OH-DPAT treatment decreased the mRNA expression of the 5-HT_{1A} receptor and BDNF in the mPFC and the $GABA_A$ receptor α2 subunit in the mPFC and the ventral hippocampus in adulthood. Therefore, we found some correlative changes between adult anxiety and depression and the mRNA expression of the $GABA_A$ receptor α subunits and BDNF in response to postnatal treatment with fluoxetine and 8-OH-DPAT.

Effects of postnatal treatment with fluoxetine and 8-OH-DPAT on body weight

5-HT regulates food intake (Voigt and Fink, 2015) and it was reported that acute and chronic treatment with fluoxetine decreased food intake and body weight in the adult rodents (Yen

and Fuller, 1992). A previous clinical study reported that prenatal SSRI exposure through depressed mother's milk increased preterm birth and decreased birth weight (Olivier et al., 2011). However, the present study showed that treatment with fluoxetine and 8-OH-DPAT during the postnatal period had no effects on the body weight of the mice throughout the life. Thus, it is suggested that fluoxetine and 8-OH-DPAT during the postnatal period had no effects on the food intake and the growth of the mice' bodies.

Effects of postnatal treatment with fluoxetine and 8-OH-DPAT on anxiety-like behavior in adulthood

SSRI is widely used as an antidepressant for adult patients and has both antidepressant and anxiolytic effects in adult mice (Olivier et al., 2011). In contrast, the effects of SSRI administration during the development are controversial. Long-term effects of prenatal and postnatal SSRI administration have been studied in rodents. Some studies reported that SSRI administration during the prenatal and postnatal periods increases both anxiety (Ansorge et al., 2004; Rebello et al., 2014; Sarkar et al., 2014) and depression-like behaviors (Karpova et al., 2009; Ko et al., 2014; Rebello et al., 2014; Sarkar et al., 2014), whereas others reported no changes (Kiryanova et al., 2013). This may be due to the differences in methods used, such as dosage (ranging from 0.3 mg/kg/day to 25 mg/kg/day) of SSRI and methods of administration (Kiryanova et al., 2013). Interestingly, several studies reported beneficial effects of the postnatal SSRI administration in the prenatally stressed animals (Ishiwata et al., 2005; Nagano et al., 2012; Rayen et al., 2015; Boulle et al., 2016). Thus, fluoxetine administration decreased anxiety-like behavior in the prenatally stressed rats, although the same treatment showed no or even increased changes of anxiety-like behavior in the

non-stressed rats (Nagano et al., 2012; Boulle et al., 2016). In addition, fluoxetine administration decreased the number of immature neurons and increased the densities of spine and synapse in the hippocampus in the prenatally stressed rodents, although fluoxetine administration increased the number of immature neurons and decreased the densities of spine and synapse in the hippocampus in non-stressed rodents (Ishiwata et al., 2005; Rayen et al., 2015).

The present study showed that fluoxetine treatment during the postnatal period decreased anxiety-like behavior of BALB/c mice. It has been reported that BALB/c mice show increased level of anxiety and vulnerability to chronic stress (Francis et al., 2003; Uchida et al., 2011). In addition, BALB/c mice are sensitive to chronic fluoxetine treatment and show anxiolytic/antidepressant responses in adult (Dulawa et al., 2004; Popa et al., 2010), similar to human cases in which antidepressants are effective in patients with higher anxiety or depression, but not so effective in healthy individuals (Barr et al., 1997; Gelfin et al., 1998). Thus, fluoxetine treatment during the postnatal period may have had beneficial effects in BALB/c mice in the present study. However, further studies are needed to elucidate the underlying mechanism of the beneficial effects in BALB/c mice in the present study. Popa et al. have shown that 5-HT levels in the raphe and the hippocampus are increased in response to chronic fluoxetine treatment and desensitization of the 5-HT_{1A} autoreceptor particularly plays an important role in anxiolytic effects in adult BALB/c mice (Popa et al., 2010). However, it still remains to be elucidated how SSRI administration during the development affects 5-HT levels and 5-HT receptors.

The present study showed that both fluoxetine and 8-OH-DPAT during the postnatal period decreased anxiety-like behavior in adulthood. Therefore, it is possible that among

5-HT receptors, 5-HT_{1A} receptor may mediate the postnatal SSRI treatment-induced decrease in anxiety levels in adulthood. The role of the 5-HT_{1A} receptor in decreasing anxiety is consistent with previous studies on postnatal treatment with the 5-HT_{1A} receptor antagonist (Vinkers et al., 2010) and 5-HT_{1A} receptor knockout and knockdown mice (Gross et al., 2002; Donaldson et al., 2014). Both 5-HT_{1A} receptor antagonist treatment from P0 to P21 and conditional knockout of heteroreceptor and knockdown of 5-HT_{1A} autoreceptor until P21 increased adult anxiety-like behavior (Gross et al., 2002; Vinkers et al., 2010). Therefore, the present activation study also suggested that postnatal 5-HT_{1A} receptor down-regulates the development of anxiety. Further studies are needed to determine autoreceptors or heteroreceptors of 5-HT_{1A} receptors are involved in the anxiety.

Effects of postnatal 8-OH-DPAT treatment on depression-like behavior in adulthood

In contrast to the effect on anxiety, the present study showed that postnatal 8-OH-DPAT treatment increased depression-like behavior. This result is in agreement with a previous study that blockade of the 5-HT_{1A} receptor during the development alleviates depression-like behavior in adulthood (Alexandre et al., 2006).

The 5-HT_{1A} receptor is one of the main targets as the treatment of major depressive disorder. Buspirone, the 5-HT_{1A} receptor partial agonist, and vilazodone, potent SSRI and the 5-HT_{1A} receptor partial agonist are already approved for clinical uses as antidepressants (Sahli et al., 2016). In the present study, 8-OH-DPAT treatment during the postnatal period showed increase of depression-like behavior as well as decrease of the 5-HT_{1A} receptor mRNA expression in the mPFC at P71. In contrast to the anti-depressive effects during the adulthood, the 5-HT_{1A} receptor during the postnatal period acted oppositely on the

depression-like behavior in the present study. A previous study has reported that blockade of the 5-HT_{1A} receptor during the development decreases depression-like behavior in adulthood (Alexandre et al., 2006). In addition, most previous studies using 5-HT_{1A} receptor knockout mice have shown the reduced level of depression in adults (Parks et al., 1998; Ramboz et al., 1998; Richardson-Jones et al., 2010; Bortozzi et al., 2012; Ferres-Coy et al., 2013; Piszszek et al., 2013). These results suggest that the 5-HT_{1A} receptor has different effects on the depression-like behavior, depending on the age.

Taken together, these findings suggest that the activation (or blockade) of the 5-HT_{1A} receptor during the postnatal period decreases (or increases) anxiety-like behavior but increases (or decreases) depression-like behavior in adulthood. Anxiety and depression are often comorbid, but these findings suggest that these two disorders are caused by different mechanisms (see also Ressler and Nemeroff, 2000; Leonardo and Hen, 2008).

Effects of postnatal 8-OH-DPAT treatment on the mRNA expression of the 5-HT_{1A} receptor

In the present study, the postnatal treatment with 8-OH-DPAT decreased the mRNA expression of the 5-HT_{1A} receptor both in the mPFC and in the hippocampus at P22, but this reduction in the mRNA expression of the 5-HT_{1A} receptor persisted until P71 in the mPFC, but not in the hippocampus. A previous study showed that environmental factors during the perinatal period such as prenatal stress and postnatal brief maternal separation (handling) modulated the mRNA expression of 5-HT receptors in the offspring's hippocampus during the postnatal period, but not in adulthood, while the effects remained observed in the adult frontal cortex (Akatsu et al., 2015). These results suggest that the effects on 5-HT receptor

expression are maintained in the frontal cortex throughout life while the hippocampus may have plasticity to recover the changes.

The present study showed that postnatal treatment with 8-OH-DPAT decreased the mRNA expression of the 5-HT_{1A} receptor in the mPFC at P71, and concomitantly increased depression-like behavior in adulthood. On the basis of the correlations between the mRNA expression and behavior in adulthood, it is possible that the 5-HT_{1A} receptor in the adult mPFC may modulate depression-like behavior, which is supported by the previous studies (Kato et al, 2015; Sahli et al., 2016). The 5-HT_{1A} receptor is one of the main targets for the treatment of major depressive disorder in adult. Kato et al. (2015) reported that DSP-1053, a novel SSRI with 5-HT_{1A} receptor agonistic activity shows antidepressant effects in adults. In addition, buspirone, 5-HT_{1A} receptor partial agonist, and vilazodone, potent SSRI with 5-HT_{1A} receptor partial agonistic activity, have already been approved for clinical use, as antidepressants in adults (Sahli et al., 2016). Taken together, the present findings suggest that the 5-HT_{1A} receptor has opposite effects on the depression between the postnatal period and adulthood. Specifically, the activation of the 5-HT_{1A} receptor in adulthood ameliorates depression-like behavior, whereas the similar activation of the 5-HT_{1A} receptor during the postnatal period induces depression-like behavior in adulthood.

Effects of postnatal 8-OH-DPAT treatment on the mRNA expression of BDNF

In the present study, 8-OH-DPAT decreased the mRNA expression of BDNF in the mPFC at P71 and concomitantly increased adult depression-like behavior in adulthood. Besides the 5-HT_{1A} receptor, many studies including postmortem studies, pharmacological studies using antidepressants, and BDNF polymorphism studies have reported strong correlation between

reduced BDNF signaling in the adult prefrontal cortex and hippocampus and depression (Dwivedi, 2009). In addition, functional association between the 5-HT_{1A} receptor and BDNF has been suggested. The 5-HT_{1A} receptor is linked to mitogen-activated protein kinase (MAPK) signaling pathway, and the activation of the cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) in the MAPK signaling pathway upregulates the expression of BDNF in adulthood (Polter and Li, 2010; Homberg et al., 2014). Indeed, the present study showed a positive correlation between the mRNA expression of the 5-HT_{1A} receptor and BDNF in the adult mPFC ($r = 0.664$, $p < 0.001$) (Fig. 12). Therefore, reduced 5-HT_{1A} receptor in adulthood possibly downregulates the expression of BDNF in the mPFC, which induces depression-like behavior.

Effects of postnatal 8-OH-DPAT treatment on the mRNA expression of the $GABA_A$ receptor α2 subunit

In addition to the 5-HT_{1A} receptor and BDNF, the present study showed that postnatal treatment with fluoxetine and 8-OH-DPAT affected the mRNA expression of the $GABA_A$ receptor α subunits. In particular, 8-OH-DPAT treatment reduced the mRNA expression of the $GABA_A$ receptor α2 subunits in the adult mPFC and ventral hippocampus. Considering that postnatal 8-OH-DPAT treatment increased adult depression-like behavior, this finding suggests that the reduction of the $GABA_A$ receptor α2 subunits in the adult mPFC and ventral hippocampus may be involved in the 8-OH-DPAT-induced increase in depression-like behavior. This idea was supported by studies reporting that depression-like behavior is increased in $GABA_A$ receptor α2 subunit knockout mice (Rudolph and Knoflach, 2011; Vollenweider et al., 2011). In addition, the $GABA_A$ receptor α2 subunit is involved in adult

neurogenesis in the dentate gyrus of the hippocampus (Duveau et al., 2011). Since antidepressants promote adult neurogenesis (Malberg et al., 2000), it is possible that the $GABA_A$ receptor α2 subunit in the ventral hippocampus affects depression-like behavior through adult neurogenesis.

Effects of postnatal treatment with fluoxetine and 8-OH-DPAT on the mRNA expression of the $GABA_A$ receptor α3 subunit

The present study also showed that postnatal treatment with both fluoxetine and 8-OH-DPAT decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the adult mPFC and ventral hippocampus. In addition, both treatments decreased anxiety levels in adulthood. On the basis of the correlative changes of mRNA expression and behavior, the reduction of the $GABA_A$ receptor α3 subunit in the adult mPFC and ventral hippocampus may decrease anxiety levels. By treating $GABA_A$ receptor α2 subunit mutated mice with the $GABA_A$ receptor α3 subunit agonist, Dias et al. demonstrated that the $GABA_A$ receptor α3 subunit is more effective in mediating anxiolytic action than is the $GABA_A$ receptor α2 subunit (Dias et al., 2005). However, the changes in the mRNA expression of the $GABA_A$ receptor α3 subunit in the mPFC and ventral hippocampus and anxiety-like behavior in the present study were not consistent with those shown in previous studies (Attack et al., 2005; Dias et al., 2005). Those studies showed that activation of the $GABA_A$ receptor α3 subunit has an anxiolytic effect (Dias et al., 2005), whereas suppression of the $GABA_A$ receptor α3 subunit has an anxiogenic effect (Attack et al., 2005). The discrepancy between the present and previous studies suggests that the $GABA_A$ receptor α3 subunit in the mPFC and ventral hippocampus

may not play a critical role in the anxiolytic effect by fluoxetine and 8-OH-DPAT. It is possible that activation of the $GABA_A$ receptor α3 subunit in brain regions other than that of the mPFC and ventral hippocampus is required for the anxiolytic effect. In addition, in the present study, postnatal fluoxetine treatment, but not 8-OH-DPAT treatment, decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the amygdala. Therefore, other 5-HT receptors than the $5\text{-}HT_{1A}$ receptor might mediate the reduction of the $GABA_A$ receptor α3 subunit in the amygdala.

Effects of postnatal treatment with fluoxetine and 8-OH-DPAT on the mRNA expression of the GluR1 and GluR2

The GluR1 and GluR2 subunits of AMPA receptors were reported to modulate depression (Martinez-Turrillas et al., 2002; Tan et al., 2006). However, the present study showed no significant changes in the mRNA expression of the GluR1 and GluR2 induced by 8-OH-DPAT treatment, which increased the depression-like behavior. Therefore, the $5\text{-}HT_{1A}$ receptor agonist during the postnatal period may induce depression-like behavior, independently of the GluR1 or GluR2 expression in the mPFC and the hippocampus.

CONCLUSION

The effects of 5-HT and the 5-HT_{1A} receptor during the postnatal period on the adult behaviors and the underlying mechanisms were largely unknown. In the present study, I showed that 5-HT during the postnatal period had an anxiolytic effect in adulthood, probably via the 5-HT_{1A} receptor during the postnatal period. In contrast, postnatal activation of the 5-HT_{1A} receptor induced depression-like behavior in adulthood. By comparing the behavioral effects and the changes in mRNA expression, I found that postnatal 5-HT and the 5-HT_{1A} receptor may decrease adult anxiety-like behavior by reducing the $GABA_A$ receptor α3 subunit in the mPFC and ventral hippocampus in adulthood. On the other hand, the 5-HT_{1A} receptor during the postnatal period may increase adult depression-like behavior by reducing the 5-HT_{1A} receptor and BDNF in the mPFC and the $GABA_A$ receptor α2 subunit in the mPFC and the ventral hippocampus in adulthood. Although the direct causal relationship between the gene expressions and the behaviors remain to be examined, the present study may give a clue to understanding the mechanisms of anxiety and depression affected by 5-HT and the 5-HT_{1A} receptor during the postnatal period.

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REFERENCES

Akatsu S, Ishikawa C, Takemura K, Ohtani A, Shiga T. (2015) Effects of prenatal stress and neonatal handling on anxiety, spatial learning and serotonergic system of male offspring mice. Neurosci Res. 101, 15-23.

Alexandre C, Popa D, Fabre V, Bouali S, Venault P, Lesch KP, Hamon M, Adrien J. (2006) Early life blockade of 5-hydroxytryptamine 1A receptors normalizes sleep and depression-like behavior in adult knock-out mice lacking the serotonin transporter. J Neurosci. 26, 5554-5564.

Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA. (2004) Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science. 306, 879-881.

Attack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford KA, McKernan RM, Dawson GR. (2005) Anxiogenic properties of an inverse agonist selective for α3 subunit-containing $GABA_A$ receptors. Br J Pharmacol. 144, 357-366.

Bach H, Arango V, Huang YY, Leong S, Mann JJ, Underwood MD. (2011) Neuronal tryptophan hydroxylase expression in BALB/cJ and C57Bl/6J mice. J Neurochem. 118, 1067-1074.

Bannerman DM, Rawlins JNP, McHugh SB, Deacon RMJ, Yee BK, Bast T, Zhang WN,

Pothuizer HHJ, Feldon J. (2004) Regional dissociations within the hippocampus- memory and anxiety. Neurosci Behav Rev. 28, 273-283.

Baram TZ, Davis EP, Obenaus A, Sandman CA, Small SL. (2012) Fragmentation and unpredictability of early-life experience in mental disorders. Am J Psychiatry. 169, 907-915.

Barr LC, Heninger GR, Goodman W, Charney DS, Price LH. (1997) Effects of fluoxetine administration on mood response to tryptophan depletion in healthy subjects. Biol Psychiatry. 41, 949-954.

Bonnin A, Peng W, Hewlett W, Levitt P. (2006) Expression mapping of 5-HT1 serotonin receptor subtypes during fetal and early postnatal mouse forebrain development. Neuroscience. 141, 781-794.

Bortolozzi A, Castane A, Semakova J, Santana N, Alvarado G, Cortes R, Ferres-Coy A, Fernandez G, Carmona MC, Toth M, Perales JC, Montefeltro A, Artigas F. (2012) Selective siRNA-mediated suppression of 5-HT_{1A} autoreceptors evokes strong anti-depressant-like effects. Mol Psychiatry. 17, 612-623.

Boulle F, Pawluski JL, Homberg JR, Machiels B, Kroeze Y, Kumar N, Steinbusch HW, Kenis G, Van den Hove DL. (2016) Prenatal stress and early-life exposure to fluoxetine have enduring effects on anxiety and hippocampal BDNF gene expression in adult male offspring. Dev Psychobiol. 58, 427-438.

Brodkin ES. (2007) BALB/c mice: low sociability and other phenotypes that may be relevant to autism. Behav Brain Res. 176, 53-65.

Castren E, Kojima M. (2017) Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. Neurobiol Dis. 97, Part B, 119-126.

Daval G, Verge D, Becerril A, Gozlan H, Spampinato U, Hamon M. (1987) Transient expression of 5-HT1A receptor binding sites in some areas of the rat CNS during postnatal development. Int J Dev Neurosci. 5, 171-180.

Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Wafford KA, Street LJ, Castro JL, Whiting PJ, Rosahl TW, Atack JR, McKernan RM, Dawson FR, Reynolds DS. (2005) Evidence for a significant role of alpha 3-containing GABAA receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci. 25, 10682-10688.

Derkach VA, Oh MC, Guire ES, Soderling TR. (2007) Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci. 8, 101-113.

Donaldson ZR, Piel DA, Santos TL, Richardson-Jones J, Leonardo ED, Beck SG, Champagne FA, HenR. (2014) Developmental effects of serotonin 1A autoreceptors on anxiety and social behavior. Neuropsychopharmacology. 39, 291-302.

Dulawa SC, Holick KA, Gundersen B, Hen R. (2004) Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology. 29, 1321-1330.

Duveau V, Lastela S, Barth L, Gianolini F, Vogt KE, Keist R, Chandra D, Homanics GE, Rudolph U, Fritschy J. (2011) Spatiotemporal specificity of $GABA_A$ receptor-mediated regulation of adult hippocampal neurogenesis. Eur J Neurosci. 34, 362-373.

Dwivedi Y. (2009) Brain-derived neurotrophic factor: role in depression and suicide. Neuropsychiatr Dis Treat. 5, 433-449.

Eaton MJ, Staley JK, Globus MY, Whittemore SR. (1995) Developmental regulation of early serotonergic neuronal differentiation: the role of brain-derived neurotrophic factor and membrane depolalization. Dev Biol. 170, 169-182.

Englander MT, Dulawa SC, Bhansali P, Schmauss C. (2005) How stress and fluoxetine modulate serotonin 2C receptor pre-mRNA editing. J Neurosci. 25, 648-651.

Ferres-Coy A, Santana N, Castane A, Cortes R, Carmona MC, Toth M, Montefeltro A, Artigas F, Bortolozzi A. (2013) Acute 5-HT_{1A} autoreceptor knockdown increases antidepressant responses and serotonin release in stressful conditions. Psychopharmacology. 225, 61-74.

Francis DD, Szegda K, Campbell G, Martin WD, Insel TR. (2003) Epigenetic sources of behavioral differences in mice. Nat Neurosci. 6, 445-446.

Gaspar P, Cases O, Maroteaux L. (2003) The developmental role of serotonin: news from mouse molecular genetics. Nat Rev Neurosci. 4, 1002-1012.

Gelfin Y, Gorfine M, Lerer B. (1998) Effect of clinical doses of fluoxetine on psychological variables in healthy volunteers. Am J Psychiatry. 155, 290-292.

Griebel G, Holmes A. (2013) 50 years of hurdles and hope in anxiolytic drug discovery. Nat Rev Drug Discov. 12, 667-687.

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R. (2002) Serotonin$_{1A}$ receptor acts during development to establish normal anxiety-like behavior in the adult. Nature. 416, 396-400.

Guidi S, Stagni F, Bianchi P, Ciani E, Ragazzi E, Trazzi S, Grossi G, Mangano C, Calza L, Bartesaghi R. (2013) Early pharmacotherapy with fluoxetine rescues dendritic pathology in the Ts65Dn mouse model of down syndrome. Brain Pathol. 23, 129-143.

Gunther L, Rothe J, Rex A, Voigt JP, Millan MJ, Fink H, Bert B. (2011) 5-HT$_{1A}$-receptor over-expressing mice: genotype and sex dependent responses to antidepressants in the forced swim test. Neuropharmacology. 61, 433-441.

Gurevich I, Tamir H, Arango V, Dwork AJ, Mann JJ, Schmauss C. (2002) Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. Neuron. 34, 349-356.

Hackler EA, Airey DC, Shannon CC, Sodhi MS, Sanders-Bush E. (2006) 5-HT(2C) receptor RNA editing in the amygdala of C57BL/6J, DBA/2J, and BALB/cJ mice. Neurosci Res. 55, 96-104.

Hillion J, Dumas Milne-Edwards JB, Catelon J, De Vitry F, Gros F, Hamon M. (1993) Prenatal developmental expression of rat brain 5-HT_{1A} receptor gene followed by PCR. Biochem Biophys Res Commun. 191, 991-997.

Homberg JR, Molteni R, Calabrese F, Riva MA. (2014) The serotonin-BDNF duo: developmental implications for the vulnerability to psychopathology. Neurosci Behav Rev. 43, 35-47.

Huganir RL, Nicoll RA. (2013) AMPARs and synaptic plasticity: the last 25 years. Neuron. 80, 704-717.

Ishiwata H, Shiga T, Okado N. (2005) Selective serotonin reuptake inhibitor treatment of early postnatal mice reverses their prenatal stress-induced brain dysfunction. Neuroscience. 133, 893-901.

Iwamoto K, Nakatani N, Bundo M, Yoshikawa T, Kato T. (2005) Altered RNA editing of serotonin 2C receptor in a rat model of depression. Neurosci Res. 53, 69-76.

Karpova NN, Lindholm J, Pruunsild P, Timmusk T, Castren E. (2009) Long-lasting behavioural and molecular alterations induced by early postnatal fluoxetine exposure are restored by chronic fluoxetine treatment in adult mice. Eur Neuropsychoparmacol. 19, 97-108.

Kato T, Matsumoto Y, Yamamoto M, Matsumoto K, Baba S, Nakamichi K, Matsuda H, Nishimuta H, Yabuuchi K. (2015) DSP-1053, a novel serotonin reuptake inhibitor with 5-HT1A partial agonistic activity, displays fast antidepressant effect with minimal undesirable effects. Pharmacol Res Perspect. 3, e00142.

Kepser L, Homberg JR. (2015) The neurodevelopmental effects of serotonin: a behavioural perspective. Behav Brain Res. 277, 3-13.

Kiryanova V, McAllister BB, Dyck RH. (2013) Long-term outcomes of developmental exposure to fluoxetine: a review of animal literature. Dev Neurosci. 35, 437-439.

Ko MC, Lee LJ, Li Y, Lee LJ. (2014) Long-term consequences of neonatal fluoxetine exposure in adult rats. Dev Neurobiol. 74, 1038-1051.

Koike H, Chaki S. (2014) Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. Behav Brain Res 271, 111-115.

Leonardo ED, Hen R. (2008) Anxiety as a developmental disorder. Neuropsychopharmacology. 33, 134-140.

Leuner B, Shors TJ. (2013) Stress, anxiety, dendritic spines: what are the connections? Neuroscinece. 251, 108-119.

Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U. (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. Science. 290, 131-134.

Lupien SJ, McEwen BS, Gunnar MR, Heim C. (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 10, 434-445.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS. (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci. 20, 9104-9110.

Mamounas LA, Blue ME, Siuciak JA, Alter CA. (1995) Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci. 15, 7929-7939.

Martinez-Turrillas R, Frechilla D, Del Rio J. (2002) Chronic antidepressant treatment increases the membrane expression of AMPA receptors in rat hippocampus. Neuropharmacology. 43, 1230-1237.

Mogha A, Guariglia SR, Debata PR, Wen GY, Banerjee P. (2012) Serotonin 1A receptor-mediated signaling through ERK and PKC α is essential for normal synaptogenesis in neonatal mouse hippocampus. Transl Psychiatry. 2, e66.

Murrough JW. (2012) Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther. 91, 303-309.

Nagano M, Liu M, Inagaki H, Kawada T, Suzuki H. (2012) Early intervention with fluoxetine reverses abnormalities in the serotonergic system and behavior of rats exposed prenatally to dexamethasone. Neuropharmacology. 63, 292-300.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. (2002) Neurobiology of depression. Neuron, 34, 13-25.

Nishi M, Horii-Hayashi N, Sasagawa T. (2014) Effects of early life adverse experiences on the brain: implications from maternal separation models in rodents. Front Neurosci. 8, 166.

Olivier JD, Blom T, Arentsen T, Homberg JR. (2011) The age-dependent effects of selective

serotonin reuptake inhibitors in humans and rodents: A review. Prog Neuropsychopharmacol Biol Psychiatry. 35, 1400-1408.

Papp M, Willner P, Muscat R. (1991) An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology. 104, 225-259.

Park H, Poo MM. (2013) Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci. 14, 7-23.

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. (1998) Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci USA. 95, 10734-10739.

Piszczek L, Schlax K, Wyrzykowska A, Piszczek A, Audero E, Gross C. (2013) Serotonin 1A auto-receptors are not sufficient to modulate anxiety in mice. Eur J Neurosci. 38, 2621-2627.

Polter AM, Li X. (2010) 5-HT1A receptor-regulated signal transduction pathways in brain. Cell Signal. 22, 1406-1412.

Popa D, Cerdan J, Reperant C, Guiard BP, Guilloux JP, David DJ, Gardier AM. (2010) A longitudinal study of 5-HT outflow during chronic fluoxetine treatment using a new technique of chronic microdialysis in a highly emotional mouse strain. Eur J Pharmacol. 628,

83-90.

Popova NK, Naumenko VS, Tibeikina MA, Kulikov AV. (2009) Serotonin transporter, 5-HT_{1A} receptor, and behavior in DBA/2J mice in comparison with four inbred mouse strains. J Neurosci Res. 87, 3649-3657.

Porsolt RD, Bertin A, Jalfre M. (1977) Behavioural despair in mice: a primary screening test for antidepressants. Arch Intern Pharmacodyn Ther. 299, 327-336.

Porsolt RD, Brossard G, Hautbois C, Roux S. (2001) Rodent models of depression: forced swimming and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci. Chapter 8, Unit 8.10A

Raineki C, Lucion AB, Weinberg J. (2014) Neonatal handling: overview of the positive negative effects. Dev Psychobiol. 56, 1613-1625.

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R. (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA. 95, 14476-14481.

Rayen I, Gemmel M, Pauley G, Steinbusch HW, Pawluski JL. (2015) Developmental exposure to SSRIs, in addition to maternal stress, has long-term sex-dependent effects on hippocampal plasticity. Psychopharmacology. 232, 1231-1244.

Rebello TJ, Yu Q, Goodfellow NM, Caffrey Cagliostro MK, Teissier A, Morelli E, Demireva EY, Chemiakine A, Rosoklija GB, Dwork AJ, Lambe EK, Gingrich JA, Ansorge MS. (2014) Postnatal day 2 to 11 constitutes a 5-HT-sensitive period impacting adult mPFC function. J Neurosci. 34, 12379-12393.

Ressler KJ, Nemeroff CB. (2000) Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety. 12 Suppl, 2-19.

Richardson-Jones JW, Craige CP, Guiard BP, Stephen A, Metzger KL, Kung HF, Gardier AM, Dranovsky A, David DJ, Beck SG, Hen R, Leonardo ED. (2010) 5-HT_{1A} autoreceptor levels determine vulnerability to stress and response to antidepressants. Neuron. 65, 40-52.

Richardson-Jones JW, Craige CP, Nguyen TH, Kung HF, Gardier AM, Dranovsky A, David DJ, Guiard BP, Beck SG, Hen R, Leonardo ED. (2011) Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. J Neurosci. 31, 6008-6018.

Rudolph U, Knoflach F. (2011) Beyond classical benzodiazepines: novel therapeutic potential of $GABA_A$ receptor subtypes. Nat Rev. 10, 685-697.

Sahli ZT, Banerjee P, Tarazi FI. (2016) The preclinical and clinical effects of vilazodone for the treatment of major depressive disorder. Expert Opin Drug Discov. 11, 515-523.

Sarkar A, Chachra P, Vaidya VA. (2014) Postnatal fluoxetine-evoked anxiety is prevented by concomitant 5-HT2A/C receptor blockade and mimicked by postnatal 5-HT2A/C receptor stimulation. Biol Psychiatry. 76, 858-868.

Sarnyai Z, Sibille EL, Pablides C, Fenster RJ, McEwen BS, Toth M. (2000) Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin(1A) receptors. Proc Natl Acad Sci USA. 97, 14731-14736.

Siesser WB, Zhang X, Jacobsen JP, Sotnikova TD, Gainetdinov RR, Caron MG. (2010) Tryptophan hydroxylase 2 genotype determines brain serotonin synthesis but not tissue content in C57Bl/6 and BALB/c congenic mice. Neurosci Lett. 481, 6-11.

Tan CH, He X, Yang J, Ong WY. (2006) Changes in AMPA subunit expression in the mouse brain after chronic treatment with the antidepressant maprotiline: a link between noradrenergic and glutamatergic function? Exp Brain Res. 170, 448-456.

Tractenberg SG, Levandowski ML, de Azeredo LA, Orso R, Roithmann LG, Hoffmann ES, Brenhouse H, Grassi-Oliveira R. (2016) An overview of maternal separation effects on behavioural outcomes in mice: evidence from a four-stage methodological systematic review. Neurosci Biobehav Rev. 68, 489-503.

Uchida S, Hara K, Kobayashi A, Otsuki K, Yamagata H, Hobara T, Suzuki T, Miyata N,

Watanabe Y. (2011) Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. Neuron. 69, 359-372.

Vaswani M, Linda FK, Ramesh S. (2003) Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. Prog Neuropsychopharmacol Biol Psychiatry. 27, 85-102.

Vetulani J. (2013) Early maternal separation: a rodent model of depression and a prevailing human condition. Pharmacol Rep. 65, 1451-1461.

Vinkers CH, Oosting RS, van Bogaert MJV, Olivier B, Groenink L. (2010) Early-life blockade of 5-HT_{1A} receptor alters adult anxiety behavior and benzodiazepine sensitivity. Biol Psychiatry. 67, 309-316.

Voigt JP, Fink H. (2015) Serotonin controlling feeding and satiety. Behav Brain Res. 277, 14-31.

Vollenweider I, Smith KS, Keist R, Rudolph U. (2011) Anti-depressant-like properties of α2-containing $GABA_A$ receptors. Behav Brain Res. 217, 77-80.

Wilson CC, Fabor KM, Haring JH. (1998) Serotonin regulates synaptic connections in the dentate molecular layer of adult rats via 5-HT_{1a} receptors: evidence for a glia mechanism. Brain Res. 782, 235-239.

Wolff M, Costet P, Gross C, Hen R, Segu L, Buhot MC. (2004) Age-dependent effects of serotonin-1A receptor gene deletion in spatial learning abilities in mice. Mol Res Brain Res 130, 39-48.

Yamada K, Nabeshima T. (2003) Brain-derived neurotrophic factor/TrkB signaling in memory processes. J Pharmacol Sci. 91, 267-270.

Yan W, Wilson CC, Haring JH. (1997) 5-HT_{1a} receptors mediate the neurotrophic effect of serotonin on developing dentate granule cells. Dev Brain Res 98, 185-190.

Yen TT, Fuller RW. (1992) Preclinical pharmacology of fluoxetine, a serotonergic drug for weight loss. Am J Clin Nutr. 55, 177S-180S.

Yoshida H, Kanamaru C, Ohtani A, Li F, Senzaki K, Shiga T. (2011) Subtype specific roles of serotonin receptors in the spine formation of cortical neurons in vitro. Neurosci Res. 71, 311-314.

Yoshii A, Constantine-Paton M. (2010) Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev Neurobiol. 70, 304-332.

Zetterstrom TS, Pei Q, Madhav TR, Coppell AL, Lewis L, Grahame-Smith DG. (1999) Manipulations of brain 5-HT levels affect gene expression for BDNF in rat brain.

Neuropharmacology. 38, 1063-1073.

Zhang X, Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science. 305, 217.

Zheng J, Xu DF, Li K, Wang HT, Shen PC, Lin M, Cao XH, Wang R. (2011) Neonatal exposure to fluoxetine and fluvoxamine alters spine density in mouse hippocampal CA1 pyramidal neurons. Int J Clin Exp Pathol 4, 162-168.

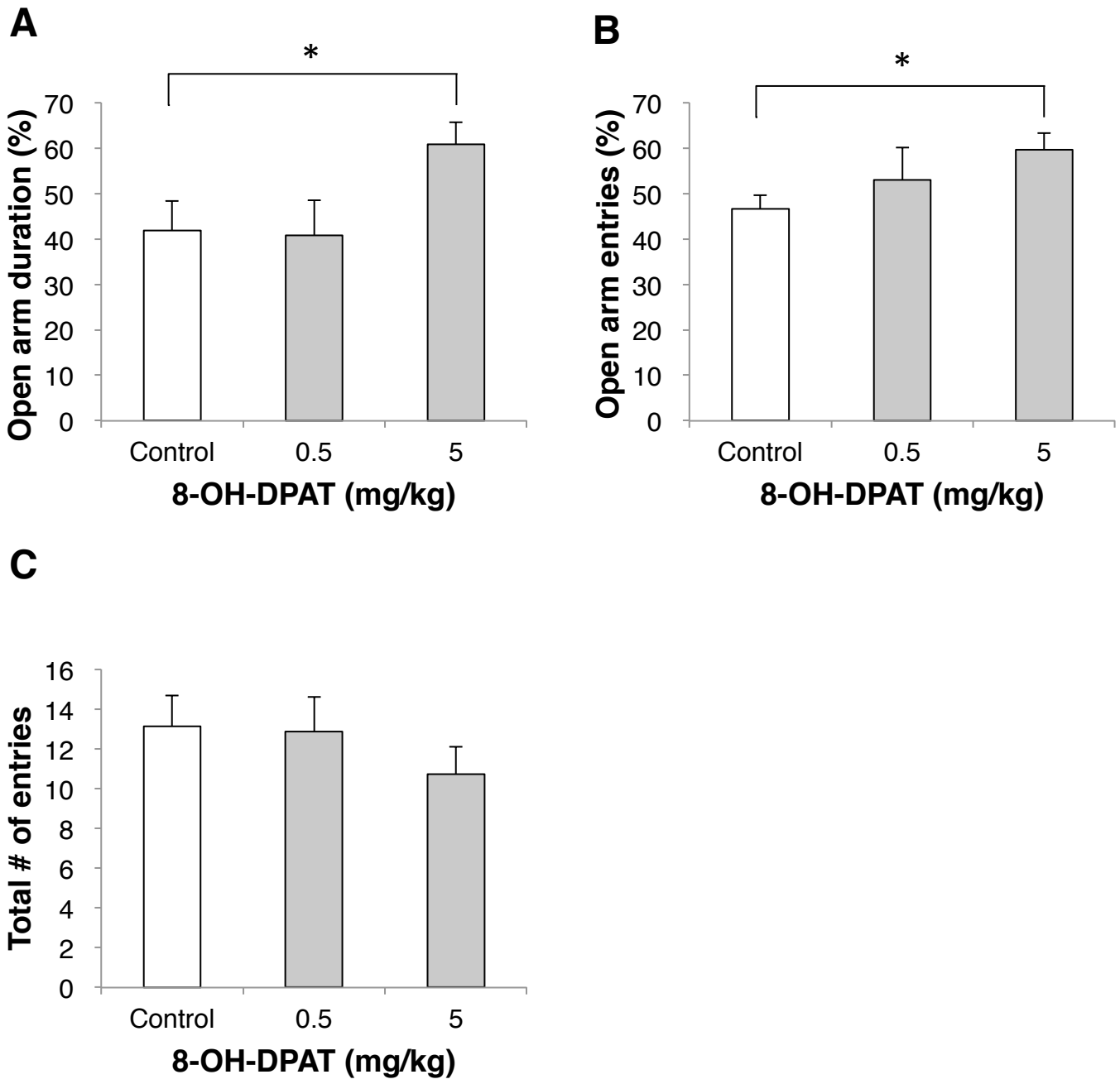

Figure 1. 8-OH-DPAT concentration

Anxiety-like behavior in the elevated plus maze test in mice treated with saline, 0.5 mg/kg 8-OH-DPAT, and 5 mg/kg 8-OH-DPAT from P1-P21. (A) Percentage of the time spent in the open arms. (B) Percentage of numbers of entries into open arms. (C) Total numbers of entries. Each bar represents the mean ± S.E.M (n=15 for control, n=7 for 0.5 mg/kg 8-OH-DPAT, n=11 for 5 mg/kg 8-OH-DPAT). * $p < 0.05$

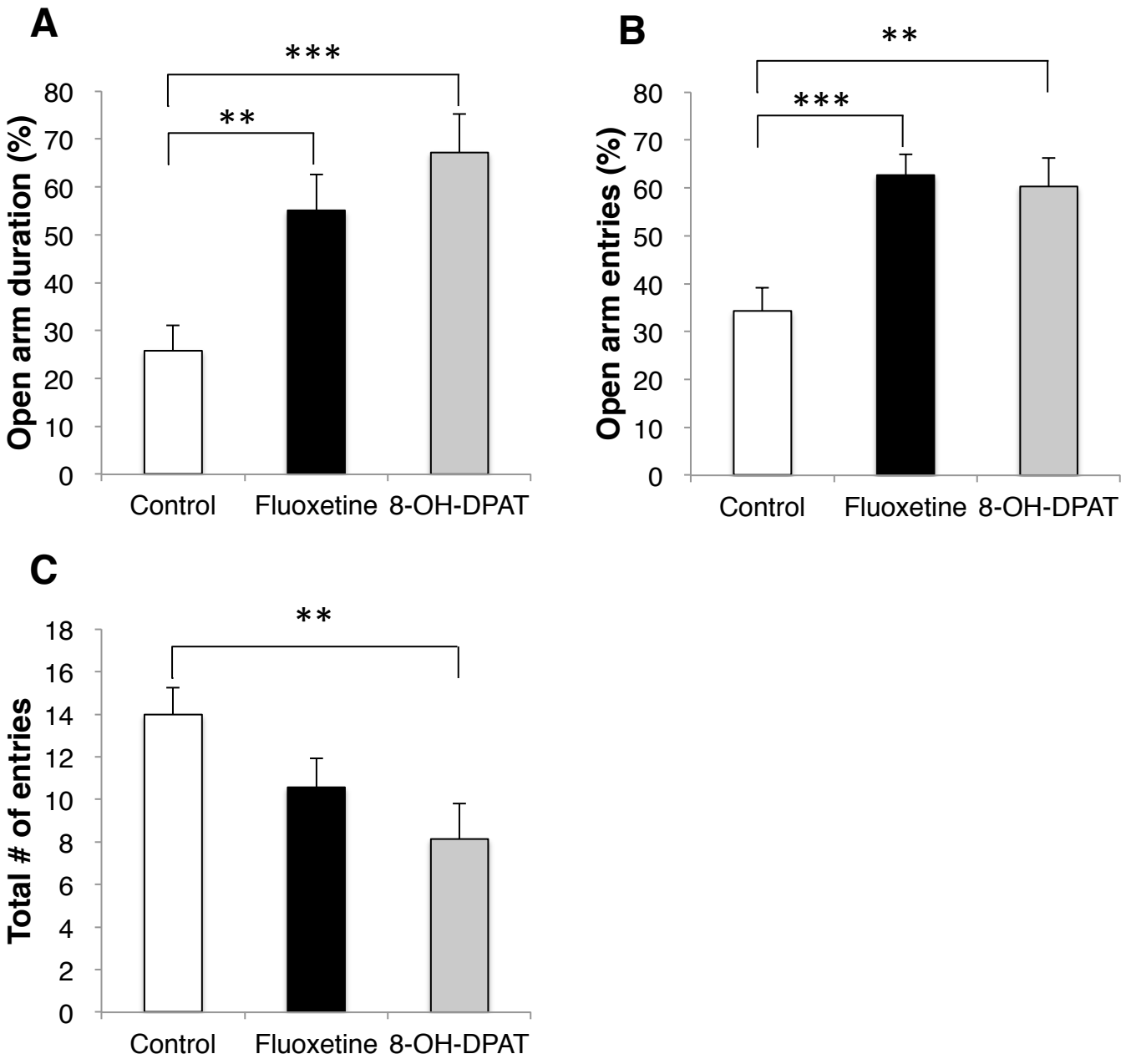

Figure 2 Anxiety-like behavior

Anxiety-like behavior in the elevated plus maze test in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) Percentage of the time spent in the open arms. (B) Percentage of numbers of entries into open arms. (C) Total numbers of entries. Each bar represents the mean ± S.E.M (n=13 for control, n=14 for fluoxetine, n=14 for 8-OH-DPAT). ** $p < 0.01$, *** $p < 0.001$

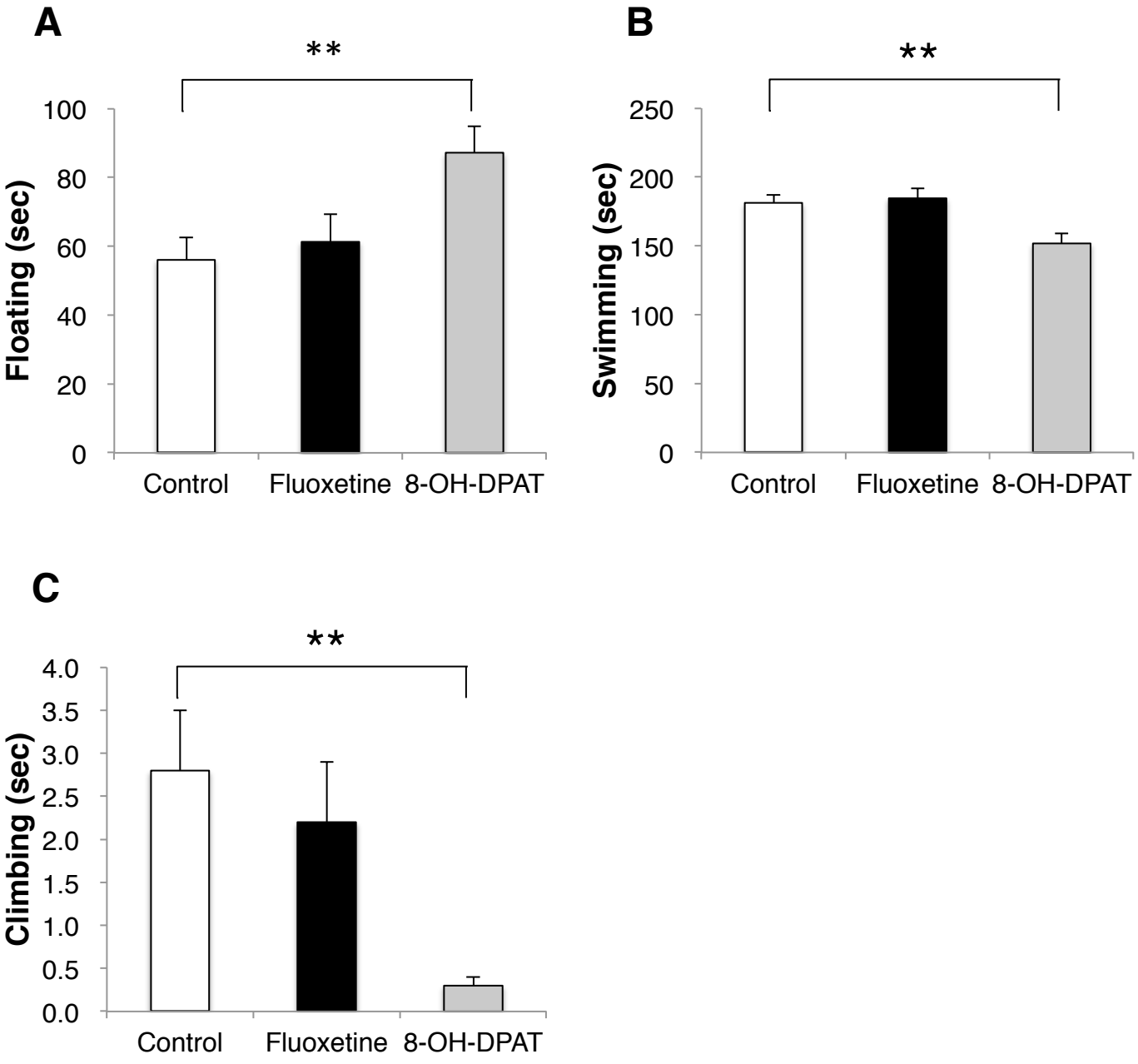

Figure 3. Behavioral despair of depression-like behavior
Depression-like behavior in the forced swim test in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) Floating time. (B) Swimming time. (C) Climbing time. Each bar represents the mean ± S.E.M (n=14 for control, n=13 for fluoxetine, n=13 for 8-OH-DPAT). ** $p < 0.01$

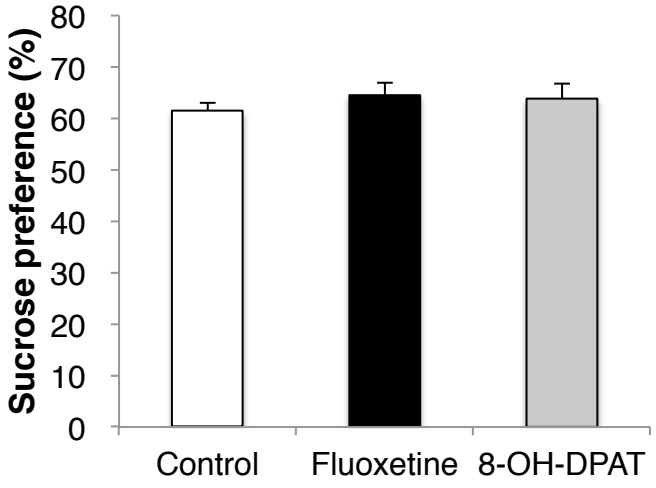

Figure 4. Anhedonia of depression-like behavior
Depression-like behavior in the sucrose preference test in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. Each bar represents the mean ± S.E.M of sucrose preference (n=14 for control, n=13 for fluoxetine, n=14 for 8-OH-DPAT).

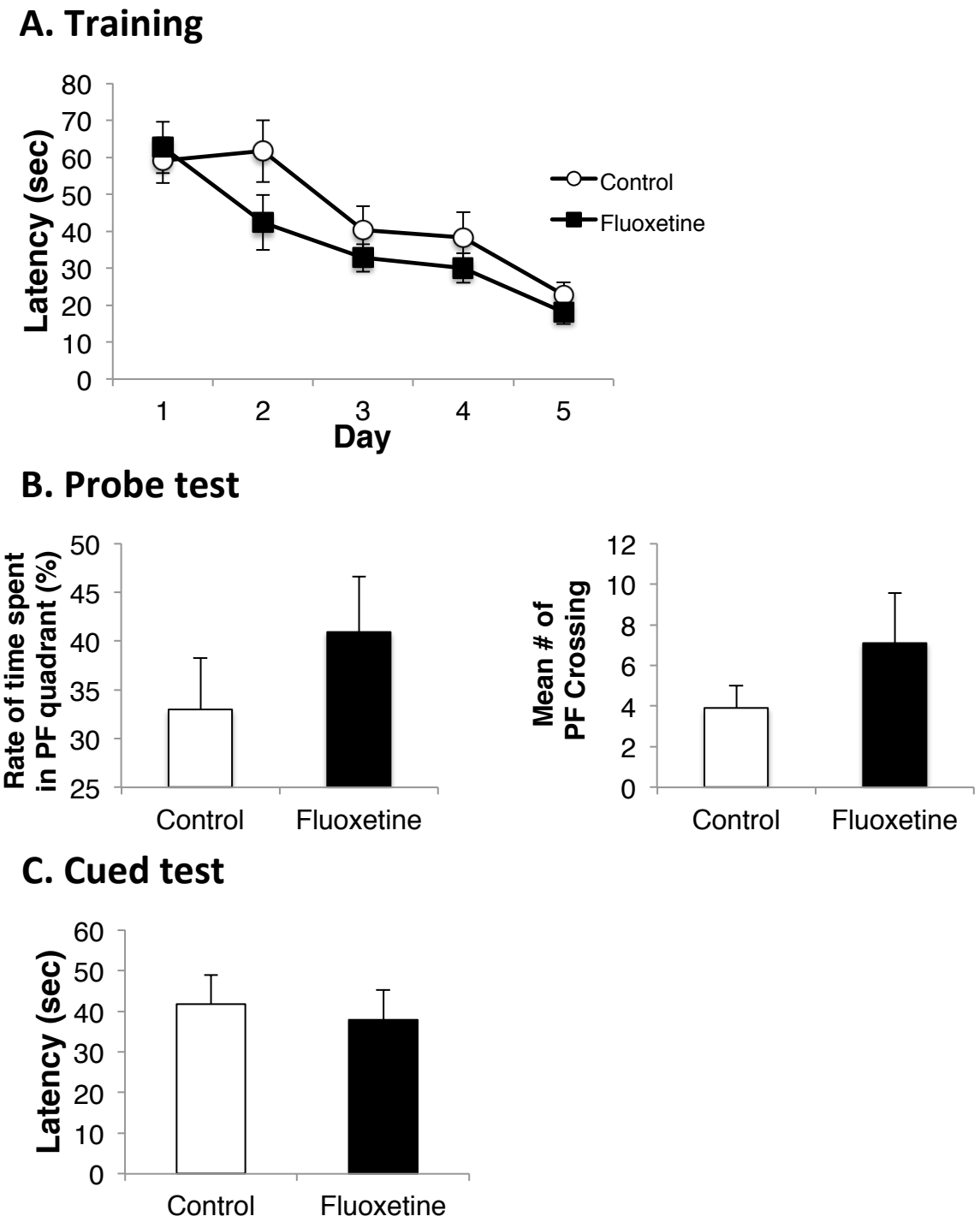

Figure 5. Spatial learning and memory in fluoxetine treated mice
Spatial learning and memory in the Morris water maze in mice treated with sucrose or fluoxetine from P1-P21. (A) Training. (B) Probe test. (C) Cued test. Each bar represents the mean ± S.E.M. (n=9 for sucrose, n=6 for fluoxetine).

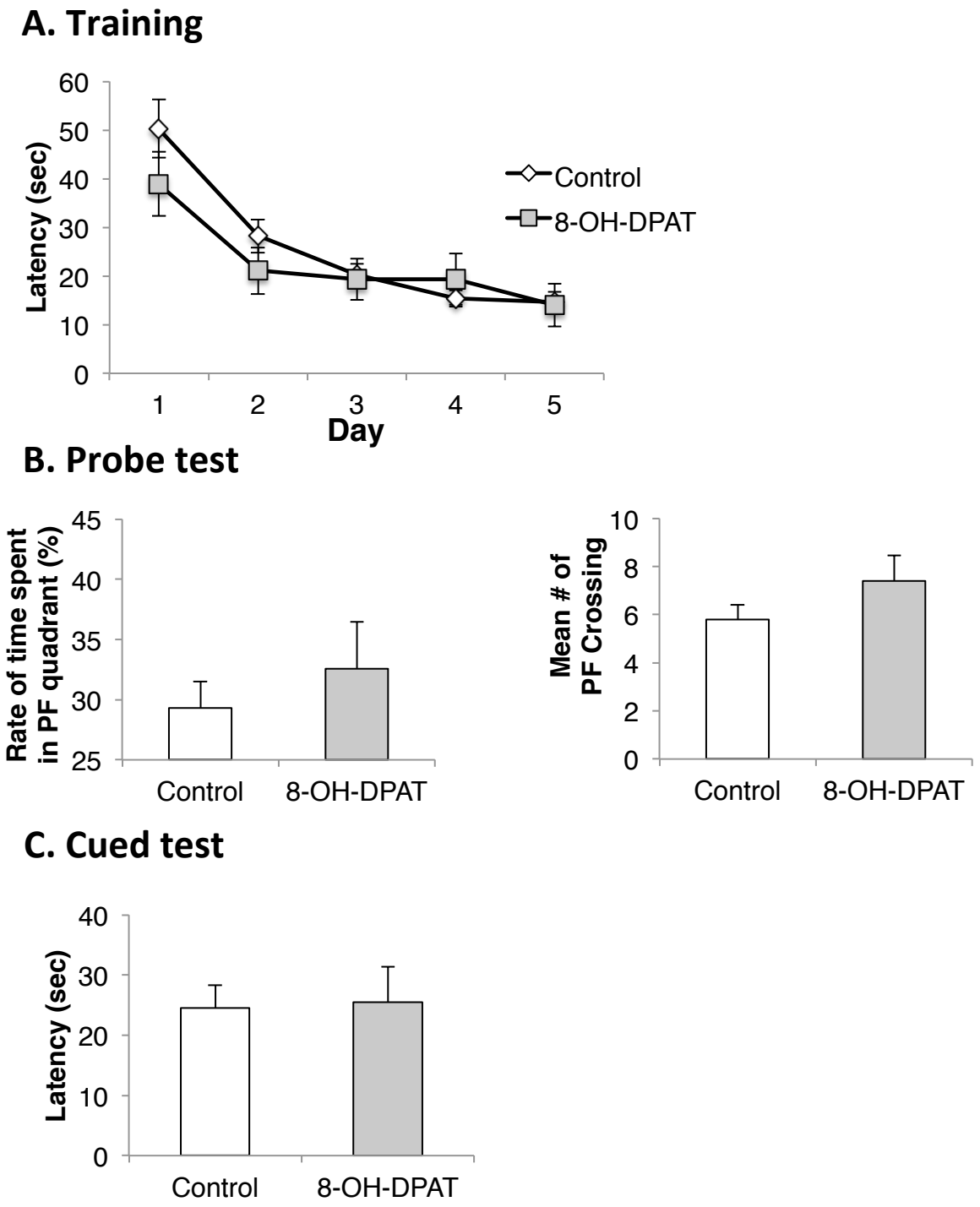

Figure 6. Spatial learning and memory in 8-OH-DPAT treated mice
Spatial learning and memory in the Morris water maze in mice treated with saline or 8-OH-DPAT from P1-P21. (A) Training. (B) Probe test. (C) Cued test. Each bar represents the mean ± S.E.M. (n=13 for saline, n=8 for 8-OH-DPAT).

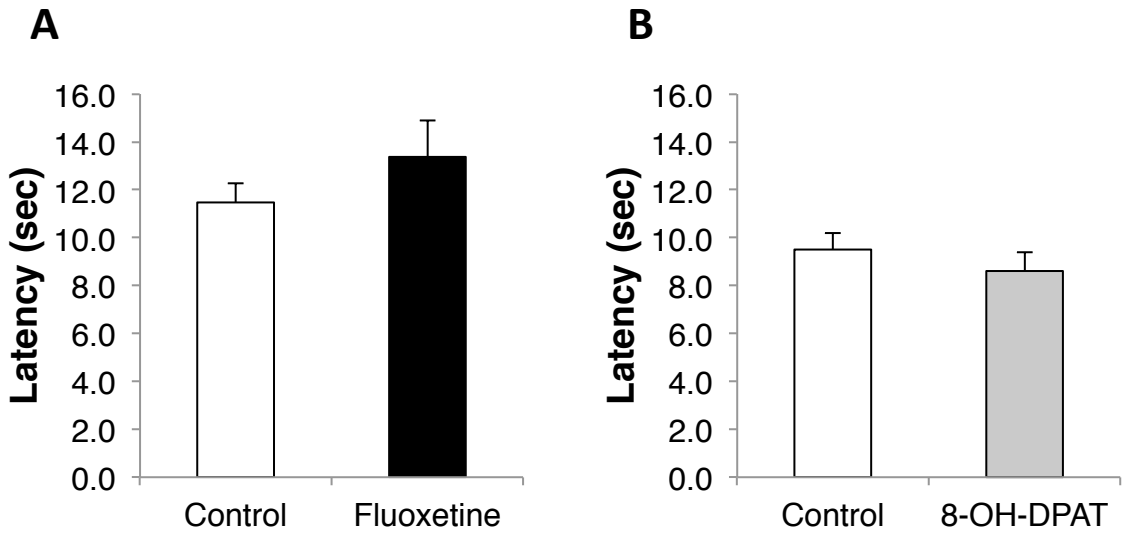

Figure 7. Pain sensitivity
Pain sensitivity in the hot plate test in mice treated with fluoxetine or 8-OH-DPAT from P1-P21. (A) The effects of fluoxetine. (n=10 for control, n=8 for fluoxetine) (B) The effects of 8-OH-DPAT (n=18 for control, n=13 for 8-OH-DPAT). Each bar represents the mean ± S.E.M of latency.

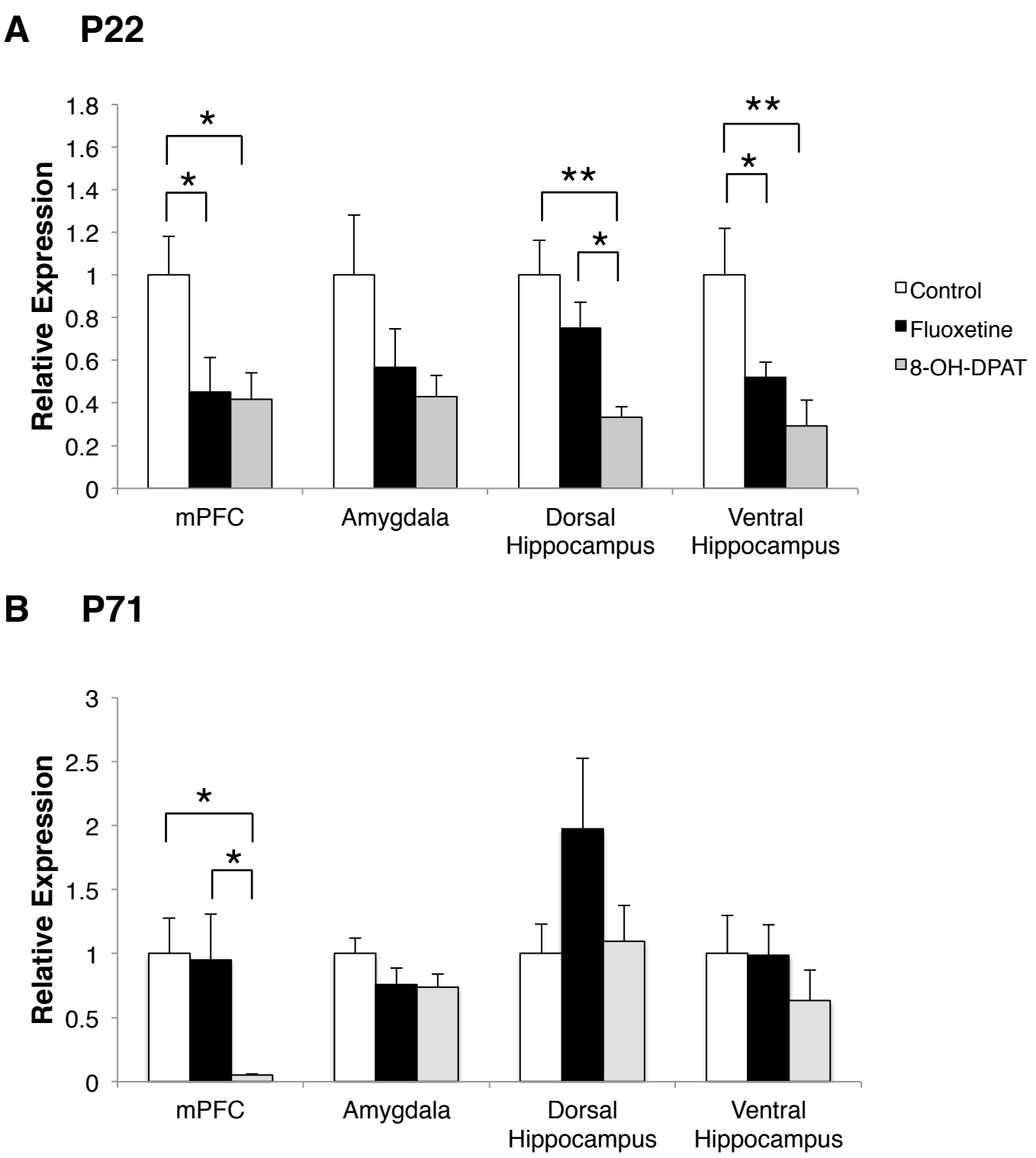

Figure 8. The mRNA expression of the 5-HT_{1A} receptor
The mRNA expression of the 5-HT_{1A} receptor in the mPFC, amygdala, dorsal hippocampus, and ventral hippocampus in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) The mRNA expression of the 5-HT_{1A} receptor at P22. (B) The mRNA expression of the 5-HT_{1A} receptor at P71. Each bar represents the mean ± S.E.M of latency (n=6~10 for each group). * $p < 0.05$, ** $p < 0.01$

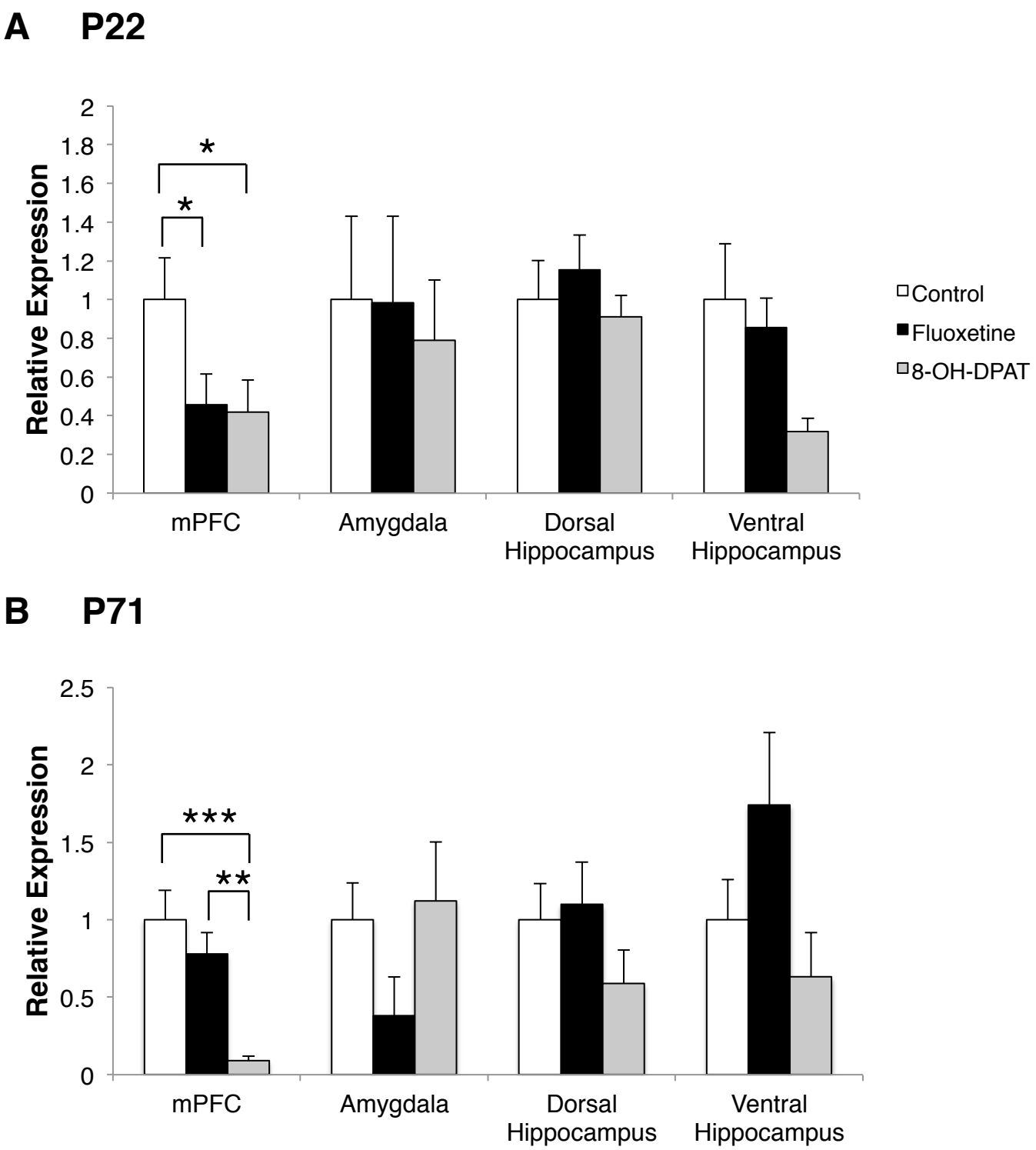

Figure 9. The mRNA expression of BDNF

The mRNA expression of BDNF in the mPFC, amygdala, dorsal hippocampus, and ventral hippocampus in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) The mRNA expression of BDNF at P22. (B) The mRNA expression of BDNF at P71. Each bar represents the mean ± S.E.M of latency (n=5~12 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

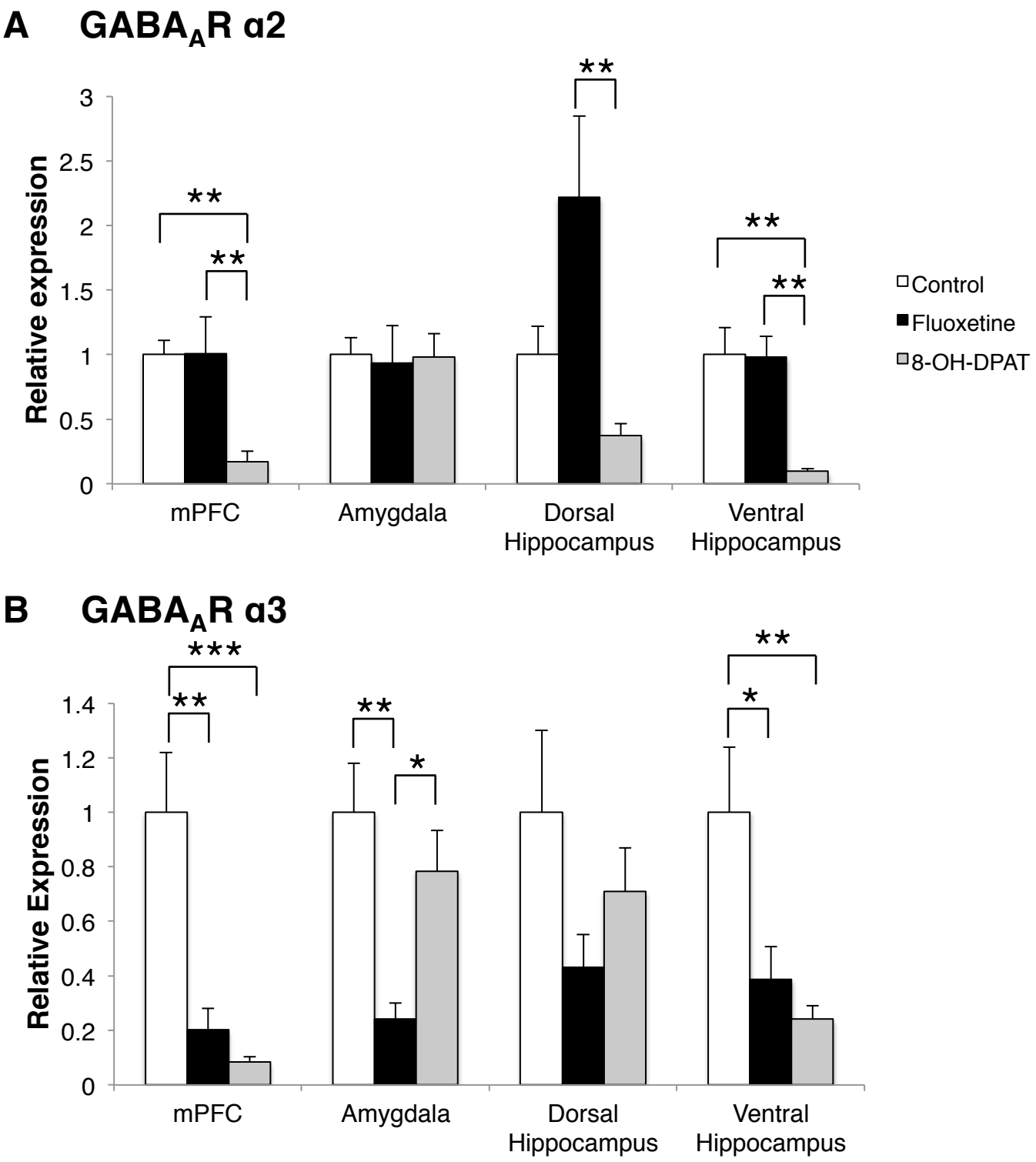

Figure 10. The mRNA expression of the $GABA_A$ receptor

The mRNA expression of the $GABA_A$ receptor α2 and α3 subunits at P71 in the mPFC, amygdala, dorsal hippocampus, and ventral hippocampus in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) The mRNA expression of the $GABA_A$ receptor α2 subunit. (B) The mRNA expression of the $GABA_A$ receptor α3 subunit. Each bar represents the mean ± S.E.M of latency (n=5~10 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

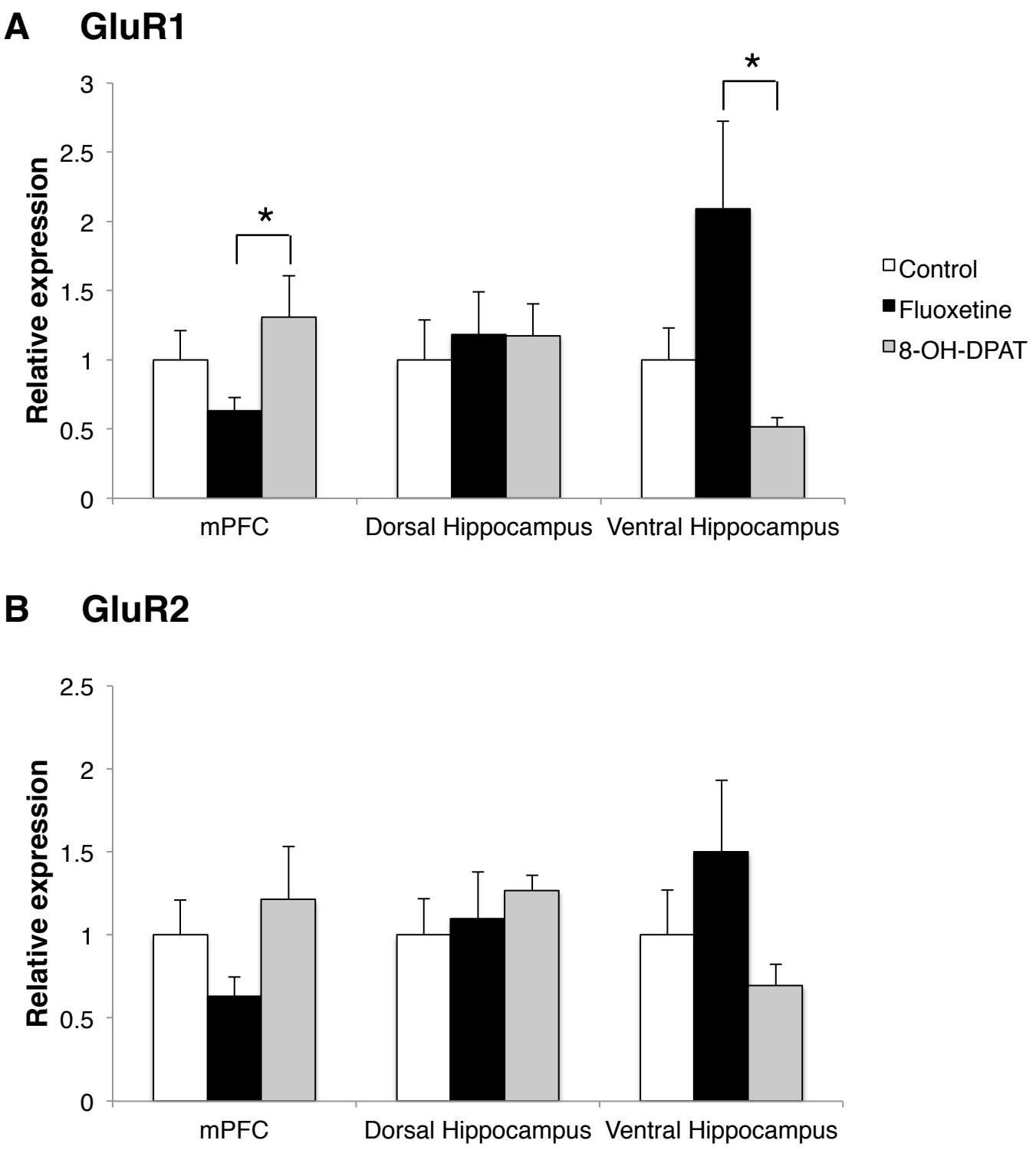

Figure 11. The mRNA expression of the GluR

The mRNA expression of the GluR1 and GluR2 at P71 in the mPFC, dorsal hippocampus, and ventral hippocampus in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) The mRNA expression of the GluR1. (B) The mRNA expression of the GluR2. Each bar represents the mean ± S.E.M of latency (n=6~9 for each group). * $p < 0.05$

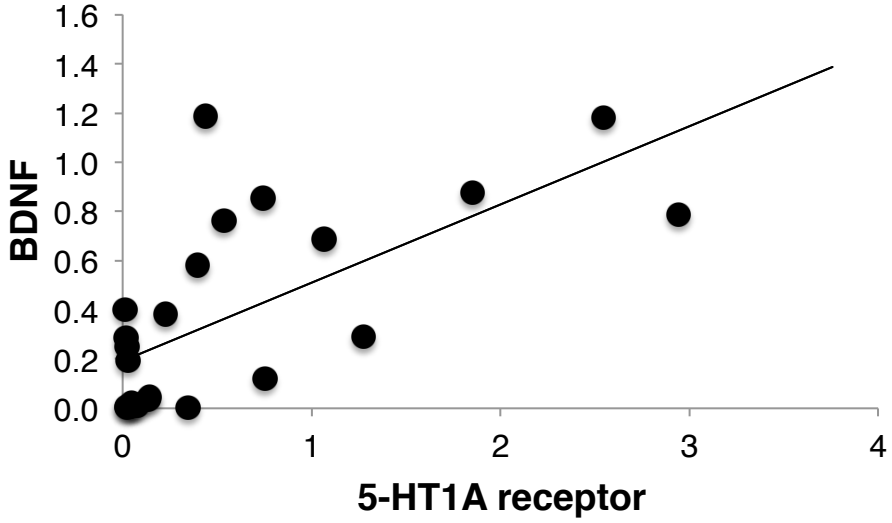

Figure 12. Correlation between the 5-HT_{1A} receptor and BDNF
Correlation between the mRNA expression of the 5-HT_{1A} receptor and BDNF in the mPFC at P71. ($r = 0.664$, $p < 0.001$)

Table 1. Primer sequences for real-time reverse transcription-PCR

Genes	Primer sequences	Tm (°C)	Length (bp)
5-HT_{1A}R	F: 5'-CCGTGAGAGGAAGACAGTGAAGAC-3' R: 5'-GGTTGAGCAGGGAGTTGGAGTAG-3'	60.5 62.2	176
BDNF	F: 5'-GACAAGGCAACTTGGCCTAC-3' R: 5'-ACTGTCACACACGCTCAGCTC-3'	58.4 60.4	353
$GABA_AR$ α2	F: 5'-GAGAATCGGTGCCAGCAAGAA-3' R: 5'-CAGTCCATGGCAGTGGCATAA-3'	64.9 64.2	118
$GABA_AR$ α3	F: 5'-TTTCAGGCCCAGTCACTTTGTTC-3' R: 5'-TTCACACCTCATTACAACTGGCATC-3'	64.7 64.4	80
GluR1	F: 5'-AGCGGACAACCACCATCTCTG-3' R: 5'-AAGGGTCGATTCTGGGATGTTTC-3'	64.8 64.6	80
GluR2	F: 5'-ATGGAACATTAGACTCTGGCTCCAC-3' R: 5'-CTGCCGTAGTCCTCACAAACACA-3'	63.9 64.6	123
18S rRNA	F: 5'-ACTCAACACGGGAAACCTCA-3' R: 5'-AACCAGACAAATCGCTCCAC-3'	56.1 53.9	123

Table 2. Body weights of mice from P1 to P71 treated postnatally with fluoxetine and 8-OH-DPAT.

Age	Control	Fluoxetine	8-OH-DPAT	P value
P1	1.7 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	ns
P7	4.9 ± 0.1	5.1 ± 0.1	4.7 ± 0.1	ns
P14	8.2 ± 0.1	8.5 ± 0.2	8.4 ± 0.1	ns
P21	9.5 ± 0.2	9.4 ± 0.2	9.4 ± 0.1	ns
P71	24.5 ± 0.5	24.5 ± 0.3	24.8 ± 0.4	ns

Unit of body weight is g.
Data are shown as the mean ± S.E.M. ns; no significant effect
P1-P21; n=33 for control, n=34 for fluoxetine, n=34 for 8-OH-DPAT. P71; n=12 for control, n=14 for fluoxetine, n=15 for 8-OH-DPAT.

Table 3. Effects on behaviors in adulthood

		Fluoxetine	8-OH-DPAT
Anxiety		↓	↓
Depression	Behavioral despair	=	↑
	Anhedonia	=	=
Spatial learning and memory		=	=
Pain sensitivity		=	=

↑, increase compared to control; ↓, decrease compared to control; =, no changes compared to control

Table 4. Effects on the mRNA expressions at P22

		Fluoxetine	8-OH-DPAT
$5\text{-}HT_{1A}R$	mPFC	↓	↓
	Amyg	=	↓
	dH	=	↓
	vH	↓	↓
BDNF	mPFC	↓	↓
	Amyg	=	=
	dH	=	=
	vH	=	=

↓, decrease compared to control; =, no changes compared to control
mPFC, medial prefrontal cortex; Amyg, amygdala; dH, dorsal hippocampus; vH, ventral hippocampus

Table 5. Effects on the mRNA expressions at P71

		Fluoxetine	8-OH-DPAT
$5\text{-}HT_{1A}R$	mPFC	=	↓
	Amyg	=	=
	dH	=	=
	vH	=	=
BDNF	mPFC	=	↓
	Amyg	=	=
	dH	=	=
	vH	=	=
$GABA_AR$ α2	mPFC	=	↓
	Amyg	=	=
	dH	=	=
	vH	=	↓
$GABA_AR$ α3	mPFC	↓	↓
	Amyg	↓	=
	dH	=	=
	vH	↓	↓
GluR1	mPFC	=	=
	Amyg	=	=
	dH	=	=
GluR2	vH	=	=
	mPFC	=	=
	Amyg	=	=

↓, decrease compared to control; =, no changes compared to control
mPFC, medial prefrontal cortex; Amyg, amygdala; dH, dorsal hippocampus; vH, ventral hippocampus