The postnatal 5-HT_{1A} receptor regulates adult anxiety and depression differently via multiple molecules

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ABSTRACT

Serotonin (5-HT) and the 5-HT_{1A} receptor during development are known to modulate anxiety and depression in later life. However, the brain mechanisms linking the postnatal 5-HT system and adult behavior remain unknown. Here, we examined the effects of pharmacological 5-HT_{1A} receptor activation during the postnatal period on anxiety and depression-like behavior in adult BALB/c male mice. To elucidate the underlying mechanisms, we measured mRNA expression of the 5-HT_{1A} receptor, brain-derived neurotrophic factor (BDNF), $GABA_A$ receptor subunits, and AMPA receptor subunits in the medial prefrontal cortex (mPFC), amygdala, and hippocampus. Treatment with the selective 5-HT reuptake inhibitor (fluoxetine) and 5-HT_{1A} receptor agonist (8-OH-DPAT) during the postnatal period decreased anxiety-like behavior in adulthood, whereas only 8-OH-DPAT treatment increased depression-like behavior. Concomitantly with the behavioral effects, postnatal treatment with fluoxetine and 8-OH-DPAT decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the mPFC and ventral hippocampus in adulthood, while 8-OH-DPAT, but not fluoxetine, 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 ventral hippocampus. On the basis of the correlative changes between behavior and mRNA

expression, these results suggest that the $GABA_A$ receptor α3 subunit in the mPFC and ventral hippocampus may regulate anxiety-like behavior. In contrast, depression-like behavior may be regulated by the $5\text{-}HT_{1A}$ receptor and BDNF in the mPFC and by the $GABA_A$ receptor α2 subunit in the mPFC and ventral hippocampus. In summary, activation of the $5\text{-}HT_{1A}$ 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.

Keywords:

$5\text{-}HT_{1A}$ receptor; $GABA_A$ receptor; BDNF; anxiety; depression

1. Introduction

Early-life experiences are known to alter brain development and behaviors in later life. For example, many studies have shown that prolonged maternal separation during the postnatal period increases both anxiety and depression levels in adulthood and impairs brain development including hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and low neurotrophin levels (Nishi et al., 2014; Tractenberg et al., 2016). In addition, early-life chronic stress induces disabilities in learning and memory in adulthood together with structural changes, including loss of dendrites, dendritic spines, and excitatory synapses (McClelland et al., 2011). In contrast to early-life adverse experiences, postnatal brief maternal separation reduces anxiety-like behavior and enhances spatial learning and memory in adulthood, which is accompanied by lower HPA axis responsiveness (Nishi et al., 2014; Raineki et al., 2014). Thus, alterations in brain structures and functions may cause behavioral changes in later life. However, the brain mechanisms (molecules and brain regions), that link early-life experiences to behavioral changes in later lifer are not well known.

Serotonin (5-hydroxytryptamine; 5-HT) regulates various brain functions, and malfunction of the 5-HT system during development is closely related to mood disorders in adulthood (Daubert and Condron, 2010). In addition to effects on behavior, 5-HT has neurotrophic activity during development to regulate the formation of neural circuits (Whitaker-Azmitia, 2001). Thus, the roles of 5-HT during development are important for the development of brain and behavior.

There are at least 14 5-HT receptors, among which the 5-HT_{1A} receptor is well

characterized. Similar to the action of 5-HT, the 5-HT_{1A} receptor modulates dendrite formation and synaptogenesis (Wilson et al., 1998; Yoshida et al., 2011; Mogha et al., 2012) and affects behavior in later life. Interestingly, previous studies suggested that the 5-HT_{1A} receptor during the postnatal period has different effects on anxiety and depression-like behavior in adulthood. 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, 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). However, the brain mechanisms linking the 5-HT_{1A} receptor during the postnatal period to anxiety or depression in adulthood are not known.

One of the candidate molecules regulating anxiety in response to 5-HT is 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 selective 5-HT reuptake inhibitors (SSRIs) show their effects after several weeks of treatment (Vaswani et al., 2003). Among the 19 $GABA_A$ receptor subunits, the α2 and α3 subunits modulate anxiety-like behavior. The diazepam-induced anxiolytic effect is absent in mice with point mutation of the α2 subunit, suggesting that the α2 subunit has an anxiolytic effect (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).

On the other hand, brain-derived neurotrophic factor (BDNF) and the glutamate receptor have been shown to be involved in depression. Similar to 5-HT, BDNF contributes to various

functions in the developing and mature brain, including survival and differentiation of neurons, dendrite formation, synaptogenesis, and synaptic plasticity (Park and Poo, 2013). Moreover, dysfunction of BDNF is related to depression (Nestler, 2002). For example, both acute and chronic stresses decrease the expression of BDNF in the hippocampus, and antidepressants increase the expression of BDNF to recover stress-induced reduction of BDNF (Nestler, 2002).

Besides the 5-HT system, glutamate receptors are known as a target of antidepressants. Recently, it was shown that ketamine has acute antidepressant activity (Murrough, 2012). Ketamine shows antidepressant effects in a few hours to several days, whereas SSRI requires a few weeks to show antidepressant effects. In addition to NMDA receptor antagonism, ketamine potentiates AMPA receptors, which have a crucial role in the antidepressant effect (Koike and Chaki, 2014). Most AMPA receptors are composed of the GluR1 and GluR2 subunits (Derkach et al., 2007), and these subunits are involved in long-term potentiation (LTP) and long-term depression (LTD) in synaptic plasticity (Huganir and Nicoll, 2013).

In the present study, we first examined the effects of postnatal treatment with SSRI and the 5-HT_{1A} receptor agonist on adult behaviors such as anxiety and depression. Next, to elucidate the underlying brain mechanisms of anxiety and depression, we measured the mRNA expression of the 5-HT_{1A} receptor, BDNF, the $GABA_A$ receptor α2 and α3 subunits, and the AMPA GluR1, and GluR2 subunits in the mPFC, amygdala, and dorsal and ventral hippocampi during the postnatal period and adulthood. By comparing the changes in behaviors with those in the mRNA expressions induced by SSRI and the 5-HT_{1A} receptor agonist, we found some candidate genes, that may regulate adult behaviors.

2. Materials and methods

2.1. 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 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.

2.2. Drug treatment

Offspring were randomly assigned to 3 groups; control group, treatment with the selective 5-HT reuptake inhibitor (SSRI) group, and treatment with the 5-HT_{1A} receptor agonist group. Pups from each group received an oral administration of 5% sucrose (control), 5 mg/kg fluoxetine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) (SSRI), 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 was decided on the basis of a previous study (Ishiwata et al., 2005).

2.3. Elevated plus maze (EPM)

All behavioral tests were conducted from postnatal week 9 to 10. To investigate the effects on anxiety-like behavior, the offspring of each experimental group were tested 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 the open arms were calculated as indices of anxiety-like behavior.

2.4. 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 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 subtracting the floating and the climbing times from the total testing period.

2.5. 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 the total volume of sucrose and water consumed during the 4-hr test.

2.6. 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) (Supplemental Fig. S1). The dorsal and ventral halves of the hippocampus were dissected out 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).

2.7. Real-time reverse transcription-PCR

Real-time reverse transcription-PCR was performed as previously described (Akatsu et al., 2015). In brief, each region of the left hemisphere 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℃ for 10 sec, followed by 50 cycles at 95℃ for 5 sec and at 60℃ for 30 sec. Table 1 lists the primer sequences. The BDNF, GluR1, and GluR2 genes have splicing variants. The primers we used 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.

2.8. Statistical analysis

Using SPSS (IBM, Armonk, NY, USA), the data were analyzed using one-way analysis of variance (ANOVA), followed by a post hoc test (Tukey or Bonferroni). $p < 0.05$ was considered as statistically significant. All data are expressed as means ± S.E.M.

3. Results

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

During the drug treatment from P1 to P21, the main-day effect on body weight was significant ($F(20,1960) = 2624.008$, $p < 0.001$), but the interaction between day and group was not significant (Supplemental Table 1). Thus, the drug treatment had no effect on the

body weight.

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

We used the EPM to examine the effects of postnatal treatment with fluoxetine and 8-OH-DPAT on anxiety-like behavior in adulthood (Fig. 1). ANOVA revealed significant main effects on the time spent in open arms ($F(2,38) = 8.788$, $p < 0.001$) (Fig. 1A), the ratios of entries into open arms ($F(2,38) = 9.152$, $p < 0.001$) (Fig. 1B), and the total number of entries into both open and closed arms ($F(2,38) = 4.104$, $p < 0.05$) (Fig. 1C). The post hoc test showed that postnatal treatment with both fluoxetine and 8-OH-DPAT increased the time spent in open arms (fluoxetine, $p < 0.05$; 8-OH-DPAT, $p < 0.01$) (Fig. 1A) and the entries into open arms (fluoxetine, $p < 0.01$; 8-OH-DPAT, $p < 0.01$) (Fig. 1B), 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 ($p < 0.05$), whereas fluoxetine treatment had no effect as compared with the control mice (Fig. 1C). Thus, these results suggest that treatment with fluoxetine and 8-OH-DPAT during the postnatal period decreased anxiety-like behavior in adulthood.

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

We used the FST (Fig.2) and the SPT (Fig. 3) to examine the effects of postnatal treatment with fluoxetine and 8-OH-DPAT on adult depression-like behavior. 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, we 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. 2A), swimming ($F(2,37) = 6.841$, $p < 0.01$) (Fig. 2B), and climbing ($F(2,37) = 4.851$, $p < 0.05$) (Fig. 2C) times. Postnatal 8-OH-DPAT treatment increased the floating time ($p < 0.05$) (Fig. 2A), whereas it decreased the swimming ($p < 0.05$) (Fig. 2B) and climbing ($p < 0.05$) (Fig. 2C) times. Postnatal fluoxetine treatment had no effects on the floating (Fig. 2A), swimming (Fig. 2B), and climbing (Fig. 2C) times.

In the SPT, ANOVA revealed no significant main effect on the sucrose preference ($F(2,38) = 0.466$, $p = 0.631$) (Fig. 3).

Thus, treatment with 8-OH-DPAT but not with fluoxetine during the postnatal period increased the the depression-like behavior of behavioral despair in adulthood.

3.4. Postnatal treatment with fluoxetine and 8-OH-DPAT did not affect spatial learning and memory and pain sensitivity in the adulthood

We also examined the effects of postnatal fluoxetine and 8-OH-DPAT on adult spatial learning and memory and pain sensitivity in adulthood (Supplemental Figs. S2-S4). In the 5 day-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 (Supplemental Figs. S2A, S3A). The probe test showed no significant effects on the time spent in the platform quadrant and the number of crossings of the platform (Supplemental Figs. S2B, S3B). The cued test also showed no significant effects (Supplemental Figs. S2C, S3C).

In the hot plate test, neither postnatal fluoxetine nor 8-OH-DPAT affected the reaction time (Supplemental Fig. S4).

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

We measured the mRNA expression of the 5-HT_{1A} receptor at P22 and P71 to examine the chronic effects of fluoxetine and 8-OH-DPAT (Fig. 4). ANOVA revealed significant main effects on the mRNA expression of the 5-HT_{1A} receptor at P22 in the mPFC ($F(2,23) = 4.228$, $p < 0.05$), the dorsal hippocampus ($F(2, 22) = 6.061$, $p < 0.01$), and the ventral hippocampus ($F(2,23) = 5.017$, $p < 0.05$), but not in the amygdala. 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 ($p < 0.05$). The treatment with 8-OH-DPAT also decreased the mRNA expression of the 5-HT_{1A} receptor in the dorsal hippocampus ($p < 0.01$) and the ventral hippocampus ($p < 0.05$) at P22 (Fig. 4A).

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 or the hippocampus. The treatment with 8-OH-DPAT decreased the mRNA expression of the 5-HT_{1A} receptor in the mPFC ($p < 0.05$) (Fig. 4B). These results suggest that the effect of postnatal treatment with 8-OH-DPAT on the mRNA expression of the 5-HT_{1A} receptor in the mPFC is maintained in adulthood.

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

Next, we investigated the mRNA expression of BDNF at P22 and P71 (Fig. 5). ANOVA revealed a marginally significant main effect on the mRNA expression of BDNF at P22 in the mPFC ($F(2,23) = 3.132$, $p = 0.063$), but not in the amygdala or the hippocampus. The post-hoc test revealed that the treatments with fluoxetine and 8-OH-DPAT during the postnatal period had some tendencies to decrease the mRNA expression of BDNF in the mPFC at P22 (fluoxetine, $p = 0.11$; 8-OH-DPAT, $p = 0.082$) (Fig. 5A).

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 or the hippocampus. The treatment with 8-OH-DPAT during the postnatal period decreased the mRNA expression of BDNF in the mPFC at P71 ($p < 0.001$) (Fig. 5B).

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

We measured the mRNA expression of the $GABA_A$ receptor α2 subunit at P71 (Fig. 6A). ANOVA revealed significant main effects on the $GABA_A$ receptor α2 subunit in the mPFC ($F(2,16) = 8.646$, $p < 0.01$), the dorsal hippocampus ($F(2,20) = 4.546$, $p < 0.05$), and the ventral hippocampus ($F(2,17) = 9.782$, $p < 0.001$), but not in the amygdala. The treatment with 8-OH-DPAT during the postnatal period decreased the mRNA expression of the $GABA_A$ receptor α2 subunit in the mPFC and the ventral hippocampus, as compared with the control (mPFC, $p < 0.01$; ventral hippocampus, $p < 0.01$) and the treatment with fluoxetine (mPFC, $p < 0.05$; ventral hippocampus, $p < 0.01$). In addition, the treatment with 8-OH-DPAT during the postnatal period decreased the $GABA_A$ receptor α2 subunit in the dorsal hippocampus, as compared with the treatment with fluoxetine ($p < 0.05$).

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

We measured the mRNA expression of the $GABA_A$ receptor α3 subunit at P71 (Fig. 6B). ANOVA revealed significant main effects 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. Both the treatment with fluoxetine and 8-OH-DPAT during the postnatal period decreased the $GABA_A$ receptor α3 subunit mRNA expression in the mPFC ($p < 0.01$) and ventral hippocampus ($p < 0.05$). In addition, only the treatment with fluoxetine decreased the $GABA_A$ receptor α3 subunit mRNA expression in the amygdala ($p < 0.01$).

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

We measured the mRNA expression of GluR1 and GluR2 at P71 as presumptive mediators of depression-like behavior (Fig. 7). ANOVA revealed a significant main effect on the mRNA expression of GluR1 in the ventral hippocampus ($F(2,21) = 3.557$, $p < 0.05$), but not in the mPFC or the dorsal hippocampus (Fig. 7A). The treatment with fluoxetine increased the mRNA expression of GluR1 in the ventral hippocampus, 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 or the dorsal and ventral hippocampi (Fig. 7B).

4. Discussion

Early-life experiences are known to alter brain structures and functions in later life. Malfunction of 5-HT and the 5-HT_{1A} receptor during development is closely related to mood disorders 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 8-OH-DPAT treatment increased the depression-like behavior in terms of behavioral despair in adulthood (Supplemental Table 2). To elucidate the brain mechanisms that regulate anxiety and depression, we examined the fluoxetine and 8-OH-DPAT-induced changes in the mRNA expression. Both the treatment with fluoxetine and 8-OH-DPAT decreased the mRNA expression of the $GABA_A$ receptor α3 subunit in the ventral hippocampus. 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 (Supplemental Table 3). Therefore, we found some correlative changes between adult anxiety and depression and the mRNA expression of $GABA_A$ receptor α subunits and BDNF in response to postnatal treatment with fluoxetine and 8-OH-DPAT.

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 mice (Gross et al., 2002). Both 5-HT_{1A} receptor antagonist treatment from P0 to P21 and conditional knockout of the 5-HT_{1A}

receptor until P21 increased adult anxiety-like behavior (Gross et al., 2002; Vinkers et al., 2010). However, there are some discrepancies in the effects of fluoxetine on anxiety-like behavior. In the previous studies, early postnatal fluoxetine treatment increased anxiety in 129/Sv mice (Ansorge et al., 2004 and 2008; Rebello et al., 2014), which is an opposite effect to that found in the present study. These differences in the effects of fluoxetine may be attributable to strain differences of tryptophan hydroxylase 2 (TPH-2). TPH-2 is the rate-limiting enzyme in brain 5-HT synthesis and a single nucleotide polymorphism (SNP) was reported in C1473G (Zhang et al., 2004). Depending on the SNP, BALB/c mice show reduced synthesis of 5-HT as compared with 129/Sv mice. Fluoxetine treatment of BALB/c mice may increase the amount of brain 5-HT suitable for the regulation of anxiety. Interestingly, different effects on depression of citalopram, another SSRI, were reported between 129/Sv and BALB/c mice: citalopram treatment reduced depression in adult 129/Sv mice but did not in BALB/c mice (Cervo et al., 2005). These results suggest that the effects of SSRI on anxiety and depression may be dependent on the TPH-2 genotype as well as on the period of the treatment. We must be careful to consider the SNP in human TPH-2 (G1463A) (Zhang et al., 2005), when treating anxiety and mood disorders.

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

In the present study, the postnatal treatment with 8-OH-DPAT decreased 5-HT_{1A} receptor mRNA expression both in the mPFC and in the hippocampus at P22, but this reduction in 5-HT_{1A} receptor mRNA expression 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 mRNA expression of the 5-HT_{1A} receptor and BDNF in the adult mPFC, and concurrently increased depression-like behavior in the adulthood. On the basis of the correlations between the mRNA expression and behavior in adulthood, it is be possible that the 5-HT_{1A} receptor and BDNF 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 reduction in 5-HT_{1A} receptor activity in the adult mPFC may increase depression-like behavior. In addition, the findings suggest that the 5-HT_{1A} receptor has opposite effects on 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.

Besides the 5-HT_{1A} receptor, many studies including postmortem studies, pharmacological studies using antidepressants, and BDNF polymorphism studies have reported strong correlations 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 the 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 ($r = 0.664$, $p < 0.001$) (Supplemental Fig. S5). Therefore, reduced 5-HT_{1A} receptor in adulthood possibly downregulates the expression of BDNF in the mPFC, which may induce depression-like behavior.

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 $GABA_A$ 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.

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 in 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 $GABA_A$ receptor α3 subunit mRNA expression in the 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). This discrepancy between the present and previous studies suggests that $GABA_A$ receptor α3 subunit in the ventral hippocampus may not play a critical role in the anxiolytic effect induced 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 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 might mediate the reduction of the $GABA_A$ receptor α3 subunit in the amygdala.

The GluR1 and GluR2 subunits of AMPA receptors were reported to modulate depression (Martinez-Turrilas et al., 2002; Tan et al., 2006). However, the present study showed no significant changes in GluR1 and GluR2 mRNA expression 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 GluR1 or GluR2 expression in the mPFC and the hippocampus.

5. Conclusion

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

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (26640024) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. We would like to thank Dr. T. Masuda, University of Tsukuba, for valuable suggestions regarding this study and we would also like to thank Ms. Flaminia Miyamasu, Medical English Communications Center, University of Tsukuba, for grammatical review and advice.

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Table

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

Genes	Primer sequences	Tm (℃)	Length
5-HT_{1A}R	F: 5'-CCGTGAGAGGAAGACAGTGAAGAC-3'	60.5	176 bp
	R: 5'-GGTTGAGCAGGGAGTTGGAGTAG-3'	62.2	
BDNF	F: 5'-GACAAGGCAACTTGGCCTAC-3'	58.4	353 bp
	R: 5'-ACTGTCACACACGCTCAGCTC-3'	60.4	
$GABA_A$Rα2	F: 5'-GAGAATCGGTGCCAGCAAGAA-3'	64.9	118 bp
	R: 5'-CAGTCCATGGCAGTGGCATAA-3'	64.2	
$GABA_A$Rα3	F: 5'-TTTCAGGCCCAGTCACTTTGTTC-3'	64.7	80 bp
	R: 5'-TTCACACCTCATTACAACTGGCATC-3'	64.4	
GluR1	F: 5'-AGCGGACAACCACCATCTCTG-3'	64.8	80 bp
	R: 5'-AAGGGTCGATTCTGGGATGTTTC-3'	64.6	
GluR2	F: 5'-ATGGAACATTAGACTCTGGCTCCAC-3'	63.9	123 bp
	R: 5'-CTGCCGTAGTCCTCACAAACACA-3'	64.6	
18S rRNA	F: 5'-ACTCAACACGGGAAACCTC-3'	56.1	123 bp
	R: 5'-AACCAGACAAATCGCTCCAC-3'	53.9	

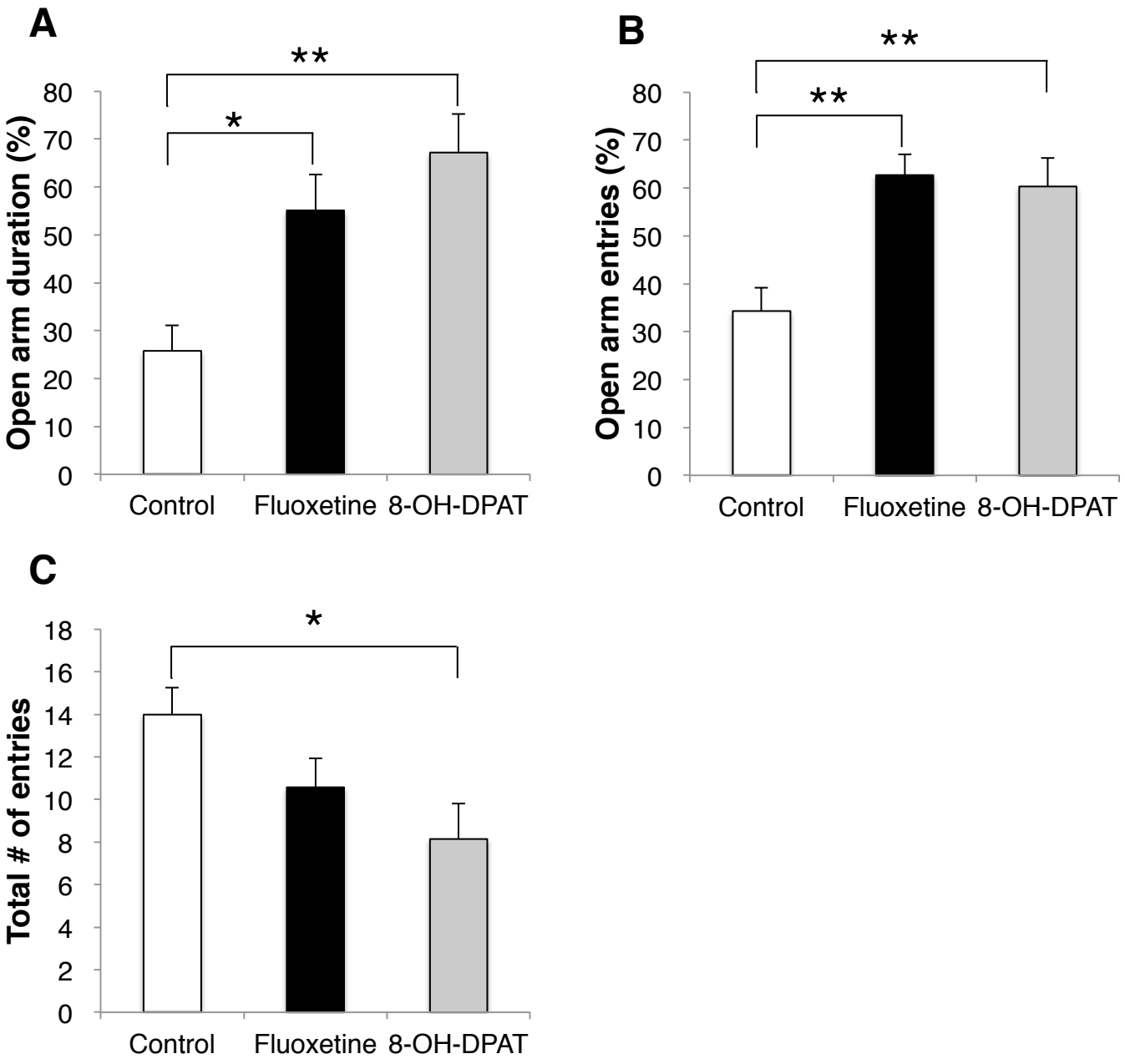

Figure 1

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). * $p < 0.05$, ** $p < 0.01$

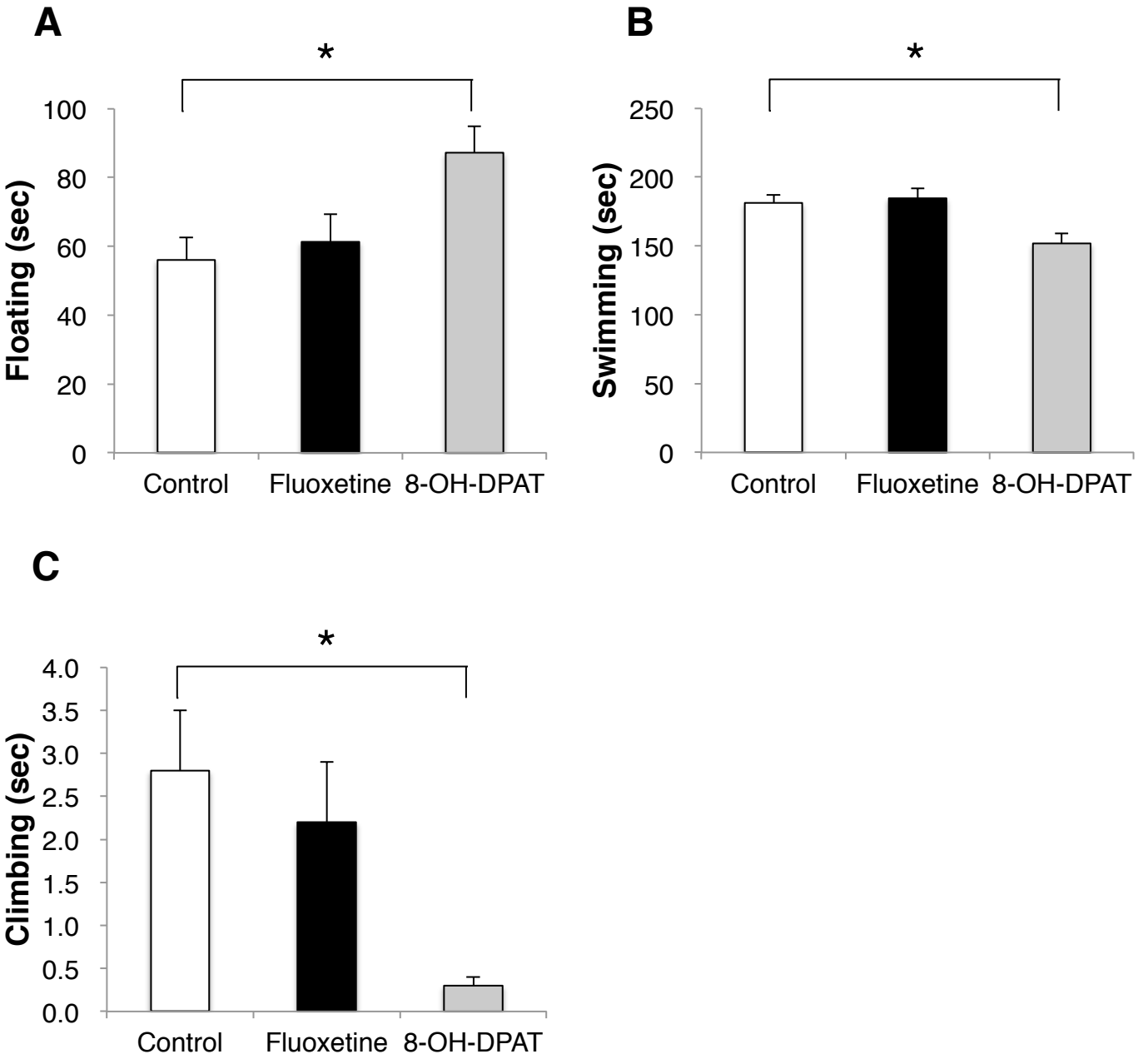

Figure 2

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). * $p < 0.05$

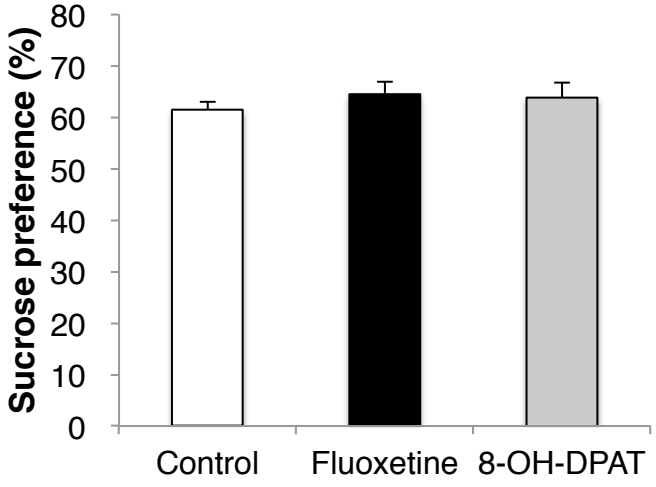

Figure 3
Depression-like behavior in the sucrose preference test in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. Each bar represents the mean ± S.E.M of sucrose preference (n=14 for control, n=13 for fluoxetine, n=14 for 8-OH-DPAT).

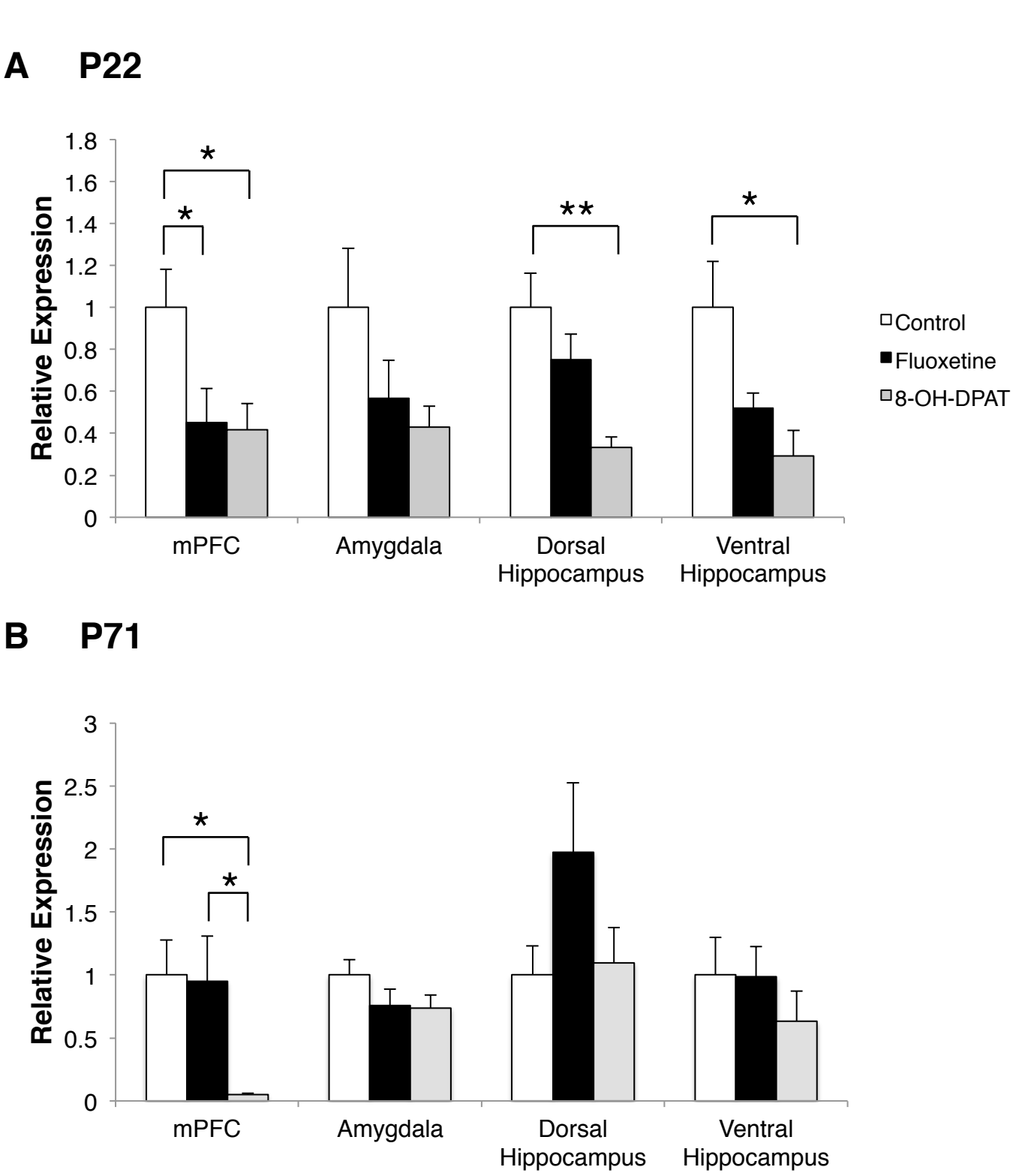

Figure 4

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). * $p < 0.05$, ** $p < 0.01$

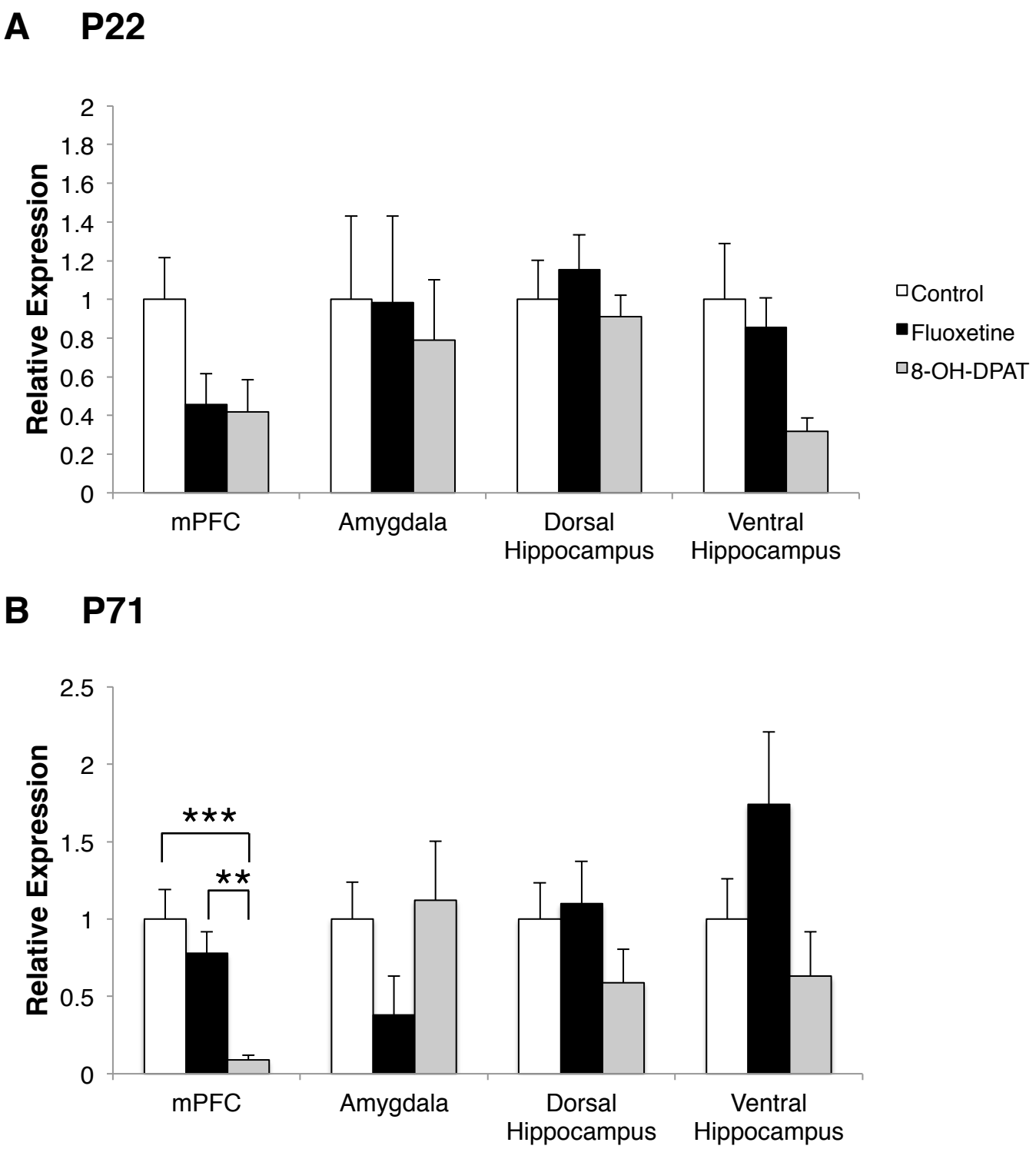

Figure 5

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). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

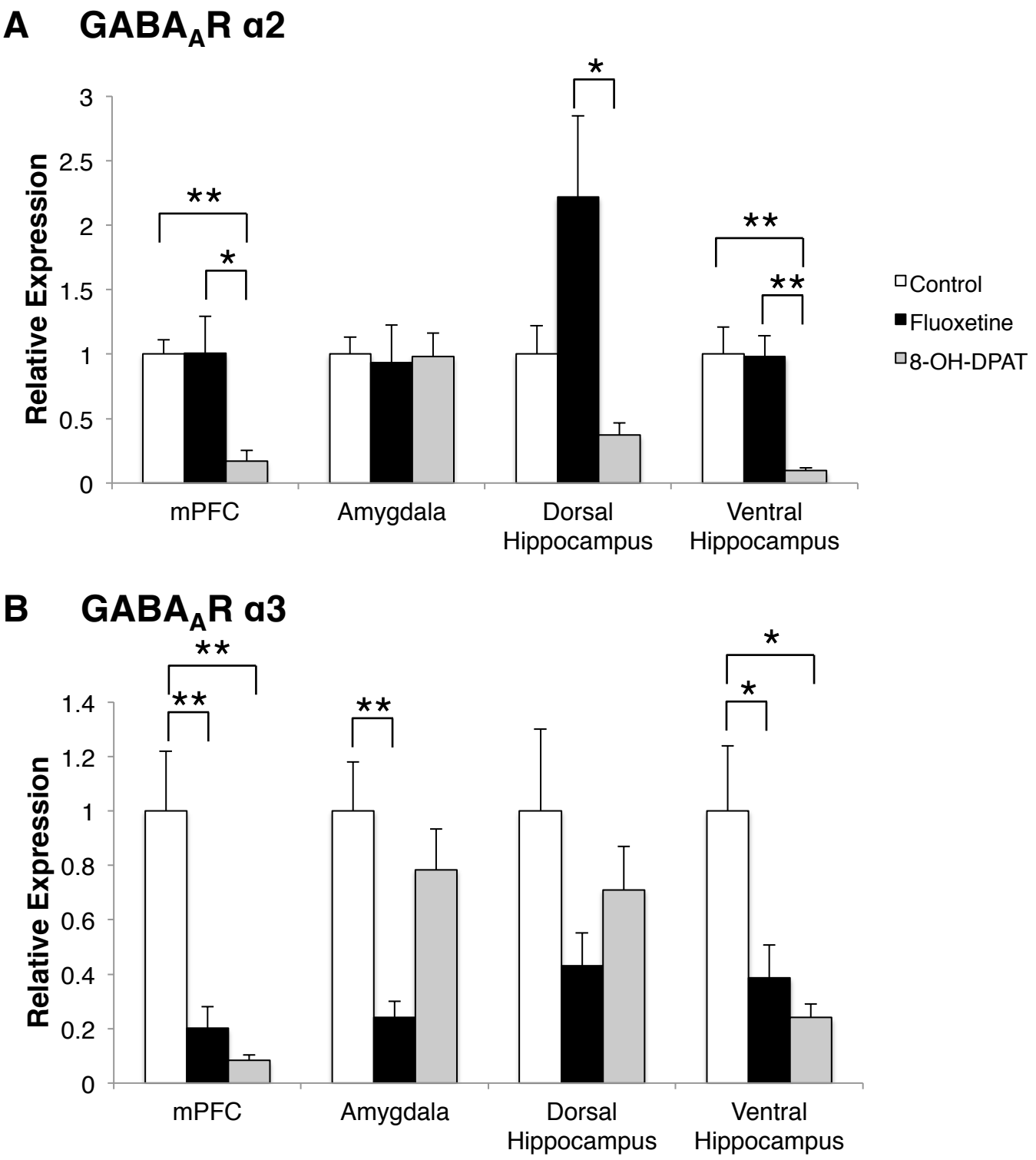

Figure 6

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). * $p < 0.05$, ** $p < 0.01$

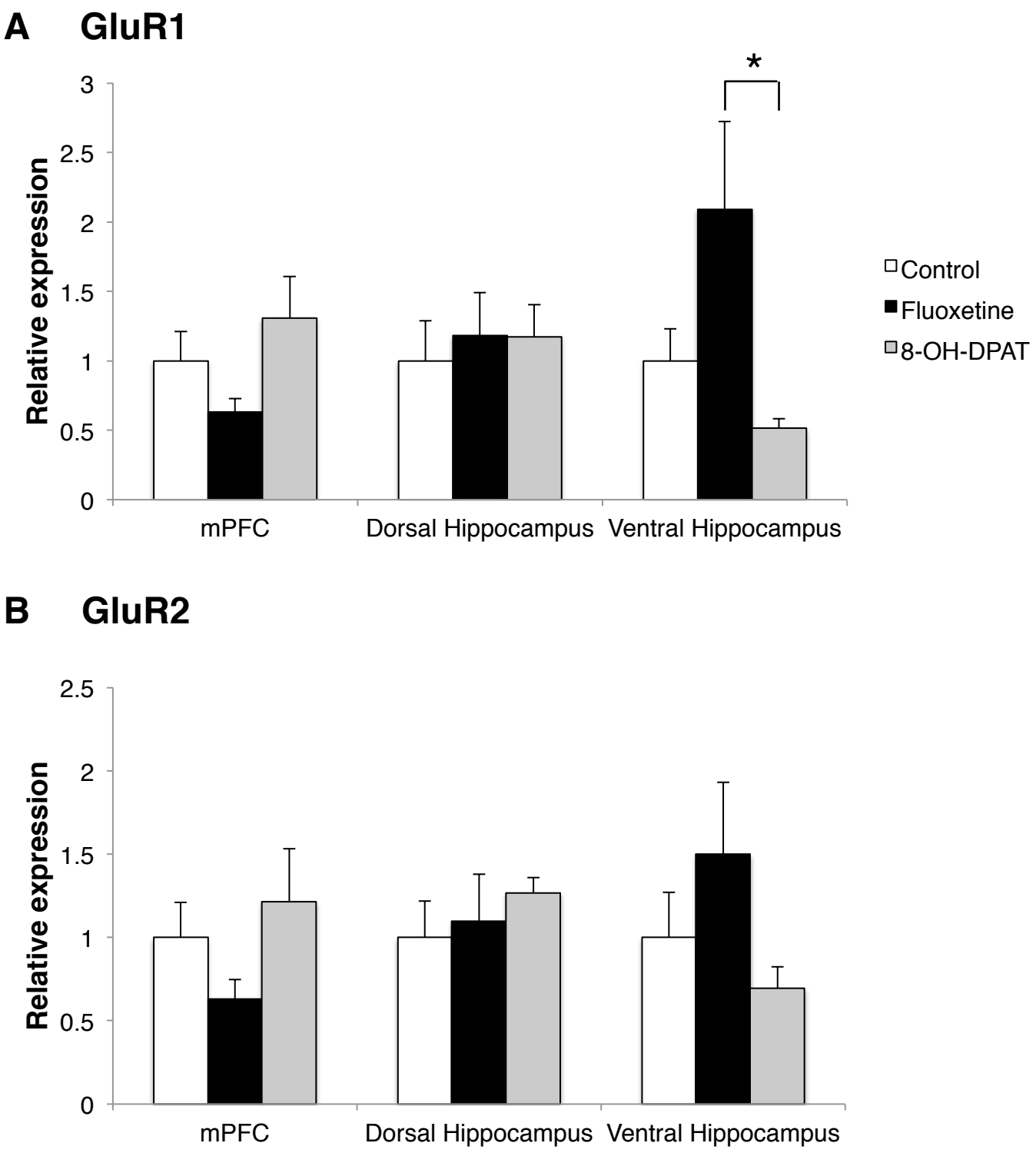

Figure 7
The mRNA expression of the GluR1 and GluR2 at P71 in the mPFC, dorsal hippocampus, and ventral hippocampus in mice treated with sucrose, fluoxetine, or 8-OH-DPAT from P1-P21. (A) The mRNA expression of the GluR1. (B) The mRNA expression of the GluR2. Each bar represents the mean ± S.E.M of latency (n=6~9 for each group). * $p < 0.05$