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Chronic Fluoxetine Selectively Upregulates Dopamine D_1-Like Receptors in the Hippocampus

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The dentate gyrus of the hippocampus has been implicated in mechanisms of action of selective serotonin reuptake inhibitors (SSRIs). We have recently demonstrated that the SSRI fluoxetine can reverse the state of maturation of the adult dentate granule cells and enhances serotonin 5-HT_4 receptor-mediated synaptic potentiation at the synapses formed by their mossy fiber axons. Here, we show that fluoxetine can induce long-lasting enhancement of dopaminergic modulation at the mossy fiber synapse. Synaptic responses arising from the mossy fiber-CA3 pyramidal cell synapse were recorded using acute mouse hippocampal slices. Dopamine potentiates mossy fiber synaptic transmission by activating D_1-like receptors. Chronic fluoxetine treatment induced a prominent increase in the magnitude of dopamine-induced synaptic potentiation, and this effect was maintained at least up to 1 month after withdrawal of fluoxetine. Quantitative autoradiography revealed that binding of the D_1-like receptor ligand [^{3}H]SCH23390 was selectively increased in the dentate gyrus and along the mossy fiber in fluoxetine-treated mice. However, binding of the 5-HT_4 receptor ligand [^{3}H]GR113808 was not significantly changed. These results suggest that chronic fluoxetine enhanced the dopaminergic modulation at least in part by upregulating expression of D_1-like receptors, while the enhanced serotonergic modulation may be mediated by modifications of downstream signaling pathways. These enhanced monoaminergic modulations would greatly increase excitatory drive to the hippocampal circuit through the dentate gyrus. The highly localized upregulation of D_1-like receptors further supports the importance of the dentate gyrus in the mechanism of action of SSRIs.

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INTRODUCTION

The central serotonergic system is the crucial target for pharmacological treatments of psychiatric disorders (Vaswani *et al*, 2003; Meltzer and Massey, 2011). Drugs that can raise extracellular serotonin levels such as selective serotonin reuptake inhibitors (SSRIs) have been widely used to treat mood and anxiety disorders (Vaswani *et al*, 2003). However, cellular mechanisms underlying both therapeutic and adverse effects of SSRIs have not been fully understood. The dentate gyrus of the hippocampus has been implicated in behavioral effects of SSRIs and other antidepressant drugs in experimental animals (Adachi *et al*, 2008; Kobayashi *et al*, 2011a; Miyamoto *et al*, 2011; Sahay and Hen, 2007; Santarelli *et al*, 2003). We have recently shown that the SSRI fluoxetine greatly changes serotonergic modulation at the synapse between the mossy fiber, the sole output of the dentate granule cell, and CA3 pyramidal cells (Kobayashi *et al*, 2008, 2010). At the mossy fiber-CA3 synapse, serotonin induces robust synaptic potentiation and small depression by activating 5-HT_4 and 5-HT_{1A} receptors, respectively (Kobayashi *et al*, 2008, 2010), and chronic fluoxetine administered at a relatively high dose causes marked enhancement of serotonin-induced synaptic potentiation (Kobayashi *et al*, 2010). We have also shown that fluoxetine reduces strong synaptic facilitation at the mossy fiber synapse to a juvenile level via a process characterized as 'dematuration' of mature granule cells (Kobayashi *et al*, 2010). In mice lacking the 5-HT_4 receptor, the serotonin-induced synaptic potentiation at the mossy fiber synapse was abolished and dematuration of granule cells was attenuated (Kobayashi *et al*, 2010), suggesting a critical role of the 5-HT_4 receptor in cellular effects of fluoxetine in the hippocampus. As the mossy fiber has an essential role in regulating excitability and associative synaptic plasticity in the CA3 pyramidal cells (Kobayashi and Poo, 2004), the fluoxetine-induced alteration of mossy fiber synaptic

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transmission is likely to have a substantial impact on functioning of the hippocampal circuit. Indeed, chronic fluoxetine caused significant changes in some forms of hippocampus-dependent behaviors, such as locomotor activity and anxiety-related behaviors, and these behavioral effects of fluoxetine were reduced in the 5-HT_4 receptor-deficient mice (Kobayashi *et al*, 2011a). Furthermore, the fluoxetine-induced reduction of mossy fiber synaptic facilitation significantly correlated with the behavioral change caused by fluoxetine in individual mice (Kobayashi *et al*, 2011a). Stress, which is generally thought to precipitate psychiatric disorders including depression, changes the structure and functions of the mossy fiber synapse (Chen *et al*, 2010; Kobayashi, 2010; Magariños *et al*, 1997). These lines of evidence suggest that the mossy fiber synapse could be an important target for SSRIs and other antidepressant drugs (Kobayashi, 2009, 2010).

The central dopaminergic system has also been suggested to be an important target for the treatment of psychiatric disorders. Dopamine has been implicated in the pathophysiology of mood disorders (Suhara *et al*, 1992) and mechanisms of action of antidepressant drugs including SSRIs (D'Aquila *et al*, 2000). Dopamine can potentiate the mossy fiber synaptic transmission, and this effect is associated with activity of mice in novel environments (Kobayashi *et al*, 2006). The dopamine-induced synaptic potentiation at the mossy fiber synapse is mediated by D_1-like receptors (Kobayashi and Suzuki, 2007). D_1-like receptors are involved in behavioral effects of antidepressants in animal models of depression (D'Aquila *et al*, 1994; Gambarana *et al*, 1995; Sampson *et al*, 1991). Given the potential involvement of the mossy fiber synapse in mechanisms of action of antidepressants, the dopaminergic modulation at the mossy fiber synapse may also be affected by antidepressant treatments. To address this issue, in the present study, we examined effects of dopamine on the mossy fiber synaptic transmission in hippocampal slices prepared from mice chronically treated with fluoxetine, and found that fluoxetine causes prominent long-lasting enhancement of the dopamine-induced synaptic potentiation. The involvement of the serotonergic system in this effect of fluoxetine was examined by using mice with lesions in the central serotonergic neurons and mice deficient for the 5-HT_4 receptor, in both of which the effectiveness of fluoxetine in inducing the granule cell dematuration has been shown to be greatly reduced in the same treatment regimen (Kobayashi *et al*, 2010). We further investigated the possibility that the enhanced monoaminergic modulation at the mossy fiber synapse is mediated by changes in expression levels of monoamine receptors that contribute to the synaptic modulation by quantitative autoradiography.

MATERIALS AND METHODS

Drug Treatment

Male C57BL/6J mice were singly housed from the age of 8 weeks in the institutional standard condition (14 : 10 light/dark cycle; lights on at 0600 h through 2000 h) at 23 ± 1 °C with food and water available ad libitum. Following 1 week of acclimation, fluoxetine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in the drinking water and orally applied as described (Kobayashi *et al*, 2010, 2011a). As fluoxetine reduced water consumption, saccharin (0.2%) was included to keep a water intake comparable to the baseline. Concentrations of fluoxetine in the drinking water were determined for individual mice everyday based on the water consumption during preceding 24 h and the body weight measured every other day. Control mice (CNT) were given water with or without saccharin, and all data were pooled. Paroxetine hydrochloride (Toronto Research Chemicals, North York, Ontario, Canada) was administered in the same way as fluoxetine. The 5-HT_4 receptor mutant mice (strain name: B6.129P2-Htr4<tm1Dgen>/J) backcrossed to the C57BL/6J background were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Male homozygous mutant mice and their wild-type littermates from heterozygous mating were treated with fluoxetine in the same way and used for electrophysiological experiments. We shared some data from control groups of both wild-type and mutant mice with our previous study (Kobayashi *et al*, 2010). All procedures were approved by the Animal Care and Use Committee of Nippon Medical School.

Electrophysiology

Mice were decapitated under deep halothane anesthesia and hippocampi were isolated. Transverse hippocampal slices (380 μm) were cut using a tissue slicer in ice-cold saline as described (Kobayashi *et al*, 2010) and maintained in a humidified interface holding chamber at room temperature (24–27°C) before recording. Electrophysiological recordings were made in a submersion-type chamber maintained at 27.0–27.5°C and superfused at 2 ml/min with saline composed of (in mM): NaCl, 125; KCl, 2.5; NaH_2PO_4, 1.0; $NaHCO_3$, 26.2; glucose, 11; $CaCl_2$, 2.5; $MgCl_2$, 1.3 (equilibrated with 95% O_2/5% CO_2). Electrical stimulation was delivered to the dentate granule cell layer, and field excitatory postsynaptic potentials (fEPSPs) arising from the mossy fiber synapses were recorded from the stratum lucidum of CA3 using a glass pipette filled with 2 M NaCl. The amplitude of fEPSPs was measured on analysis as described (Kobayashi and Suzuki, 2007). A criterion used to identify the mossy fiber input was >85% block of the fEPSP amplitude by an agonist of group II metabotropic glutamate receptors, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV, 1 μM) (Tocris Bioscience, Bristol, UK). Single electrical stimulation was delivered at a frequency of 0.05 Hz for baseline recordings. Dopamine hydrochloride was purchased from Wako Pure Chemical Industries. SCH23390 was from Tocris Bioscience. All recordings were made using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered at 2 kHz and stored in a personal computer via an interface (digitized at 5–10 kHz).

Autoradiography

Mice were killed by decapitation under deep halothane anesthesia and their brains were quickly removed. One hemisphere of the brain was frozen with powdered dry ice and cut into 20-μm-thick coronal sections with a HM560

cryotome (Carl Zeiss, Oberkochen, Germany). The other hemisphere was used for the electrophysiological experiment. The sections were mounted on slide glass (Matsunami Glass, Osaka, Japan) and stored at −80°C until use. All radiochemicals were purchased from GE Healthcare Bio-Sciences (Piscataway, NJ). Specific binding of the dopamine D_1-like receptor ligand [^{3}H]SCH233090 (1 nM) to the brain slice was analyzed by autoradiography as described (Mansour *et al*, 1990). The brain slices were pre-incubated for 15 min in 50 mM Tris-HCl buffer (pH 7.4, 25°C) containing 120 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$. The samples were then incubated at room temperature for 1 h in the same buffer containing 1 μM ketanserin and 1 nM [^{3}H]SCH23390. Nonspecific binding was assessed in the presence of 1 μM of cis-flupentixol. Specific binding of serotonin 5-HT_4 receptor ligand [^{3}H]GR113808 was analyzed as described (López-Giménez *et al*, 2002). The brain slices were pre-incubated for 20 min in 50 mM HEPES buffer (pH 7.4, 25°C) containing 10 μM pargyline and 0.01% ascorbic acid. The samples were then incubated at room temperature for 1 h in the same buffer containing 0.1 nM [^{3}H]GR113808. Nonspecific binding was assessed in the presence of 100 μM of serotonin. Specific binding of the serotonin 5-HT_{1A} receptor ligand [^{3}H]8-OHDPAT was analyzed as described (Vergé *et al*, 1986). The brain slices were pre-incubated for 30 min in 170 mM Tris-HCl buffer (pH 7.4, 25°C) containing 4 mM $CaCl_2$, 10 μM pargyline, and 0.01% ascorbic acid. The samples were then incubated at room temperature for 1 h in the same buffer containing 2 nM [^{3}H]8-OHDPAT. Nonspecific binding was assessed in the presence of 100 μM of serotonin. Following the incubation, the samples were rinsed with ice-cold buffer, and desalted with ice-cold distilled water. The slices were subsequently dried under warm blowing air and exposed to a BAS-TR2025 imaging plate (Fuji Film, Tokyo, Japan). Exposure time for [^{3}H]SCH233090, [^{3}H]8-OHDPAT, and [^{3}H]GR113808 was 5, 7, and 28 days, respectively. The imaging plate was subsequently scanned with a BAS5000 system (Fuji Film). Regions of interest (ROIs) were defined on the images using a Multi Gauge software (Fuji Film), and densitometric assay for each ROI was performed using autoradiographic [^{3}H]micro-scales (GE Healthcare Bio-Sciences).

5,7-Dihydroxytryptamine (DHT) Lesion

To lesion the central serotonergic system, the serotonergic neurotoxin DHT(MP Biomedicals, OH, USA) was intracerebroventricularly injected as previously described (Kobayashi *et al*, 2010). Briefly, mice were anesthetized with pentobarbital (60 mg/kg i.p.) and were also injected with desipramine (25 mg/kg i.p.) to block the uptake of DHT by noradrenergic terminals. A total of 100 μg of DHT was dissolved in 10 μl of 0.9% sterile saline supplemented with 0.1% ascorbic acid, and slowly infused into the lateral cerebral ventricle. After the injections, mice were allowed to recover for 8–11 days and then treated with fluoxetine, as above.

Statistics

All data are presented as mean ± SEM. The number of data (n) represents the number of mice unless otherwise specified. Statistical tests were performed using GraphPad Prism version 3.03 for Windows (GraphPad Software, San Diego, CA, USA) with the significance level $p<0.05$. The two-tailed t test was used to compare two groups, and the Bonferroni's multiple comparison test or Dunn's multiple comparison test was used to compare three groups or more.

RESULTS

Fluoxetine Enhances Dopamine-Induced Potentiation at Mossy Fiber Synapse

We first examined effects of chronic fluoxetine treatment on the dopaminergic modulation at the mossy fiber-CA3 synapse. Mice were treated with fluoxetine at a dose of 22 mg/kg per day for 4 weeks, a regimen sufficient for the induction of the granule cell dematuration and enhancement of the serotonergic modulation (Kobayashi *et al*, 2010). Using acute hippocampal slices, fEPSPs arising from the mossy fiber synapses were recorded. Bath-applied dopamine (10 μM) induced robust potentiation of fEPSPs (to 171 ± 7% of baseline, $n=8$) as in previous studies (Kobayashi *et al*, 2006; Kobayashi and Suzuki, 2007). In fluoxetine-treated mice (FLX), the magnitude of dopamine-induced potentiation was strongly enhanced (to 348 ± 28% of baseline, $n=11$, $p<0.001$) (Figures 1a and b). At 14 mg/kg per day, fluoxetine had no significant effects on the dopamine-induced potentiation (Figure 1b). The effect of fluoxetine at 22 mg/kg per day was already evident after 2 weeks of treatment (Figure 1b) and could be observed at least up to 4 weeks after withdrawal of fluoxetine (Figure 1c). Chronic treatment with another SSRI paroxetine similarly enhanced the effect of dopamine (Figure 1b). As reported previously (Kobayashi and Suzuki, 2007), dopamine increased the amplitude of the presynaptic fiber volley component of the field potentials. Although this effect was also slightly augmented in the FLX, there was no statistically significant difference between two groups (control: 118 ± 3% of baseline; fluoxetine: 125 ± 6% of baseline; $p=0.2810$). The potentiating effect of dopamine at the mossy fiber synapse is mediated by D_1-like receptors (Kobayashi and Suzuki, 2007). In the FLX, the effect of dopamine was nearly completely suppressed when slices were pretreated with the D_1-like receptor antagonist SCH23390 (30 nM) (control slice: to 304 ± 29% of baseline, $n=6$ slices; pretreated slice: to 105 ± 2% of baseline, $n=6$ slices; $p=0.001$). These results indicate that chronic fluoxetine can induce long-lasting enhancement of the potentiating effect of dopamine mediated by D_1-like receptors at the hippocampal mossy fiber-CA3 synapse.

Requirement of Serotonergic System for Fluoxetine-Induced Enhancement of Dopaminergic Modulation

As the primary target of fluoxetine is the serotonin transporter, we examined the involvement of the serotonergic system in the fluoxetine-induced enhancement of the dopaminergic modulation. To lesion the central serotonergic system, mice were intracerebroventricularly injected with the serotonergic neurotoxin DHT. In vehicle-treated mice, chronic fluoxetine induced robust enhancement of dopamine-induced potentiation as in normal mice

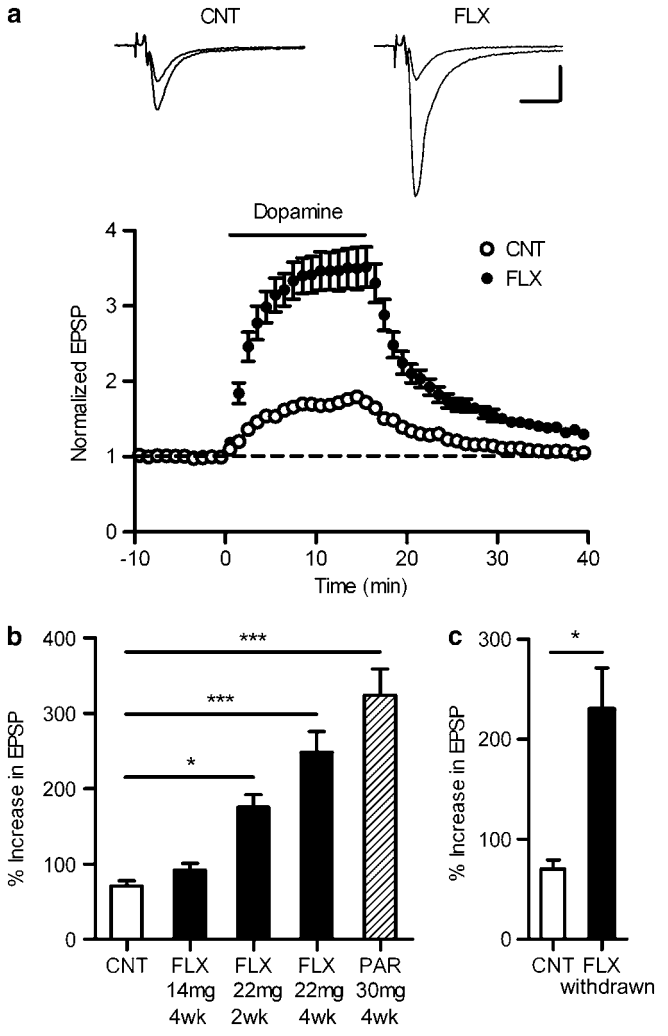

Figure 1 Chronic fluoxetine induces long-lasting enhancement of dopaminergic synaptic modulation. (a) Bath-applied dopamine induced reversible potentiation of mossy fiber synaptic transmission. The magnitude of potentiation was clearly increased in fluoxetine-treated mice (FLX) as compared with control mice (CNT). Sample traces show averages of 15 consecutive field excitatory postsynaptic potentials (fEPSPs) before and during dopamine application. Scale bar: 10 ms, 0.2 mV. (b) Effects of fluoxetine and paroxetine (PAR) on dopaminergic synaptic modulation. Dopamine-induced potentiation was significantly increased after 2 weeks ($n=5$, $p<0.05$) and 4 weeks ($n=11$, $p<0.001$, Bonferroni's multiple comparison test) of fluoxetine treatments at 22 mg/kg per day and 4 weeks of PAR treatment at 30 mg/kg per day ($n=4$, $p<0.001$), but not after 4 weeks of fluoxetine treatment at 14 mg/kg per day ($n=8$). (c) Dopamine-induced potentiation remained enhanced for at least 1 month after withdrawal of fluoxetine ($n=6$ each, $p=0.0133$). *$p<0.05$; ***$p<0.001$.

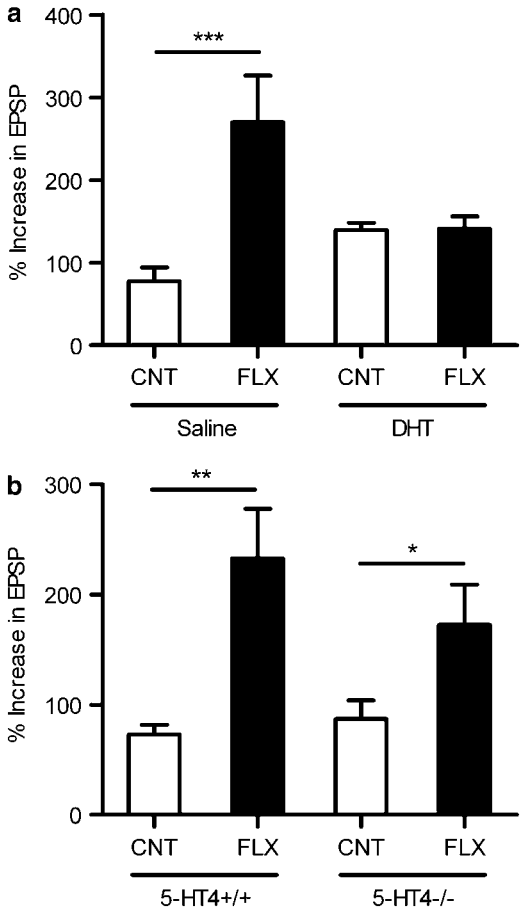

Figure 2 Serotonin dependence of fluoxetine-induced enhancement of dopaminergic modulation. (a) Chronic fluoxetine significantly increased the magnitude of dopamine-induced potentiation in saline-injected mice (control mice (CNT): $n=6$, fluoxetine-treated mice (FLX): $n=3$, $p<0.001$, Bonferroni's multiple comparison test), but not in 5,7-dihydroxytryptamine (DHT)-injected mice (CNT: $n=5$, FLX: $n=4$). There was no significant difference between saline- and DHT-injected control groups. (b) Chronic fluoxetine significantly increased the magnitude of dopamine-induced potentiation in both wild-type (5-HT_4+/+, CNT: $n=6$, FLX: $n=8$, $p<0.01$) and mutant mice (5-HT_4-/-, CNT: $n=10$, FLX: $n=9$, $p<0.05$, Dunn's multiple comparison test). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

(Figure 2a). In DHT-treated mice, dopamine-induced potentiation was slightly increased in magnitude in the control condition, and chronic fluoxetine did not affect the magnitude of potentiation (Figure 2a), suggesting that the integrity of the serotonergic system is required for the effect of fluoxetine on the dopaminergic modulation. We have previously shown that the serotonin 5-HT_4 receptor has an important role in denaturation of the granule cell by fluoxetine (Kobayashi *et al*, 2010). We examined whether this receptor also contributes to the enhancement of dopamine-induced potentiation using mice lacking the 5-HT_4 receptor. In both wild-type and mutant mice, fluoxetine caused significant enhancement of dopamine-induced potentiation (Figure 2b). Therefore, the 5-HT_4 receptor is not essential for the enhancement of the potentiating effect of dopamine.

Fluoxetine Increases D_1-like Receptor Ligand Binding in the Dentate Gyrus and CA3

As shown above, the dopamine-induced synaptic potentiation in the FLX required activation of D_1-like receptors as in normal mice. The prominent enhancement of the effect of dopamine by fluoxetine might be mediated by an increase in expression of the D_1-like receptors at the mossy fiber synapse. To test this possibility, we examined binding of the D_1-like receptor ligand [^{3}H]SCH23390 by quantitative autoradiography. In CNT, strong [^{3}H]SCH23390 binding was seen in the striatum, and relatively weak binding was

detected in the hippocampus (Figure 3a). Chronic fluoxetine significantly increased the [^{3}H]SCH23390 binding in the dentate gyrus and CA3 region (Figures 3a and b). After the treatment, the signal was visible along the mossy fiber pathway (Figure 3a). There was no significant change in the binding in the hippocampal CA1 region and other brain regions including striatum (Figure 3b). These results suggest that chronic fluoxetine caused selective upregulation of the dopamine D_1-like receptor expression in the dentate gyrus and along the mossy fiber pathway.

In order to test a possible involvement of changes in serotonin receptor expression in the fluoxetine-induced enhancement of the serotonergic modulation, we examined binding of the 5-HT_4 receptor-specific ligand [^{3}H]GR113808. Chronic fluoxetine caused an overall decrease in the [^{3}H]GR113808 binding (Figure 4a), which is consistent with previous results (Licht *et al*, 2009; Vidal *et al*, 2009). The decrease was evident in the striatum, amygdala, and substantia nigra, but did not reach the statistical significance in the hippocampus (Figure 4b). In addition to 5-HT_4 receptor-mediated synaptic potentiation, serotonin can cause weak synaptic inhibition via activation of the 5-HT_{1A} receptor at the mossy fiber synapse (Kobayashi *et al*, 2008). We also examined a possible change in expression of the 5-HT_{1A} receptor at the mossy fiber synapse by analyzing binding of the 5-HT_{1A} ligand [^{3}H]8-OHDPAT. Although fluoxetine significantly decreased the [^{3}H]8-OHDPAT binding in the CA1 region, it had no significant effect on the binding in the dentate gyrus or CA3 region (Figure 5). These results suggest that chronic fluoxetine had no significant effects on the expression of these serotonin receptors at the mossy fiber synapse.

DISCUSSION

The present study has demonstrated that chronic fluoxetine treatment causes long-lasting strong enhancement of dopamine D_1-like receptor-dependent synaptic potentiation at the hippocampal mossy fiber synapse and also selectively upregulates the binding of the D_1-like receptor ligand in the dentate gyrus and along the mossy fiber pathway. These results suggest that the enhanced dopaminergic synaptic modulation caused by fluoxetine is at least in part mediated by increased expression levels of D_1-like receptors. On the other hand, there were no significant changes in the binding of specific ligands for serotonin receptors that are involved in the modulation of mossy fiber synaptic transmission. Therefore, the enhanced serotonergic modulation by fluoxetine shown previously is likely mediated by modifications of receptor functioning or intracellular pathways downstream of the receptor activation.

Dopamine has been implicated in neuronal bases of action of antidepressant drugs including SSRIs (D'Aquila *et al*, 1994, 2000; Gambarana *et al*, 1995; Sampson *et al*, 1991). Consistent with our finding, chronic fluoxetine has been shown to increase mRNA levels of the D_1 receptor in the hippocampus (Miller *et al*, 2008). Increased D_1 receptor

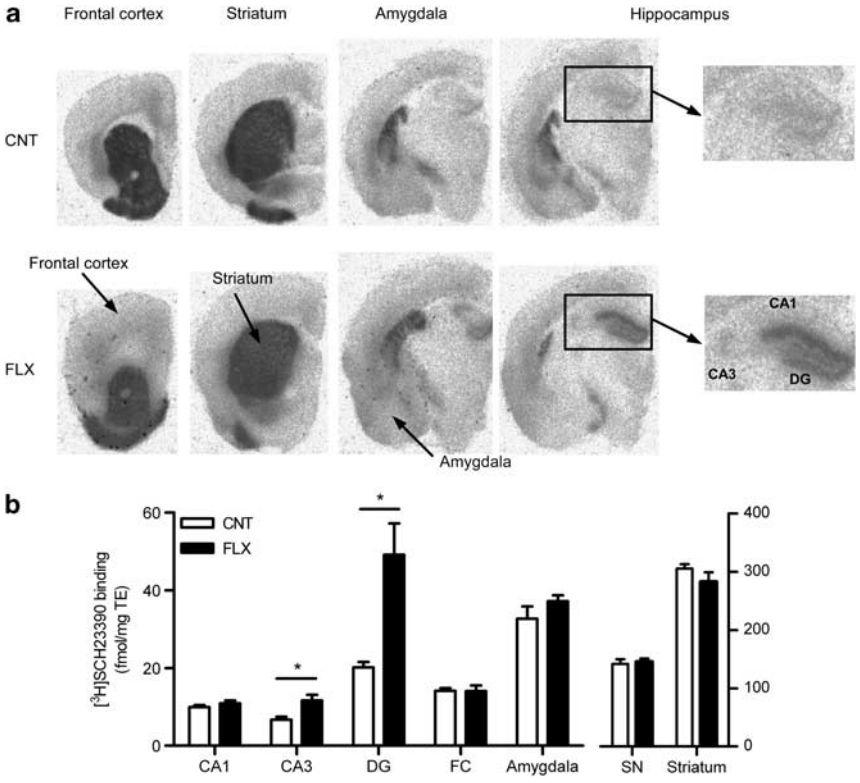

Figure 3 Selective increase in D_1-like ligand binding in dentate gyrus and CA3 in fluoxetine-treated mice (FLX). (a) Representative autoradiograms of [^{3}H]SCH23390 binding at four different section levels. (b) Summary data showing significant increases in [^{3}H]SCH23390 binding after fluoxetine treatment in CA3 ($p = 0.019$) and dentate gyrus ($p = 0.0121$) (control mice (CNT): $n = 6$, FLX: $n = 7$). DG, dentate gyrus; FC, frontal cortex; SN, substantia nigra. *$p < 0.05$.

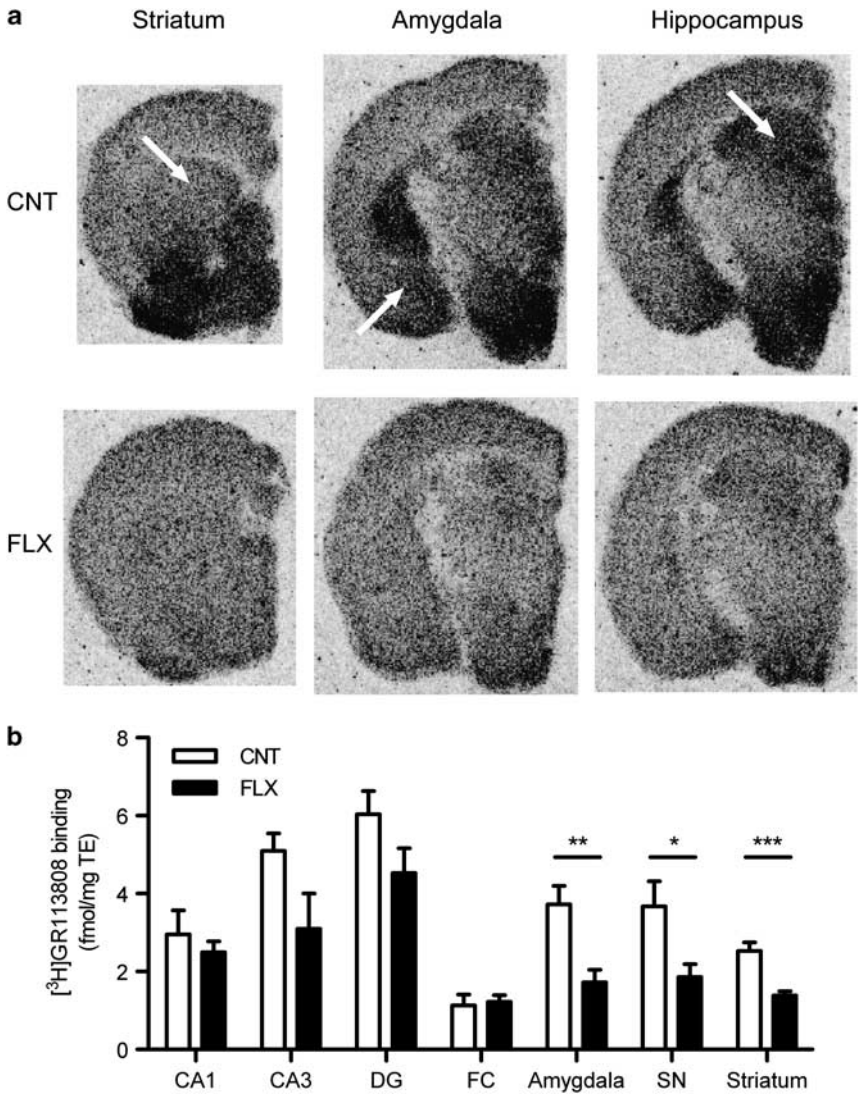

Figure 4 Reduced 5-HT_4 ligand binding in fluoxetine-treated mice (FLX). (a) Representative autoradiograms of [^{3}H]GR113808 binding in the striatum, amygdala and hippocampus (indicated by arrows). (b) Fluoxetine significantly reduced [^{3}H]GR113808 binding in amygdala ($p = 0.0043$), striatum ($p = 0.0005$) and substantia nigra ($p = 0.0239$) (control mice (CNT): $n = 6$, FLX: $n = 7$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

mRNA has also been demonstrated in the striatum and nucleus accumbens after repeated treatments with the SSRI sertraline (Huzarska *et al*, 2006). However, other studies have reported no effect of SSRIs on D_1 mRNA levels in the striatum and nucleus accumbens (Ainsworth *et al*, 1998; Dziedzicka-Wasylewska *et al*, 1997). In membrane preparations of the striatum, D_1-like ligand binding did not change (Deslandes *et al*, 2002) or decreased after SSRI treatments (Klimek and Nielsen, 1987). Decreased D_1-like ligand binding has also been demonstrated in the membrane preparation of the limbic system (Klimek and Nielsen, 1987). In the present study, the D_1-like ligand binding was selectively increased in the dentate gyrus and hippocampal CA3 region. This highly localized hippocampus-specific increase in D_1-like ligand binding is in agreement with the lack of an increase in D_1 mRNA levels or D_1-like ligand binding in the brain regions other than the hippocampus in most previous studies. We have previously demonstrated a similar marked increase in D_1-like ligand binding that is restricted to the dentate gyrus and mossy fiber tract in mice heterozygous for α-calcium/calmodulin-dependent protein kinase II (Yamasaki *et al*, 2008). We have also shown that the dopaminergic modulation at the mossy fiber synapse is clearly enhanced in a subpopulation of mice lacking the schizophrenia susceptible gene dysbindin-1 (Kobayashi *et al*, 2011b). Our present results demonstrate that, in addition to these genetic factors, environmental factors can converge on this D_1-like receptor-mediated dopaminergic modulation to regulate mossy fiber synaptic transmission. The resultant changes in potentiation of synaptic transmission by dopamine might lead to alterations of hippocampus-dependent behaviors such as locomotor activity in novel environments (Kobayashi *et al*, 2006).

In contrast to marked upregulation of the D_1-like ligand binding, chronic fluoxetine did not cause any increase in the 5-HT_4 ligand binding in the hippocampus. Although the 5-HT_{1A} receptor binding was reduced in the CA1 region, there were no detectable changes in the dentate gyrus and

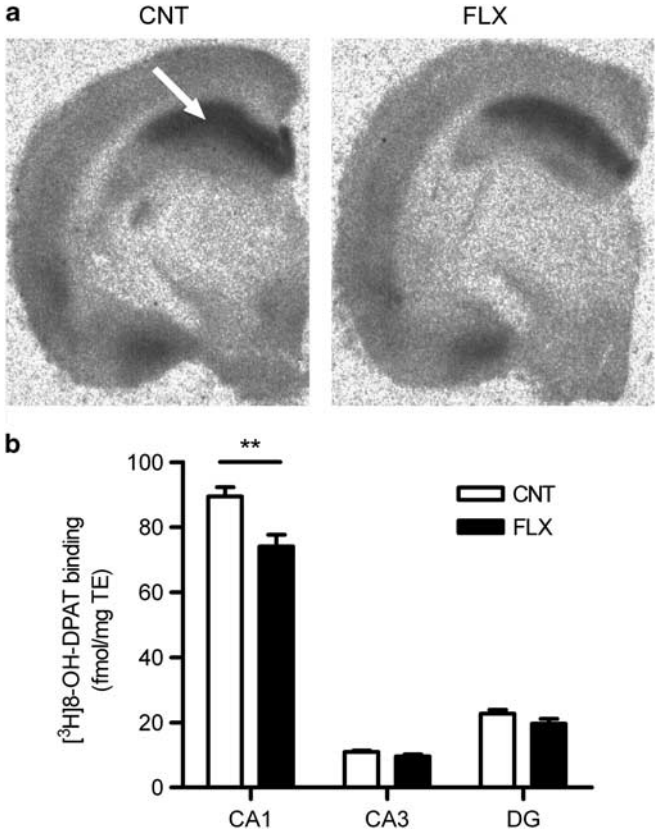

Figure 5 Reduced 5-HT_{1A} ligand binding in fluoxetine-treated mice (FLX). (a) Representative autoradiograms of [^{3}H]8-OHDPAT binding. The arrow indicates the hippocampal CA1 region. (b) Fluoxetine significantly reduced [^{3}H]8-OHDPAT binding in CA1 ($p = 0.007$) (control mice (CNT): $n = 6$, FLX: $n = 7$). $^{**}p < 0.01$.

CA3 region. Therefore, the enhanced serotonin-induced potentiation at the mossy fiber synapse caused by fluoxetine cannot be ascribed to either upregulation of receptors mediating synaptic potentiation or downregulation of receptors mediating synaptic depression. Both 5-HT_4 and D_1-like receptors are coupled to Gs-adenylate cyclase pathways, and the monoaminergic modulation at the mossy fiber synapse depends on cAMP (Kobayashi and Suzuki, 2007; Kobayashi *et al*, 2008). Antidepressant drugs have been shown to facilitate interaction between Gs and adenylate cyclase (Donati and Rasenick, 2003). In some conditions, fluoxetine can reduce activity or expression of phosphodiesterase that metabolizes cAMP (Fatemi *et al*, 2010; Korff *et al*, 2009). Such altered downstream signaling may account for the enhanced 5-HT_4-dependent synaptic modulation in the FLX and may also contribute to the enhancement of the dopaminergic modulation at the mossy fiber synapse.

As the SSRI fluoxetine can inhibit dopamine reuptake at high concentrations (Sánchez and Hyttel, 1999), it is possible that the enhanced dopaminergic modulation could be caused by its direct action on the dopaminergic neurons. We showed that the effect of fluoxetine was abolished in mice treated with the serotonergic neurotoxin DHT. It has been shown that DHT injected into the brain region that is rich in dopaminergic terminals could change dopamine levels as well as serotonin at the injected region (Ludwig and Schwarting, 2007). However, intracerebroventricular injection of DHT as in the present study has been reported to generally spare dopaminergic neurons (Fischette *et al*, 1987; Reader and Gauthier, 1984; Winstanley *et al*, 2003). Therefore, the result of our DHT experiment suggests that the integrity of the serotonergic system is essential for the effect of fluoxetine on the dopaminergic modulation. In addition, whereas inhibition of dopamine reuptake generally causes immediate locomotor hyperactivity (Uhl *et al*, 2002), fluoxetine tends to reduce locomotor activity (Kobayashi *et al*, 2008, 2011a). Thus, although we cannot exclude the possibility of direct action of fluoxetine on the dopaminergic system, a contribution of such action, if any, to the effect of fluoxetine demonstrated in the present study is supposed to be small. Fluoxetine is known to antagonize the serotonin 5-HT_{2C} receptor (Sánchez and Hyttel, 1999). We showed that another SSRI paroxetine, which has a much lower affinity for 5-HT_{2C} (Sánchez and Hyttel, 1999), similarly augmented the dopaminergic synaptic modulation, confirming the importance of the serotonin reuptake inhibition rather the 5-HT_{2C} antagonism in the effect of fluoxetine. The mechanism that links the serotonin reuptake inhibition to the D_1-like receptor-dependent synaptic modulation is unknown. Brain-derived neurotrophic factor (BDNF) has been implicated in antidepressant action (Adachi *et al*, 2008; Malberg and Blendy, 2005). Chronic fluoxetine can increase both mRNA and mature protein levels of BDNF in the hippocampus (Musazzi *et al*, 2009), and BDNF has been shown to greatly increase the expression of D_1 receptor mRNA in the catecholaminergic CAD cell line (Do *et al*, 2007). Thus, it is possible that BDNF mediates the fluoxetine-induced increase in the D_1-like receptor expression levels in the hippocampus. Activation of cAMP response element-binding protein has been suggested to mediate an antidepressant-induced increase in BDNF transcription (Conti *et al*, 2002; Malberg and Blendy, 2005). Therefore, the upregulation of the 5-HT_4 receptor-dependent signaling coupled to cAMP elevation might be critically involved in the enhancement of D_1-like receptor-dependent synaptic modulation. However, in 5-HT_4-deficient mice, chronic fluoxetine significantly enhanced the dopaminergic modulation as in wild-type mice. The serotonin 5-HT_6 and 5-HT_7 receptors, which are also coupled to Gs-cAMP cascades, have been reported to be expressed in the dentate gyrus (Gérard *et al*, 1997; Neumaier *et al*, 2001; Vizuete *et al*, 1997). These receptors might have compensated for the lack of the 5-HT_4 receptor in the mutant mice. In the present study, we did not further examine the subtype of serotonin receptors mediating the effect of fluoxetine. Other subtypes of serotonin receptors are also expressed in the dentate gyrus (Klempin *et al*, 2010). The 5-HT_{1A} receptor mediates depression of mossy fiber synaptic transmission by serotonin (Kobayashi *et al*, 2008) and is involved in facilitation of the adult neurogenesis in the dentate gyrus by fluoxetine (Santarelli *et al*, 2003). The immunohistochemical analysis demonstrated abundant 5-HT_{2C}-like immunoreactivity in the granule cell layer (Klempin *et al*, 2010). These serotonin receptors might also have a role in mediating the effect of fluoxetine on the dopaminergic modulation at the mossy fiber synapse.

The dentate gyrus has been implicated in behavioral effects of SSRIs and other antidepressant drugs (Adachi

et al, 2008; Kobayashi *et al*, 2011a; Miyamoto *et al*, 2011; Santarelli *et al*, 2003). The selective upregulation of D_1-like ligand binding in the dentate gyrus-mossy fiber system by fluoxetine further supports the importance of the dentate gyrus in antidepressant action. Facilitated adult neurogenesis in the dentate gyrus has been suggested to be a candidate cellular process mediating antidepressant action (Malberg *et al*, 2000; Sahay and Hen, 2007; Santarelli *et al*, 2003). However, newly generated neurons constitute only a small fraction of dentate granule cells, and a recent study has shown that an increase in adult neurogenesis alone did not cause antidepressant-like behavioral effects (Sahay *et al*, 2011). We have previously shown that chronic SSRI treatments induce dematuration of dentate granule cells in adult mice (Kobayashi *et al*, 2010). The granule cell dematuration is induced in a large population of mature granule cells, significantly changes somatic excitability and synaptic plasticity at both input and output synapse of the granule cells, and is accompanied by marked enhancement of the serotonergic modulation at the mossy fiber synapse (Kobayashi *et al*, 2010). The present study has demonstrated that the potentiating effect of dopamine at the mossy fiber synapse is increased by about threefold after the fluoxetine treatment. These enhanced monoaminergic modulations would greatly increase excitatory drive to CA3 pyramidal cells by the mossy fibers. Furthermore, increased D_1-like receptor expression in the granule cells may facilitate the induction of long-term potentiation at the perforant path granule cell synapse (Hamilton *et al*, 2010; Kusuki *et al*, 1997). Together with changes in intrinsic functional properties of granule cells caused by dematuration, the altered monoaminergic synaptic modulation is likely to have a significant impact on propagation of neuronal signals through the dentate gyrus, thereby possibly contributing to neuronal bases for mechanisms of action of SSRIs.

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DISCLOSURE

The authors declare no conflict of interest.

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