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Fluoxetine Modulates Corticostriatal Synaptic Transmission through Postsynaptic Mechanism

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Fluoxetine, widely used for the treatment of depression, is known to be a selective serotonin reuptake inhibitor (SSRI), however, there are also reports that fluoxetine has direct effects on several receptors. Employing whole-cell patch clamp techniques in rat brain slice, we studied the effects of fluoxetine on corticostriatal synaptic transmission by measuring the change in spontaneous excitatory postsynaptic currents (sEPSC). Acute treatment of rat brain slice with fluoxetine (10 μM) significantly decreased the amplitude of sEPSC (84.1±3.3%, n=7), but did not alter its frequency (99.1±4.7%, n=7). Serotonin (10 μM) also significantly decreased the amplitude (81.2±3.9%, n=4) of sEPSC, but did not affect its frequency (105.8±8.0, n=4). The effect of fluoxetine was found to have the same trend as that of serotonin. We also found that the inhibitory effect of fluoxetine on sEPSC amplitude (93.0±1.9%, n=8) was significantly blocked, but not serotonin (84.3±1.6%, n=4), when the brain slice was incubated with p-chloroamphetamine (10 μM), which depletes serotonin from the axon terminals and blocks its reuptake. These results suggest that fluoxetine inhibits corticostriatal synaptic transmission through postsynaptic, and that these effects are exerted through both serotonin dependent and independent mechanism.

Key Words: Striatum, Fluoxetine, Spontaneous EPSC, Synaptic transmission

INTRODUCTION

Fluoxetine is widely used in the treatment of a variety of brain disorders such as mental depression, panic disorder, obesity, and alcoholism. It is generally believed that fluoxetine exerts its therapeutic effects by enhancing serotonergic transmission, exclusively through inhibition of serotonin (5-hydroxytryptamine, 5-HT) transporters with minimal or no effects on other neurotransmitter receptors (Cusack et al, 1994; Wong et al, 1995). Recent studies show that fluoxetine decreases immobility and increases swimming behavior in a forced swimming test, which is often used to determine if pharmacological compounds exhibit antidepressant activity, and this effect of fluoxetine is dependent on endogenous 5-HT (Kirby & Lucki, 1997; Page et al, 1999; Slattery et al, 2005). However, it has also been shown that fluoxetine has a direct effect on certain ion channels and receptors such as γ-aminobutyric acid $(GABA)_A$ receptors, 5-HT receptors, K^+ channels, Na^+ channels, and Ca^{2+} channels (Garcia-Colunga et al, 1997; Tytgat et al, 1997; Pancrazio et al, 1998; Hahn et al, 1999; Tunnicliff et al, 1999; Deak et al, 2000; Choi et al, 2003; Robinson et al, 2003).

Striatum is the major input nuclei in the basal ganglia and involved in motor control, therefore, it has been thought that changes in the synaptic transmission or synaptic plasticity of the striatum may contribute to the changes of the motor function. Striatum receives convergent glutamatergic inputs from the cortex and thalamus, dopaminergic inputs from the substantia nigra *pars compacta* (Ronesi & Lovinger, 2005), and 5-HT inputs from the raphe nuclei (Yakel et al, 1988). Many studies have shown that 5-HT has an important role in the function of both peripheral and central nervous systems, including sensory and motor regulation, cortical function, and emotional and mental illnesses, such as depression, schizophrenia, generalized anxiety disorder, and obsessive compulsive disorder (Jones & Blackburn, 2002).

These earlier studies suggest that fluoxetine might modulate corticostriatal synaptic transmission in the striatum, however, it is still not clear how fluoxetine acts, especially in electrophysiological studies. Therefore, in the present study, we studied the effects of fluoxetine on the corticostriatal synaptic transmission by measuring the changes of spontaneous excitatory postsynaptic currents (sEPSC) with whole-cell patch clamp techniques in corticostriatal rat brain slice.

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine, serotonin; sEPSC, spontaneous excitatory postsynaptic current; aCSF, artificial cerebrospinal fluid; PCA, p-chloroamphetamine; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; GABA, γ-aminobutyric acid.

METHODS

Slice preparation

Brain slices were prepared from 14- to 20-day-old Sprague-Dawley rats using previously described techniques (Sung et al, 2001). Rats were killed by decapitation, and the brains were removed and placed in ice-cold, modified artificial cerebrospinal fluid (aCSF) containing (in mM) 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose, pH adjusted to 7.4 by bubbling with 95% O_2/5% CO_2. Coronal slices (300 μm thick) were cut using a manual vibratome (Campden Instruments, Loughborough, UK). Brain slices were transferred to aCSF containing (in mM) 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose, pH adjusted to 7.4 by bubbling with 95% O_2/5% CO_2 at room temperature. Slices were used for electrophysiological experiments,

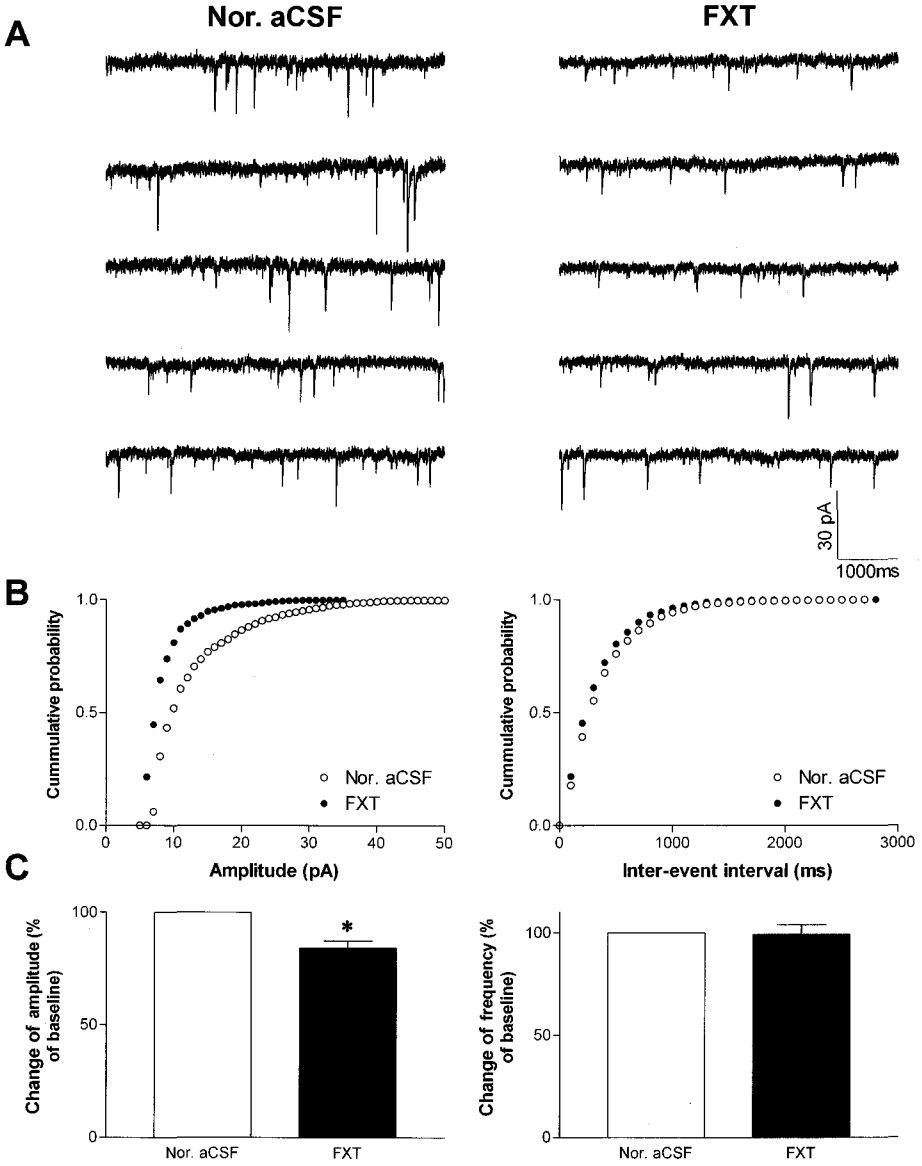

Fig. 1. Fluoxetine (FXT) decreases corticostriatal spontaneous excitatory postsynaptic currents (sEPSC) amplitude. (A) Representative traces of sEPSC shown in the presence of normal artificial cerebrospinal fluid (aCSF left column) and 10 μM FXT (right column). (B) Cumulative amplitude and inter-event interval histogram. The plot of the cumulative amplitude shows a left shift by FXT treatment, which decreased amplitude with no changes in inter-event interval. Kolmogorov-Smirnov two-sample test; amplitude, $P<0.005$; inter-event interval, $P=0.1372$. (C) Bar graphs showing the averaged changes of normalized amplitude (84.1$\pm$3.3%, n=7, $P<0.01$) and frequency (99.1$\pm$4.7%, n=7, $P=0.8621$) by the treatment of corticostriatal slices with FXT. Note significant decrease of only the amplitude by FXT treatment. *$P<0.01$, compared with before fluoxetine treatment.

beginning 1 hour after the end of slice preparation. A hemi-slice containing the cortex and striatum was completely submerged in a recording chamber and continuously superfused (at a flow rate of 2~3 ml/min) with aCSF that was constantly bubbled with 95% O_2/5% CO_2. The temperature of the bath solution was kept at 30~32°C. For 5-HT depletion, brain slices were incubated with aCSF, containing 10 μM p-chloroamphetamine (PCA), for at least 1 hour, and this PCA containing aCSF was used during the entire experiment.

Whole-cell recording

Whole-cell voltage-clamp recordings were performed to record the spontaneous excitatory postsynaptic currents (sEPSC) at striatal synapses. Slices were placed in the

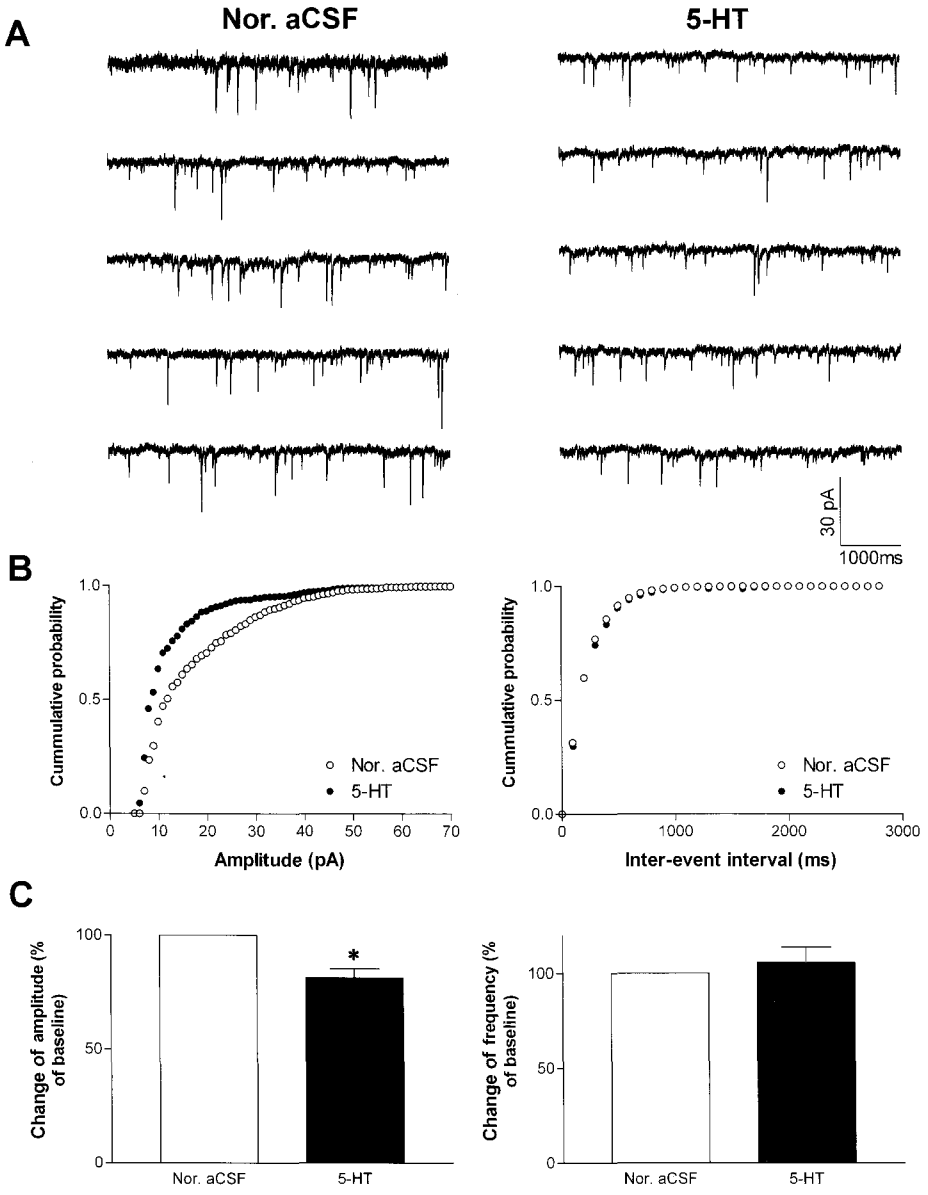

Fig. 2. Serotonin (5-HT) also decreases sEPSC amplitude. (A) Representative traces of sEPSC shown in the presence of normal artificial cerebrospinal fluid (aCSF left column) and 10 μM 5-HT (right column). (B) Cumulative amplitude and inter-event interval histogram. Cumulative amplitude and inter-event interval distribution display same trend, compared with FXT treatment only, shown in Fig. 1. Kolmogorov-Smirnov two-sample test; amplitude, $P<0.005$; inter-event interval, $P=0.1323$. (C) Bar graphs showing averaged change of normalized amplitude (81.2±3.9%, n=4, $P<0.05$) and frequency (105.8±8.0, n=4, $P=0.5234$) by the treatment of corticostriatal slices with 5-HT. 5-HT significantly decreased only the amplitude, which showed same trend as FXT. *$P<0.05$, compared with before 5-HT treatment.

recording chamber and superfused with aCSF as described previously. Tight-seal whole-cell recordings were obtained using pipettes made from borosilicate glass capillaries pulled on a P-97 micropipette puller (Sutter Instruments, Novato, CA). Pipette resistance ranged from 4 to 7 MΩ, and it was filled with internal solution containing (in mM) 120 $CsMeSO_3$, 5 NaCl, 10 tetraethylammonium chloride, 10 HEPES, 5 lidocaine N-ethyl bromide (QX-314) (Br^{2+} salt), 1.1 EGTA, 4 ATP (Mg^{2+} salt), 0.3 GTP (Na^{+} salt), pH adjusted to 7.2 with CsOH, and osmolarity adjusted to 290~300 mOsm with sucrose. Medium-sized neurons within two or three layers below the surface of the slice were identified using an Olympus BX50WI (Tokyo, Japan) differential interference contrast (DIC) microscope. Neurons were voltage-clamped at -70 mV, and sEPSC recorded with an Axopatch 1D patch-clamp amplifier (Axon Instruments, Foster City, CA) were filtered at 5 kHz, digitized at 20 kHz using a Digidata 1322A (Axon Instruments), and stored on a personal computer using pClamp 9.2 software (Axon Instruments).

Spontaneous synaptic events were analyzed off-line using the Mini Analysis Program (Synaptosoft, Decatur, GA). The threshold amplitude for the detection of an event was generally adjusted twice the RMS noise level (typically 7 pA). Events were visually inspected following automated analysis to prevent false positive identification and false negative rejection. This software was used to calculate sEPSC amplitude and frequency for each event. Frequencies were expressed as number of events per second (in Hz).

Data analysis

All averaged data were presented as means$\pm$SE. Statistical significance was determined by the Student's *t*-test. The criterion for significance was $P<0.05$. Data for cumulative histograms were compared statistically using the Kolmogorov-Smirnov test, where the statistical criterion for significance was $P<0.005$.

Drugs and chemicals

All chemicals, including fluoxetine, 5-HT, and PCA, were purchased from Sigma (St. Louis, MO). Agonists and other drugs were diluted in aCSF from stock solutions. 5-HT and PCA were dissolved in distilled water, and fluoxetine was dissolved in DMSO as 10 mM stock solution. The concentration of DMSO in the final dilution was less than 0.1%, and this DMSO concentration had no effect on sEPSC.

RESULTS

Effect of fluoxetine on corticostriatal synaptic transmission

As presented in Fig. 1, 10 μM fluoxetine significantly inhibited corticostriatal sEPSC amplitude, but not its frequency. Compared with control, fluoxetine decreased the amplitude and frequency to $84.1\pm3.3\%$ (n=7, $P<0.01$) and $99.1\pm4.7\%$ (n=7, P=0.8621) of the baseline, respectively. According to the quantal hypothesis, changes of amplitude indicate that there is a postsynaptic change of sensitivity to glutamate, and changes of frequency signify that there is a presynaptic change involving glutamate release (Muramatsu et al, 1998; Bennett & Kearns, 2000). Therefore, the above result auggests that fluoxetine acted on the postsynaptic site. To clarify whether the synaptic inhibition by fluoxetine was mediated by 5-HT through a selective serotonin reuptake inhibitior (SSRI) action or fluoxetine itself, we studied the effect of 5-HT (10 μM) on corticostriatal sEPSC. Fig. 2 shows that the amplitude was decreased to $81.2\pm3.9\%$ (n=4, $P<0.05$) and the frequency was increased only slightly to $105.8\pm8.0\%$ (n=4, P=0.5234) of the baseline. Therefore, both fluoxetine and 5-HT appears to show the same tendency, suggesting that the effect of fluoxetine might be based on a SSRI action.

Incubation of brain slices with PCA blocks the effect of fluoxetine

PCA depletes 5-HT from the axon terminals and blocks its reuptake (Sprague et al, 1996; Scholze et al, 2000). To confirm our results that the effect of fluoxetine on corticostriatal synaptic transmission might be due to SSRI action, we added 10 μM PCA to the aCSF to deplete 5-HT from the brain slice during the incubation period as well as throughout the entire experiment, and found that the inhibitory effect of fluoxetine on sEPSC amplitude was still significant under this condition: The amplitude was $93.0\pm1.9\%$ (n=8, $P<0.01$) and frequency was $94.3\pm4.1\%$ (n=8, P=0.2708) of the baseline (Fig. 3). The effect of 5-HT treatment on corticostriatal sEPSC under this condition was also significant, showing that the amplitude decreased to $84.3\pm1.6\%$ (n=4, $P<0.01$) of the baseline while the frequency was $97.3\pm5.8\%$ (n=4, P=0.6659) of the baseline (Fig. 4). Both fluoxetine and 5-HT significantly inhibitied the amplitude of sEPSC. Next, we compared these results with those obtained in the absence of PCA. Although the inhibitory activity of fluoxetine in the presence of PCA was significant, nevertheless, the effect of fluoextine was significantly blocked by PCA ($P<0.05$), but PCA did not block the effect of 5-HT (P=0.5055) (Fig. 5). These results suggest that the effect of fluoxetine is dependent on the amount of 5-HT present in the brain slice, however, there is also 5-HT independent effect.

DISCUSSION

The present study showed that fluoextine inhibits corticostriatal synaptic transmission by decreasing the amplitude of sEPSC, however it did not affect the frequency. As suggested, changes in the amplitude of sEPSC indicate a change in postsynaptic sensitivity to glutamate, and changes in the frequency of sEPSC alter the release of glutamate from the presynaptic axon terminals (Muramatsu et al, 1998; Bennett & Kearns, 2000), indicating that fluoxetine modulates corticostriatal synaptic transmission through postsynaptic mechanisms. In the present study, both 5-HT and fluoxetine showed similar trend of effect on corticostriatal sEPSC, suggesting that the effect of fluxoetine is mediated by a SSRI action. Therefore, we further confirmed this with depleting 5-HT from the brain slice by using PCA. Under such condition, the effect of 5-HT on corticostriatal sEPSC had no significant difference from that carried out without PCA, but the effect of fluoxetine was significantly blocked. Nevertheless, the effect of fluoxetine still remained significant. These results show that the decrease of corticostriatal sEPSC amplitude by fluoxetine is primarily mediated by a SSRI action which enhances the serotonergic trans-

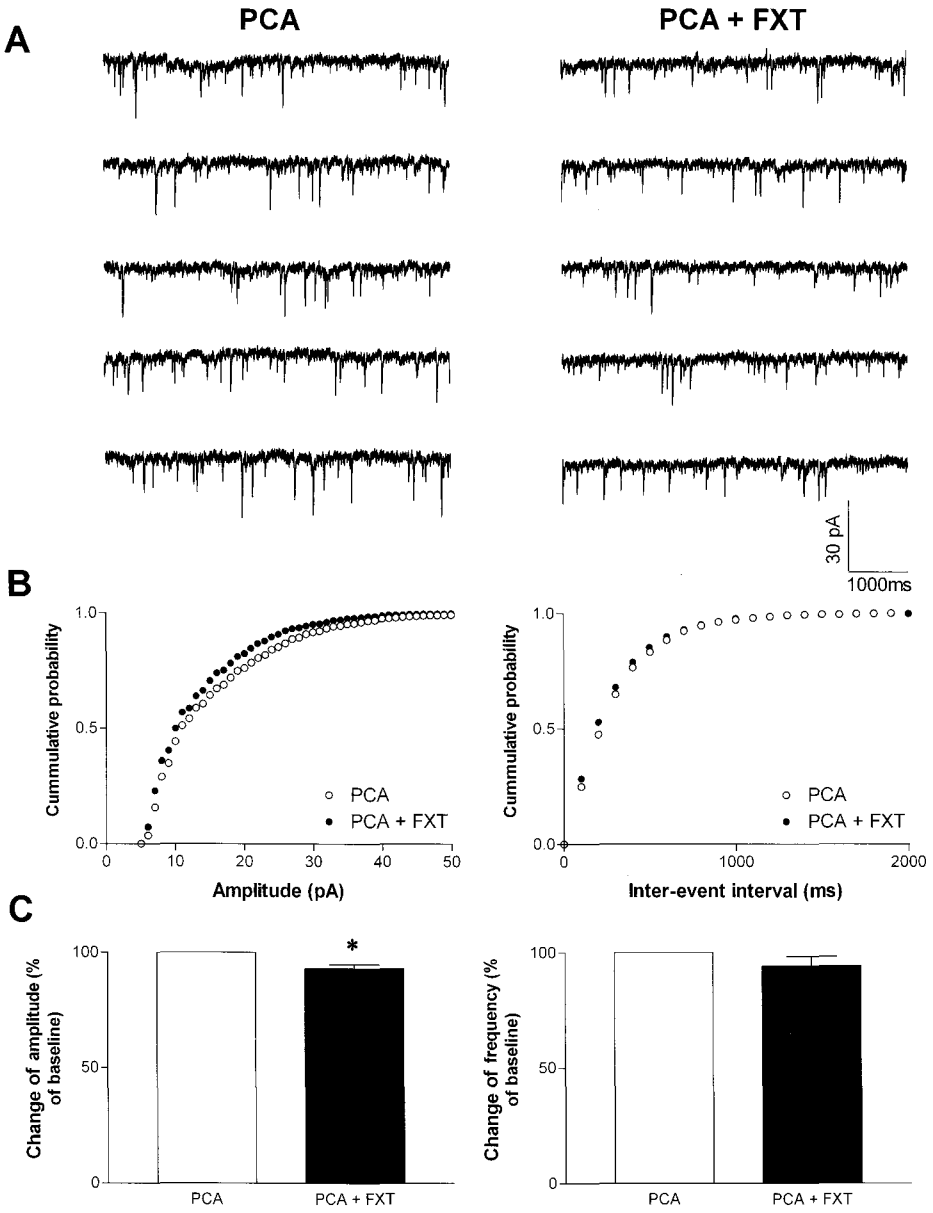

Fig. 3. The effect of 5-HT depletion by p-chloroamphetamine (PCA) on FXT treatment. (A) Representative trace showing prior to and following FXT in the presence of 10 M PCA (left and right column, respectively). (B) Cumulative amplitude and inter-event interval histogram. The plot of the cumulative amplitude still shows a left shift by FXT treatment in the presence of PCA. Kolmogorov-Smirnov two-sample test; amplitude, $P<0.005$; inter-event interval, $P=0.1772$. (C) Bar graphs showing averaged change of normalized amplitude ($93.0\pm1.9\%$, n=8, $P<0.01$) and frequency ($94.3\pm4.1\%$, n=8, $P=0.2078$) by the treatment of corticostriatal slices with FXT in the presence of PCA. Note the decrease by FXT treatment is still significant. But, the effect of FXT has also been blocked significantly by PCA (see Fig. 5). $^*P<0.01$, compared with before fluoxetine treatment.

mission, but there may also be another mechanism that inhibits corticostriatal sEPSC amplitude independent of 5-HT.

Various subtypes of 5-HT receptors (5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_{5A}, and 5-HT_6) exist in the striatum (Barnes & Sharp, 1999), and we recently demonstrated that 5-HT inhibits glutamatergic synaptic transmission in the rat corticostriatal region, especially on α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor mediated synapytic transmission (Choi et al, 2003; Cho et al, 2005). Dopamine- and cAMP-regulated phosphoprotein, M_r 32 kDa (DARPP-32), which plays an important role in AMPA receptor channel phosphorylation in the striatum, is affected by fluoxetine and, in this study, 5-HT showed the same effect as fluoxeine did (Svenningsson et al, 2002; Svenningsson et al, 2004). Furthermore, in the

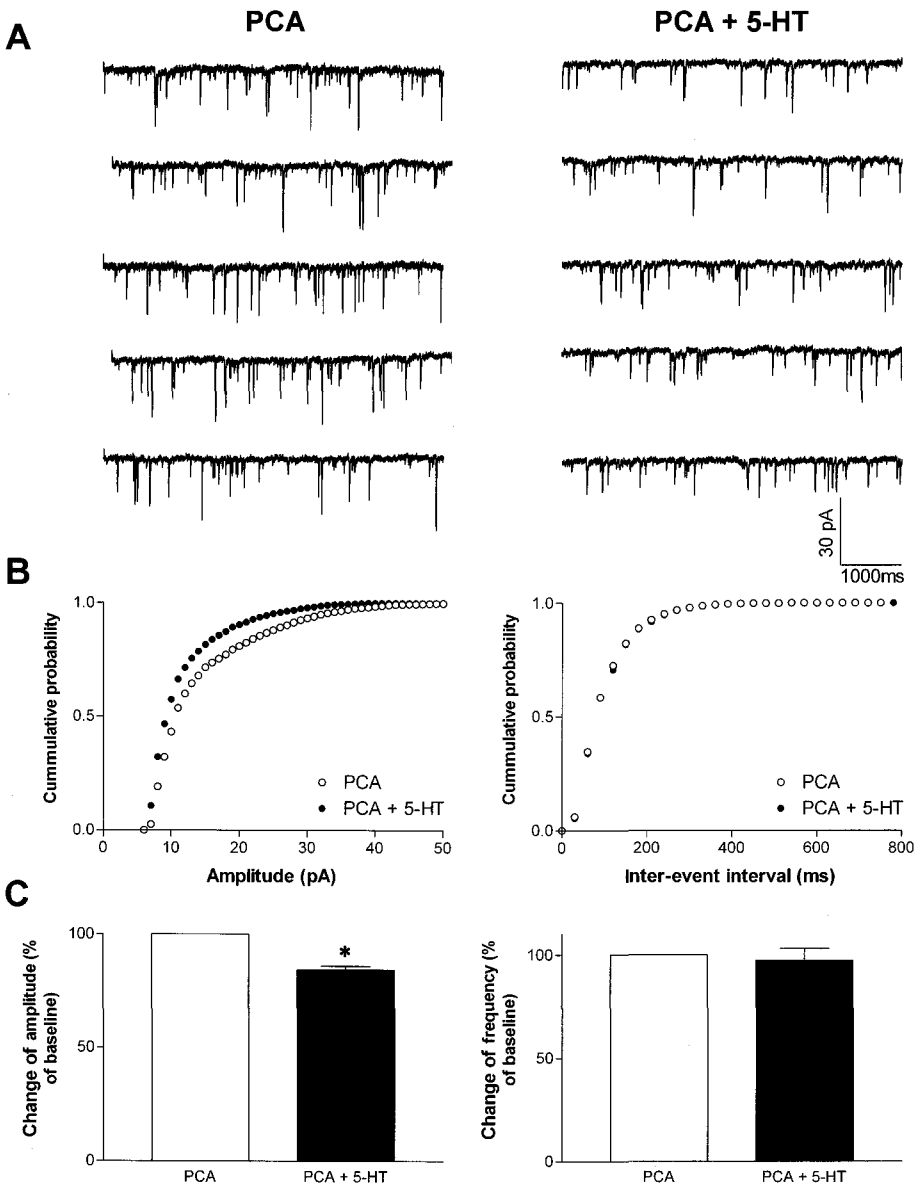

Fig. 4. The effect of 5-HT depletion by p-chloroamphetamine (PCA) incubation on 5-HT treatment. (A) Representative trace shows the prior to and following 5-HT in the presence of 10 μM PCA (left and right column, respectively). (B) Cumulative amplitude and inter-event interval histogram. The plot of the cumulative amplitudeshows the same trend compared with only 5-HT treatment, shown in Fig. 2. Kolmogorov-Smirnov two-sample test; amplitude, $P<0.005$; inter-event interval, $P=0.1342$. (C) Bar graphs showing averaged change of normalized amplitude ($84.3\pm1.6\%$, n=4, $P<0.01$) and frequency ($97.3\pm5.8\%$, n=4, $P=0.6659$) by the treatment of corticostriatal slices with 5-HT in the presence of PCA. PCA had no significant effect on 5-HT (also see Fig. 5). $^*P<0.01$, compared with before 5-HT treatment.

forced swimming test, which is a test to measure antidepressant activity (Porsolt et al, 1977; Porsolt et al, 1978), recent studies showed that fluoxetine increased the motility of rats and elevated the level of extracellular 5-HT (Kirby & Lucki, 1997; Page et al, 1999; Slattery et al, 2005). These results are in good agreement with our results that the effect of fluoxetine was mediated by 5-HT through a SSRI action. However, there are reports that fluoxetine has an effect on receptors or channels with a non-SSRI action: Fluoxetine inhibits the activity of nicotinic acetylcholine receptors and 5-HT_3 receptors (Garcia-Colunga et al, 1997; Choi et al, 2003). On the other hand, fluoxetine increases dopamine and norepinephrine levels (Koch et al, 2004), and enhances $GABA_B$ receptor activity (Sands et al, 2004). Especially, fluoxetine increases GABAergic synaptic transmission by increasing $GABA_A$ receptor activity (Robinson et al, 2003). These non-SSRI effects of fluoxetine may explain our results that fluxoetine still significantly decreased corticos-

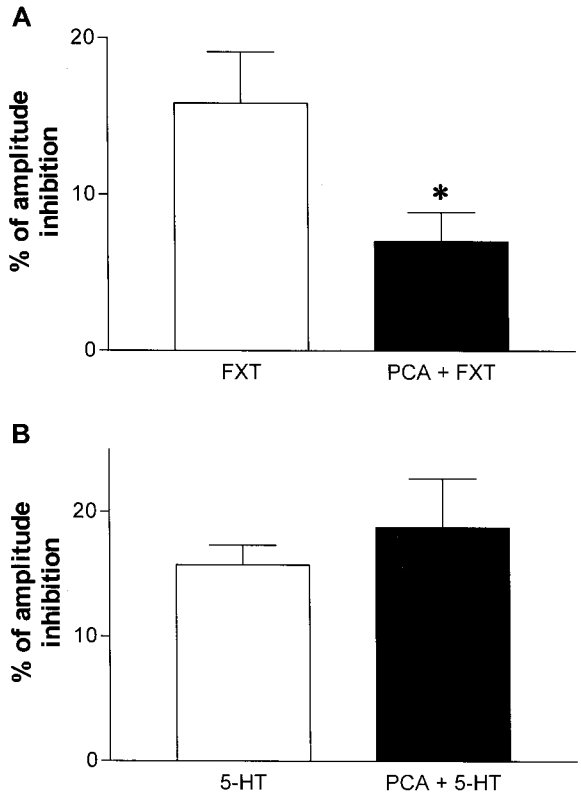

Fig. 5. The effect of PCA on the inhibition by FXT and 5-HT. (A) Bar graphs shows that the inhibitory effect of FXT was significantly blocked by the presence of PCA. (B) The effect of 5-HT on corticostriatal sEPSC was not affected by PCA. $^*P<0.05$, compared with the group without PCA treatment.

triatal sEPSC, when 5-HT in the brain slice was depleted by PCA. To obtain more evidence for the modulation of fluoxeitne on corticostriatal synaptic transmission, further study is needed by using selective 5-HT receptor antagonists to untangle which 5-HT receptor is involved in the action of fluoxetine as a SSRI. We might be able to figure out the mechanisms of the non-SSRI effects of fluxoetine, using GABA receptor antagonists and dopamine receptor antagonists.

Taken together, this study demonstrated that fluoxetine can suppress corticostriatal synaptic transmission via both 5-HT dependent and independent mechanisms, and might shed more light to the pharmacological mechanisms of fluoxetine.

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