CHRONIC ANTIDEPRESSANT TREATMENT ALTERS SEROTONERGIC
REGULATION OF GABA TRANSMISSION IN PREFRONTAL CORTICAL
PYRAMIDAL NEURONS

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Abstract—The serotonin system is highly involved in the
pathophysiology of mood disorders such as depression and
anxiety. Currently, the most widely used treatment for these
ilnesses is selective serotonin (5-HT) reuptake inhibitors,
such as fluoxetine. Because of the multiplicity of 5-HT recep-
tors and their different adaptive properties, the chronic ef-
fects of fluoxetine have remained unclear. In this study, we
investigated the alteration of 5-HT functions by long-term
antidepressant treatment in pyramidal neurons of prefrontal
cortex (PFC), a brain region crucial for the control of emotion
and cognition. One prominent function of serotonin in PFC is
to regulate GABAergic inhibitory transmission. Application
of 5-HT induced a large, desensitizing enhancement of the
amplitude and frequency of spontaneous inhibitory postsyn-
aptic currents (sIPSC), as well as a potent reduction of elec-
trically evoked IPSC (eIPSC). Chronic fluoxetine treatment
did not alter basal sIPSC, but reduced eIPSC in response to
different stimulus strengths. Moreover, chronic (but not
acute) fluoxetine treatment caused a much faster desensiti-
zation of the 5-HT effect on sIPSC, and significantly attenu-
ated the 5-HT effect on eIPSC. Application of a 5-HT₂ receptor
agonist produced similar effects as 5-HT on sIPSC and
eIPSC, and these effects were similarly altered by long-term
fluoxetine treatment. These electrophysiological results sug-
gest that chronic antidepressant treatment resulted in a
down-regulation of the synaptic function of forebrain 5-HT₂
receptors. Given the key role of GABAergic inhibitory trans-
mission in controlling PFC functions, its altered regulation by
serotonin after chronic fluoxetine treatment may provide a
mechanism underlying the therapeutic action of antidepressants. © 2004 IBRO. Published by Elsevier Ltd. All
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Key words: serotonin, 5-HT₂ receptors, desensitization,
imhibitory postsynaptic currents, fluoxetine, depression.

Serotonin in the CNS is a powerful modulator of emotional
processes. Tremendous evidence supports that dysfunc-
tion of serotonergic neurotransmission is implicated in the
pathogenesis of mood disorders including depression and
anxiety (Deakin, 1988; Dubovsky and Thomas, 1995;
Maes and Meltzer, 1995; Griebel, 1995; Stockmeier,
1997). One of the main target structures of the serotoner-
gic system is prefrontal cortex (PFC), a brain region highly
associated with the control of emotion and cognition
(Miller, 1999). Specific changes of the PFC serotonin sys-
tem found in patients with mental disorders (Stockmeier,
1997; Dean et al., 1999; Meyer et al., 1999) suggest that
serotonin plays a crucial and unique role in PFC.

Molecular cloning experiments have identified at
least 13 G protein-coupled serotonin receptor subtypes.
Serotonin can have both inhibitory and excitatory func-
tions in neuronal networks through the coupling of dif-
ferent 5-hydroxytryptamine (5-HT) receptors to distinct
cellular targets (Andrade, 1998). One mechanism
through which serotonin modulates PFC functions is via
the regulation of GABAergic inhibitory synaptic trans-
mission (Zhou and Habilitz, 1999; Feng et al., 2001; Cai
et al., 2002; Yan, 2002). The GABA system plays a key
role in regulating PFC functions (Constantinidis et al.,
2002). Alterations in the GABA system have been impli-
cated in mental illnesses associated with PFC dysfunc-
tion (Benes et al., 1996; Ohnuma et al., 1999; Dean et
al., 1999; Lewis, 2000).

Currently, the most widely prescribed antidepressant
drugs are selective serotonin reuptake inhibitors (SSRIs),
such as fluoxetine. Molecular and cellular mechanisms
that underlie the therapeutic action of these drugs have
remained unclear. One reason is that a multiplicity of 5-HT
receptors co-exists in neurons and each of their function is
complex and obscure. Chronic treatment with fluoxetine
can induce different adaptive changes in these 5-HT re-
cessors in distinct brain regions (Blier et al., 1988; Blier and
Bouchard, 1994; Haddjeri et al., 1998), making it difficult to
delineate the effects of long-term antidepressant treat-
ment.

In this study, we investigated the 5-HT regulation of
GABA transmission in PFC pyramidal neurons from rats
exposed to chronic fluoxetine treatment. Rats were
treated repeatedly with the SSRI fluoxetine for 21 days.
The effect of 5-HT or 5-HT receptor agonists on sponta-
neous inhibitory postsynaptic currents (IPSC [sIPSC])
and electrically evoked IPSC (eIPSC) was measured in
PFC slices from fluoxetine-treated rats using the whole-
cell patch-clamp recording technique. Our data on the
antidepressant-induced alteration of 5-HT functions may
provide a framework within which the role of serotonin in
normal mental functions and affective disorders can be
better understood.
**EXPERIMENTAL PROCEDURES**

**Electrophysiological recordings in slices**

Young adult (4 to 6 weeks old) Sprague–Dawley rat slices containing PFC were prepared as previously described (Zhong et al., 2003). All experiments were carried out with the approval of State University of New York at Buffalo Animal Care Committee. The number of animals used and their potential suffering was minimized. In brief, animals were anesthetized by inhalation of 2-bromo-2-chloro-1,1,1-trifluoroethane (1 ml/100 g; Sigma, St. Louis, MO, USA) and decapitated; brains were quickly removed, iced and then blocked for slicing. The blocked tissue was cut in 300–400 µm slices with a Vibratome while bathed in a low Ca²⁺ (100 µM) HEPES-buffered salt solution (in mM: 140 Na iodide, 2 KCl, 4 MgCl₂, 0.1 CaCl₂, 23 glucose, 15 HEPES, 1 kynurenic acid, pH=7.4, 300–305 mOsm). Slices were then incubated for 1–6 h at room temperature (20–22 °C) in a NaHCO₃-buffered saline bubbled with 95% O₂, 5% CO₂ (in mM): 126 NaCl, 2.5 KCl, 2 CaCl₂, 2 MgCl₂, 6 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 1 pyruvic acid, 0.05 glutathione, 0.1 N⁷-nitro-L-arginine, 1 kynurenic acid, pH=7.4, 300–305 mOsm.

To evaluate the regulation of sIPSC in PFC slices, the whole-cell patch technique was used for voltage-clamp recordings using patch electrodes (5–9 MΩ) filled with the following internal solution (in mM): 100 CsCl, 30 N-methyl-d-glucamine, 10 HEPES, 1 MgCl₂, 4 NaCl, 5 EGTA, 0.8 QX314, 12 phosphocreatine, 2 MgATP, 0.2 Na₃GTP, 0.1 leupeptin, pH=7.2–7.3, 265–270 mOsm. The slice (300 µm) was placed in a perfusion chamber attached to the fixed-stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial cerebrospinal fluid. All recordings were made at the room temperature. For blocking glutamate transmission, the AMPA/KA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (20 µM) was positioned approximately 100 µm from the pyramidal tissue. All recordings were made at the room temperature. 

To determine the identity of 5-HT receptors in the serotoninergic regulation of sIPSC, we treated PFC slices with the GABAergic antagonist bicuculline (10 µM) and were anesthetized by inhalation of 2-bromo-2-chloro-1,1,1-trifluoroethane (1 ml/100 g; Sigma, St. Louis, MO, USA) and decapitated; brains were quickly removed, iced and then blocked for slicing. The blocked tissue was cut in 300–400 µm slices with a Vibratome while bathed in a low Ca²⁺ (100 µM) HEPES-buffered salt solution (in mM: 140 Na iodide, 2 KCl, 4 MgCl₂, 0.1 CaCl₂, 23 glucose, 15 HEPES, 1 kynurenic acid, pH=7.4, 300–305 mOsm). Slices were then incubated for 1–6 h at room temperature (20–22 °C) in a NaHCO₃-buffered saline bubbled with 95% O₂, 5% CO₂ (in mM): 126 NaCl, 2.5 KCl, 2 CaCl₂, 2 MgCl₂, 6 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 1 pyruvic acid, 0.05 glutathione, 0.1 N⁷-nitro-L-arginine, 1 kynurenic acid, pH=7.4, 300–305 mOsm.

**RESULTS**

**5-HT enhancement of spontaneous GABA transmission desensitizes faster after chronic fluoxetine treatment**

To test the potential impact of long-term administration of antidepressants on serotonin functions, we compared the 5-HT regulation of GABAergic inhibitory transmission in PFC neurons from rats treated with fluoxetine (10 mg/kg, 21 days) or saline. All recordings were performed in pyramidal neurons located at the deep layers (IV–V) of rat medial PFC. Chronic fluoxetine treatment did not significantly alter the basal spontaneous GABA transmission, as reflected on sIPSC mean amplitude (saline-treated: 35.0±2.3 pA, n=20, 13 rats; fluoxetine-treated: 37.8±2.7 pA, n=16, 10 rats) and frequency (saline-treated: 267±31.2 events/min, n=20; fluoxetine-treated: 278.3±37.5 events/min, n=16), as well as mIPSC mean amplitude (saline-treated: 22.5±2.1 pA, n=6, four rats; fluoxetine-treated: 23.4±1.9 pA, n=8, five rats). The kinetics of mIPSC, as measured with a double exponential fitting of the decay of averaged mIPSC, was also not significantly changed by long-term fluoxetine treatment (saline-treated: t₁: 10.2±0.8 ms, r₂: 54.2±10.6 ms, n=6; fluoxetine-treated: t₁: 10.7±0.3 ms, r₂: 52.1±13.1 ms, n=5). The responsiveness of PFC neurons to 5-HT was evaluated by exogenous application of this neurotransmitter directly onto these neurons. In PFC pyramidal neurons from saline-treated rats, bath application of 5-HT (20 µM) caused a reversible increase in the amplitude and frequency of mIPSC. The increase reached a peak and then started to decline within minutes during extended application of 5-HT. A representative example is shown in Fig. 1A–C. In neurons from fluoxetine-treated rats, the 5-HT-induced increase of mIPSC was observable, but it desensitized much faster (Fig. 1D–F).

To determine the identity of 5-HT receptors in the serotoninergic regulation of sIPSC, we treated PFC slices
with the selective 5-HT₂ antagonist ketanserin (10 μM).
Ketanserin treatment (n=6) abolished the effect of 5-HT on 
siPSC amplitude (Fig. 2A) and frequency (Fig. 2B), 
suggesting that 5-HT₂A/2C are the primary receptors mediating 
the 5-HT effect on siPSC.

The time courses of the 5-HT regulation of siPSC amplitude and frequency in a sample of PFC pyramidal neurons from rats treated with saline or fluoxetine are summarized in Fig. 2A–D. After long-term exposure (21-day) to fluoxetine, the duration of 5-HT effects on siPSC was 1.5±0.2 min (Fig.
2E, \( n=16, \) 10 rats), which was significantly \( (P<0.001, \) ANOVA) shorter than that in control cells (Fig. 2E, 4.2\pm0.5 \text{ min,} \ n=20, \) 13 rats). In contrast to the faster desensitization of 5-HT effects, chronic fluoxetine treatment did not significantly alter the enhancing effect of 5-HT on sIPSC amplitudes (saline-treated: 108.7\pm13.4\%, \ n=20; fluoxetine-treated:

Fig. 2. Summary data showing the effect of 5-HT on sIPSC in neurons from saline- or fluoxetine-treated rats. (A, B) Plot of sIPSC amplitude (A) and frequency (B) against time and agonist (5-HT, 20 \( \mu \text{M} \)) application in a sample of neurons from saline-treated rats treated with or without selective 5-HT\(_2\) antagonist ketanserin (10 \( \mu \text{M} \)). (C, D) Plot of sIPSC amplitude (C) and frequency (D) against time and agonist (5-HT, 20 \( \mu \text{M} \)) application in a sample of neurons from fluoxetine-treated rats. Filled points (A–D) indicated the points recorded during 5-HT application. Bin width (A–D): 15 s. (E) Histograms (mean\pmS.E.M.) showing the duration of 5-HT effects on sIPSC in PFC neurons from rats treated with saline, acute fluoxetine (12 h), chronic fluoxetine (21 days), or 2 days’ withdrawal from chronic fluoxetine. The number of tested neurons in different conditions is indicated in each bar. ** \( P<0.001, \) ANOVA.
96.4±14.3%, n=16; P>0.01, ANOVA) or sIPSC frequencies (saline-treated: 283.5±38.6%, n=20; fluoxetine-treated: 250.3±36.7%, n=16; P<0.01, ANOVA). To discern whether the shorter duration of 5-HT effects on sIPSC is due to changes in 5-HT receptor sensitivity or to the effect of remaining fluoxetine in brain, we also examined rats 2 days after the last fluoxetine administration to allow for drug clearance from the tissue. As shown in Fig. 2E, in these 2-day withdrawal fluoxetine-treated rats (n=3), the duration of 5-HT effects on sIPSC was also significantly shorter (1.7±0.3 min, n=5, P<0.001, ANOVA), while the enhancing effect of 5-HT on sIPSC amplitudes (93.5±21.9%, n=5) and sIPSC frequencies (242.3±49.4%, n=5) was not altered. Acute (12 h) treatment with fluoxetine did not alter the 5-HT effect on sIPSC (Fig. 2E, duration: 4.0±0.7 min, amplitude increase: 98.3±16.9%, frequency increase: 270.7±51.2%, n=5, 3 rats). These results indicate that chronic fluoxetine treatment caused a faster desensitization of the 5-HT effect on spontaneous GABA transmission.

Long-term fluoxetine treatment reduces evoked GABA transmission and its regulation by 5-HT

We next compared the GABAergic synaptic transmission evoked by electrical stimulation in PFC neurons from saline- or fluoxetine-treated rats. Stimuli with different intensities (200–500 μA) were delivered to neighboring GABAergic interneurons to trigger action potentials, which induced the release of GABA at synaptic terminals and evoked IPSC in the pyramidal neuron under recording. As shown in Fig. 3A, when the stimuli intensity increased from 300 μA to 400 μA, eIPSC amplitudes increased almost linearly, however, in neurons from fluoxetine-treated rats (n=5), IPSC amplitudes evoked by these stimuli were significantly smaller (25–42%, n=9; P<0.01, ANOVA), compared with neurons (n=8) from saline-treated rats (n=4). With further increase of stimulus strengths, eIPSC amplitudes approached a plateau, but eIPSC amplitudes in neurons from fluoxetine-treated rats were still smaller (15–25%, n=9; P<0.01, ANOVA) than those neurons (n=8) from saline-treated rats. The kinetics of ePSC (fitted with a double exponential equation) was not significantly changed by long-term fluoxetine treatment (saline-treated: τ1: 14.9±0.7 ms, τ2: 46.2±2.2 ms, n=5; fluoxetine-treated: τ1: 13.8±0.8 ms, τ2: 43.7±1.9 ms, n=5). These results suggest that chronic treatment with fluoxetine reduced the efficacy of GABAergic synaptic transmission.

We then compared the regulation of eIPSC by exogenously applied 5-HT in PFC neurons from saline- or fluoxetine-treated rats. As shown in Fig. 3B, a low concentration of 5-HT (2 μM) produced a potent reduction of eIPSC in the neuron from a saline-treated rat, while this 5-HT effect was markedly attenuated in the neuron from a fluoxetine-treated rat. The percentage reduction of eIPSC by different concentrations of 5-HT in neurons from rats treated with saline or fluoxetine is summarized in Fig. 3C. A high concentration of 5-HT (20 μM) reduced eIPSC amplitude by 56.2±2.4% (n=6) in neurons from saline-
treated rats \( n=4 \), and this 5-HT effect in neurons from fluoxetine-treated rats \( n=3 \) was significantly smaller \( (39.5 \pm 4.1\% \), \( n=5 \), \( P<0.01 \), ANOVA) \. More substantial difference was found with a low concentration of 5-HT (2 \( \mu M \)) which reduced eIPSC amplitude by 41.2 \( \pm 1.9\% \) \( (n=8) \) in neurons from saline-treated rats \( n=4 \), but produced a significantly smaller reduction \( (23.8 \pm 1.3\% \), \( n=5 \), \( P<0.001 \), ANOVA) \) in neurons from chronic fluoxetine-treated rats \( n=3 \). In the 2-day withdrawal fluoxetine-treated rats \( n=3 \), the reducing effect of 5-HT (2 \( \mu M \)) on eIPSC was also significantly smaller \( \text{(Fig. 3C, 25.6} \pm 1.1\% \), \( n=6 \), \( P<0.001 \), ANOVA) \. The 5-HT (2 \( \mu M \))-induced reduction of eIPSC amplitude was unaltered \( (\text{Fig. 3C, 40.0} \pm 3.2\% \), \( n=10) \) in acute fluoxetine-treated rats \( n=6 \). These results indicate that chronic treatment with fluoxetine reduced the serotonergic regulation of evoked GABA transmission.

5-HT \(_2\) receptor-mediated regulation of spontaneous and evoked GABA transmission is also altered by chronic fluoxetine treatment

To test whether the fluoxetine-induced alteration of 5-HT regulation of GABA transmission is attributable to changes of some 5-HT receptors, we examined the regulation of GABA transmission by 5-HT receptor agonists in neurons from fluoxetine-treated rats. Application of \( \alpha\)-methyl-5-HT \( (\alpha\)-Me-5-HT; 20 \( \mu M \)), a selective 5-HT \(_{2A/2C}\) agonist, produced a large, desensitizing enhancement of sIPSC amplitude and frequency in approximately 60% \( (21 \text{ of } 35) \) of PFC pyramidal neurons from saline-treated rats \( n=12 \). A representative example is shown in Fig. 4A and B. However, \( \alpha\)-Me-5-HT exhibited an enhancing effect on sIPSC only in \(<40\%\) (seven of 18) of PFC pyramidal neurons from fluoxetine-treated rats \( n=6 \). Moreover, the \( \alpha\)-Me-5-HT effect desensitized much faster after fluoxetine treatment (an example shown in Fig. 4A and D). As summarized in Fig. 4E–H, after long-term exposure to fluoxetine, the duration of \( \alpha\)-Me-5-HT effects on sIPSC was 1.4 \( \pm 0.2 \) min \( (n=7) \), six rats), which was significantly \( (P<0.001 \), ANOVA) shorter than that in control cells \( (4.1 \pm 0.6 \) min, \( n=21 \), 12 rats) \. Chronic fluoxetine treatment did not significantly alter the enhancing effect of \( \alpha\)-Me-5-HT on sIPSC amplitudes \( (\text{saline-treated: 70.2}\% \pm 10.3\% \), \( n=21 \); fluoxetine-treated: 55.3 \( \pm 9.2\% \), \( n=7 \), \( P>0.01 \), ANOVA) or sIPSC frequencies \( (\text{saline-treated: 175.6}\% \pm 23.4\% \), \( n=21 \); fluoxetine-treated: 132.7 \( \pm 18.5\% \), \( n=7 \), \( P>0.01 \), ANOVA) \. These results suggest that long-term fluoxetine treatment facilitates the desensitization of 5-HT \(_2\) receptors in the responsive PFC neurons.

We then compared the regulation of eIPSC by activation of 5-HT \(_2\) receptors with \( \alpha\)-Me-5-HT in PFC neurons from saline- or fluoxetine-treated rats. Similar to what was found with 5-HT \( (2 \, \mu M \), Fig. 3B), \( \alpha\)-Me-5-HT \( (20 \, \mu M \)) produced a potent reduction of eIPSC in the neuron from a saline-treated rat, while this effect was markedly attenuated in the neuron from a fluoxetine-treated rat \( (\text{Fig. 5A, B}) \). As summarized in Fig. 5C, in neurons from fluoxetine-treated rats \( n=4 \), \( \alpha\)-Me-5-HT \( (20 \, \mu M \)) reduced eIPSC amplitude by 19.2 \( \pm 1.1\% \) \( (n=7) \), which was significantly \( (P<0.001 \), ANOVA) smaller than the effect of \( \alpha\)-Me-5-HT in neurons \( (39.8\% \pm 1.3\% \), \( n=12 \)) from saline-treated rats \( n=7 \). After acute treatment with fluoxetine, the effect of \( \alpha\)-Me-5-HT on eIPSC was not attenuated \( (40.5\% \pm 2.1\% \), \( n=5 \)). These results indicate that chronic treatment with fluoxetine reduced the capability of 5-HT \(_2\) receptors to regulate GABA transmission.

To test whether chronic SSRI treatment induced a tonic activation of 5-HT \(_2\) receptors in PFC neurons, we also examined the effect of the non-selective 5-HT receptor antagonist methysergide \( (10 \, \mu M \)) and the selective 5-HT \(_2\) antagonist ketanserin \( (20 \, \mu M \)) on eIPSC. We found that both methysergide and ketanserin had little effect on eIPSC in PFC neurons \( (6.5\% \pm 1.5\% \), \( n=7 \)) from fluoxetine-treated rats \( n=3 \), similar to the lack of effect with methysergide or ketanserin in PFC neurons \( (4.6\% \pm 1.5\% \), \( n=4 \)) from saline-treated rats \( n=3 \).

DISCUSSION

An enhanced 5-HT synaptic transmission is believed to be a common end result of long-term administration of antidepressants \( (Blier et al., 1988; Artigas et al., 1996) \). Several lines of evidence suggest that adaptive changes in the 5-HT system may play a pivotal role in the therapeutic effect of antidepressant treatments \( (Blier and de Montigny, 1994) \). Different 5-HT receptors exhibit distinct adaptive properties. Long-term treatment with SSRIs results in the desensitization of somatodendritic 5-HT \(_{1A}\) autoreceptors in dorsal raphe nucleus \( (Blier et al., 1988) \), while causes a tonic activation of postsynaptic 5-HT \(_{1B/1D}\) receptors in dorsal hippocampus \( (Haddjeri et al., 1998) \). Moreover, chronic SSRI treatment desensitizes terminal 5-HT \(_{1B/1D}\) autoreceptors \( (Blier and Bouchard, 1994) \). In contrast to the well-documented alterations of 5-HT, family receptors by antidepressant treatments, evidence showing the functional changes of forebrain 5-HT \(_2\) family receptors induced by long-term SSRI treatment has been lacking.

Serotonin exerts a powerful and complex impact on GABAergic synaptic transmission in frontal cortical neurons \( (Zhou and Hablitz, 1999; Feng et al., 2001; Cai et al., 2002; Yan, 2002) \). On the one hand, 5-HT induces a large and desensitizing increase in the amplitude and frequency of sIPSC, which is mediated primarily by 5-HT \(_2\) receptors \( (Zhou and Hablitz, 1999) \). On the other hand, 5-HT causes a potent and non-desensitizing reduction of the amplitude of eIPSC. It suggests that the 5-HT regulation of sIPSC (transient) vs. 5-HT regulation of eIPSC (sustained) may be mediated by different mechanisms. The 5-HT effects on sIPSCs could be due to a 5-HT-induced elevation of GABAergic interneuron excitability, while the 5-HT effects on eIPSCs could be due to a 5-HT-induced inhibition of action potential-dependent GABA release from axon terminals.

After chronic fluoxetine treatment, several alterations were found regarding the serotonergic regulation of GABA transmission. First, the 5-HT \(_2\) regulation of sIPSC desensitizes much faster. The effect of 5-HT or \( \alpha\)-Me-5-HT usually declines within 4 min in the continued presence of the agonist. However, in PFC slices from fluoxetine-treated
Fig. 4. The enhancing effect of a 5-HT$_2$ receptor agonist on sPSC desensitized faster after chronic fluoxetine treatment. (A–D) Plot of sPSC amplitude (A, C) and frequency (B, D) against time and agonist (α-Me-5-HT, 20 μM) application in a neuron from a saline-treated rat (A, B) and a neuron from a fluoxetine-treated rat (C, D). Bin width: 2 s. Frequency: events/second (Hz). (E–H) Plot of sPSC amplitude (E, G) and frequency (F, H) against time and agonist (α-Me-5-HT, 20 μM) application in a sample of neurons from saline-treated rats (E, F, $n=7$) or fluoxetine-treated rats (G, H, $n=21$). Filled points indicated the points recorded during α-Me-5-HT application. Bin width: 15 s.
animals, the serotonergic regulation of siPSC lasts much shorter (1.5 min). It suggests that 5-HT₂ receptors are more easily desensitized after long-term SSRI treatment. Second, the strength of GABAergic synaptic transmission evoked by electrical stimulation is reduced, suggesting that the SSRI-induced elevation of extracellular 5-HT concentration leads to a tonic inhibition of eIPSC. Third, the regulation of eIPSC by 5-HT or α-Me-5-HT is significantly attenuated. It suggests that 5-HT₂ receptors are partially desensitized after long-term SSRI treatment, therefore resulting in the diminished response to an acute challenge with 5-HT (low-concentration) or a 5-HT₂ receptor agonist. This desensitization could result from the sustained activation of synaptic 5-HT₂ receptors due to increased levels of 5-HT after prolonged SSRI treatment. In addition to the potential change in 5-HT₂ receptor properties, long-term SSRI treatment may also cause a change in the 5-HT-induced intracellular signaling cascade, which leads to the altered regulation of siPSC and eIPSC by exogenously applied 5-HT or α-Me-5-HT. All these alterations were only observed with chronic, but not acute, administration of fluoxetine, consistent with its delayed therapeutic effect. In addition to the altered 5-HT₂-mediated regulation of inhibitory transmission, it is possible that fluoxetine treatment may have also affected 5-HT₂₅A-mediated regulation of excitatory inputs to pyramidal neurons (Aghajanian and Marek, 1997), which is not included in this study, because all recordings were made in the presence of AMPA and NMDA receptor blockers.

Using radioligand binding, previous studies have given inconsistent results about the effect of chronic fluoxetine treatment on 5-HT₂ receptor numbers, with either no change (Cadogan et al., 1993; Goodnough and Baker, 1994) or an increase (Hrdina and Vu, 1993; Klimek et al., 1994) being reported. More precise measurement of the expression of 5-HT₂ receptors in PFC pyramidal neurons and GABAergic interneurons with or without long-term fluoxetine treatment needs to be done. At this point, we think that the SSRI-induced functional changes of 5-HT₂ receptors are likely to be caused by the alteration of their properties or coupled signaling.

In addition to the alterations we found with serotonergic regulation of GABA transmission in fluoxetine-treated animals, an electrophysiological study shows that chronic, but not acute, treatment with fluoxetine significantly suppresses the firing and excitability of PFC neurons (Gronier and Rasmussen, 2003). This effect could be due to the reduced 5-HT suppression of GABAergic inhibition following sustained fluoxetine treatment. A neurochemical study indicates that fluoxetine treatment (21 days) increases 5-HT-stimulated, but not GTPγS-stimulated, PLC activity in PFC, suggesting a supersensitivity at the level of 5-HT₂₅A receptor or receptor-G protein interaction (Damjanoska et al., 2003). Our functional studies suggest that chronic fluoxetine treatment causes 5-HT₂ receptors to desensitize faster (as seen with the regulation of siPSC by exogenous application of 5-HT or 5-HT₂ agonists) or partially desensitized (as seen with the regulation of eIPSC by exogenous application of 5-HT or 5-HT₂ agonists). One possible mechanism that may explain the discrepancy is that different kinds of receptor-G protein interaction are involved in serotonergic regulation of PLC activity vs. serotonergic regulation of GABA transmission. One gets supersensitized and the other gets desensitized after chronic fluoxetine treatment.

What is the functional consequence of the 5-HT-induced bi-directional regulation of GABA transmission at basal and activated states? Under basal conditions, the tonic firing of a few GABAergic interneurons maintains a weak inhibitory circuit, and activation of 5-HT receptors triggers a transient potentiation of the weak inhibition. Under active conditions, the excitation of a large number of GABAergic interneurons forms a strong inhibitory circuit, and activation of 5-HT receptors produces a potent suppression of the strong inhibition. Therefore, serotonin serves as a stabilizing agent, which assists in returning the neuronal activity to its homeostatic set point. 5-HT deficit in depression could lead to the dyregulation of GABA transmission and thus unbalanced inhibitory circuits. By blocking 5-HT reuptake and elevating extracellular 5-HT levels, long-term SSRI antidepressant treatments will endow the 5-HT system with different adaptive changes, leading to the restoration of normal 5-HT functions. These adaptive changes may have also affected 5-HT₂₅A-mediated regulation of excitatory inputs to pyramidal neurons (Aghajanian and Marek, 1997), which is not included in this study, because all recordings were made in the presence of AMPA and NMDA receptor blockers.

**Fig. 5.** Chronic fluoxetine treatment reduced the effect of 5-HT₂ receptors on eIPSC. (A) Representative eIPSC traces recorded on a neuron from a saline-treated rat (left) and a neuron from a fluoxetine-treated rat (right) under control condition and during application of α-Me-5-HT (20 μM). Scale bars = 100 pA, 50 ms. (C) Histograms (mean ± S.E.M.) showing the percentage reduction of eIPSC amplitudes by α-Me-5-HT (20 μM) in neurons from saline- or fluoxetine-treated rats. The number of tested neurons is indicated in each bar. *P < 0.01, ANOVA.
changes include sensitization/desensitization of different 5-HT receptors in different brain regions. Since selective alterations in the GABA system have been discovered in PFC of patients with mental disorders (Benes et al., 1996; Ohnuma et al., 1999; Dean et al., 1999, Lewis, 2000), our study provides functional evidence suggesting that one of the possible mechanisms by which SSRIs alleviate depression is to desensitize forebrain 5-HT$_2$ receptors and thus change the serotonergic regulation of GABA transmission.

Acknowledgments—We would like to thank Xiaqing Chen for technical assistance. This work was supported by NIH grants MH63128, AG21923, and NSF grant IBN-0117026 (Z.Y.).

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(Accepted 30 June 2004)

(Available online 1 September 2004)