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

P. ZHONG AND Z. YAN*

Department of Physiology and Biophysics, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, 124 Sherman Hall, Buffalo, NY 14214, USA

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 illnesses is selective serotonin (5-HT) reuptake inhibitors, such as fluoxetine. Because of the multiplicity of 5-HT receptors and their different adaptive properties, the chronic effects 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 postsynaptic currents (sIPSC), as well as a potent reduction of electrically 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 desensitization of the 5-HT effect on sIPSC, and significantly attenuated the 5-HT effect on eIPSC. Application of a 5-HT_2 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 suggest that chronic antidepressant treatment resulted in a down-regulation of the synaptic function of forebrain 5-HT_2 receptors. Given the key role of GABAergic inhibitory transmission in controlling PFC functions, its altered regulation by serotonin after chronic fluoxetine treatment may provide a mechanism underlying the therapeutic action of antidepressants.

Key words: serotonin, 5-HT_2 receptors, desensitization, inhibitory postsynaptic currents, fluoxetine, depression.

Serotonin in the CNS is a powerful modulator of emotional processes. Tremendous evidence supports that dysfunction 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 serotonergic 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 system 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 functions in neuronal networks through the coupling of different 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 transmission (Zhou and Hablitz, 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 implicated in mental illnesses associated with PFC dysfunction (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 receptors 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 treatment.

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 spontaneous 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.

*Corresponding author. Tel: +1-716-829-3058; fax: +1-716-829-2699.
E-mail address: zhenyan@buffalo.edu (Z. Yan).

Abbreviations: eIPSC, electrically evoked inhibitory postsynaptic current; IPSC, inhibitory postsynaptic currents; IR, infrared; α-Me-5-HT, α-methyl-5-HT; PFC, prefrontal cortex; sIPSC, spontaneous inhibitory postsynaptic current; SSRI, selective serotonin reuptake inhibitors; 5-HT (serotonin), 5-hydroxytryptamine.

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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 inhaling 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 Ca^{2+} (100 μM), HEPES-buffered salt solution (in mM: 140 Na isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, 1 kynurenic acid, pH=7.4, 300–305 mOsm/l). Slices were then incubated for 1–6 h at room temperature (20–22 °C) in a $NaHCO_3$-buffered saline bubbled with 95% O_2, 5% CO_2 (in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 glucose, 1 pyruvic acid, 0.05 glutathione, 0.1 N^G-nitro-L-arginine, 1 kynurenic acid, pH=7.4, 300–305 mOsm/l.

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 $MgCl_2$, 4 NaCl, 5 EGTA, 0.8 QX314, 12 phosphocreatine, 2 MgATP, 0.2 Na_3GTP, 0.1 leupeptin, pH=7.2–7.3, 265–270 mOsm/l. 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) and NMDA receptor antagonist D(−)-2-amino-5-phosphonopetanoic acid (20 μM) were added to the recording solution. Cells were visualized with a 40× water-immersion lens and illuminated with near infrared (IR) light and the image was detected with an IR-sensitive CCD camera. A Multiclamp 700A amplifier (Axon Instruments, Union City, CA, USA) was used for these recordings. Tight seals (2–10 GΩ) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction and the whole-cell configuration was obtained. The access resistances ranged from 13 to 18 MΩ and were compensated 50–70%. Cells were held at −70 mV for the recording of spontaneous IPSC. eIPSC was generated with a 50 μs pulse from a stimulation isolation unit controlled by a S48 pulse generator (Astro-Med, Inc., West Warwick, RI, USA). A bipolar stimulating electrode (FHC, Inc., Bowdoinham, ME, USA; offset: 100 μm, spacing: 115 μm, shank diameter: 75 μm, extension: 2 mm) was positioned approximately 100 μm from the pyramidal neuron under recording. IPSCs were evoked by extracellular stimulation of GABAergic interneurons at the neighborhood. Application of the $GABA_A$ receptor antagonist bicuculline (10 μM) completely blocked the sIPSC and eIPSC, indicating that these synaptic currents are mediated by $GABA_A$ receptors.

Mini Analysis Program (Synaptosoft, Decatur, GA, USA) was used to analyze spontaneous synaptic activity. Individual synaptic events with fast onset and exponential decay kinetics were captured with threshold detectors in Mini Analysis software. All quantitative measurements were taken 4–6 min after drug application. Neurons used for statistical analysis were required to have synaptic events with stable frequencies and amplitudes during both control and drug application. The detection parameters for analyzing synaptic events in each cell in the absence or presence of drugs were the same. The effects of 5-HT on sIPSC amplitude and frequency were measured by comparing 1 min of recorded events before and during early application of 5-HT (when the maximal effect emerged and before desensitization occurred). The duration data were calculated from the time point that the 5-HT effect started to show up to the time point that the 5-HT effect declined to baseline. Statistical comparisons of the frequency and amplitude of synaptic currents were made using the Kolmogorov-Smirnov test. Numerical values were expressed as mean±S.E.M. Clampfit Program (Axon Instrument) was used to analyze evoked synaptic activity. The amplitude of eIPSC was calculated by taking the mean of a 2–4 ms window around the peak and comparing with the mean of a 4–8 ms window immediately before the stimulation artifact.

Antidepressant treatment

Young male rats were administered intraperitoneally either the antidepressant fluoxetine (10 mg/kg) or saline for 21 days (once daily). The 21-day fluoxetine treatment regimen has been widely used as an effective way of chronic antidepressant treatment (Caccia et al., 1992; Contreras et al., 2001; Schenberg et al., 2001; Gronier and Rasmussen, 2003). This regimen can cause behavioral, biochemical and physiological changes that are associated with anti-depression efficacy of fluoxetine. Experimental groups were matched such as a fluoxetine-treated rat and saline-treated control rat were killed on the same day and tissue was processed in parallel. To test the effect of acute administration of fluoxetine, rats were i.p. injected with fluoxetine or saline 12 h before the electrophysiological experiments.

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 sIPSC, as measured with a double exponential fitting of the decay of averaged sIPSC, was also not significantly changed by long-term fluoxetine treatment (saline-treated: τ1: 10.2±0.8 ms, τ2: 54.2±10.6 ms, n=6; fluoxetine-treated: τ1: 10.7±0.3 ms, τ2: 52.1±13.1 ms, n=5). The responsiveness of PFC neurons to 5-HT was evaluated by exogenous application of this neuromodulator 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 sIPSC. 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 sIPSC was observable, but it desensitized much faster (Fig. 1D–F).

To determine the identity of 5-HT receptors in the serotonergic regulation of sIPSC, we treated PFC slices

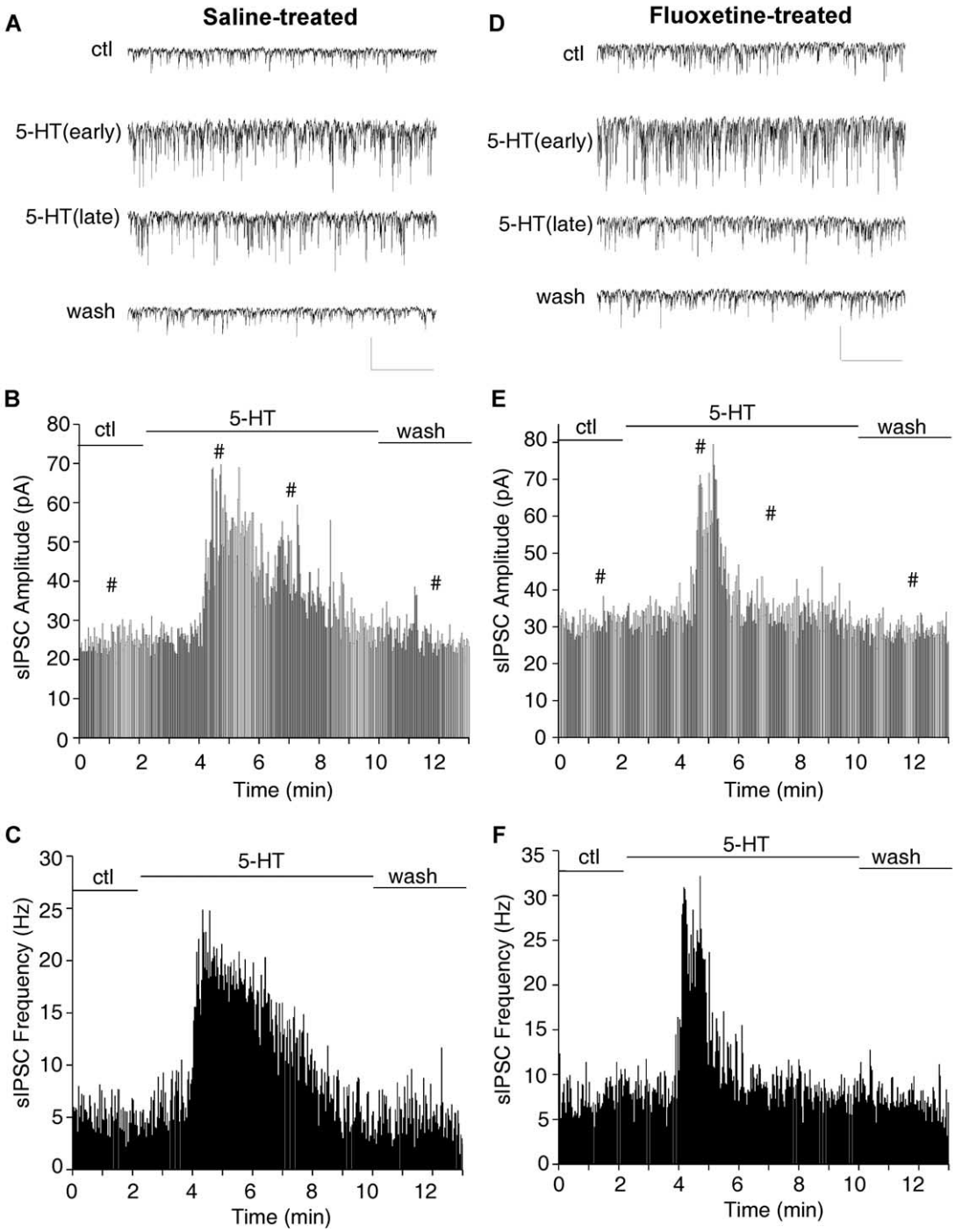

Fig. 1. The enhancing effect of 5-HT on sIPSC desensitized faster in PFC pyramidal neurons from rats exposed to chronic fluoxetine treatment. (A, D) Representative sIPSC traces recorded on a neuron from a saline-treated rat (A) and a neuron from a fluoxetine-treated rat (D) under control condition, during early (when the peak response emerged) and late (3 min after peak response) application of 5-HT and after washing off the agonist (at time points denoted by #). Scale bars=100 pA, 3 s. (B, C, E, F) Plot of sIPSC amplitude (B, E) and frequency (C, F) against time and agonist (5-HT, 20 μM) application in the neuron from a saline-treated rat (B, C) and the neuron from a fluoxetine-treated rat (E, F). Bin width: 2 s. Frequency: events/second (Hz).

with the selective 5-HT_2 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-$HT_{2A/2C}$ 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.

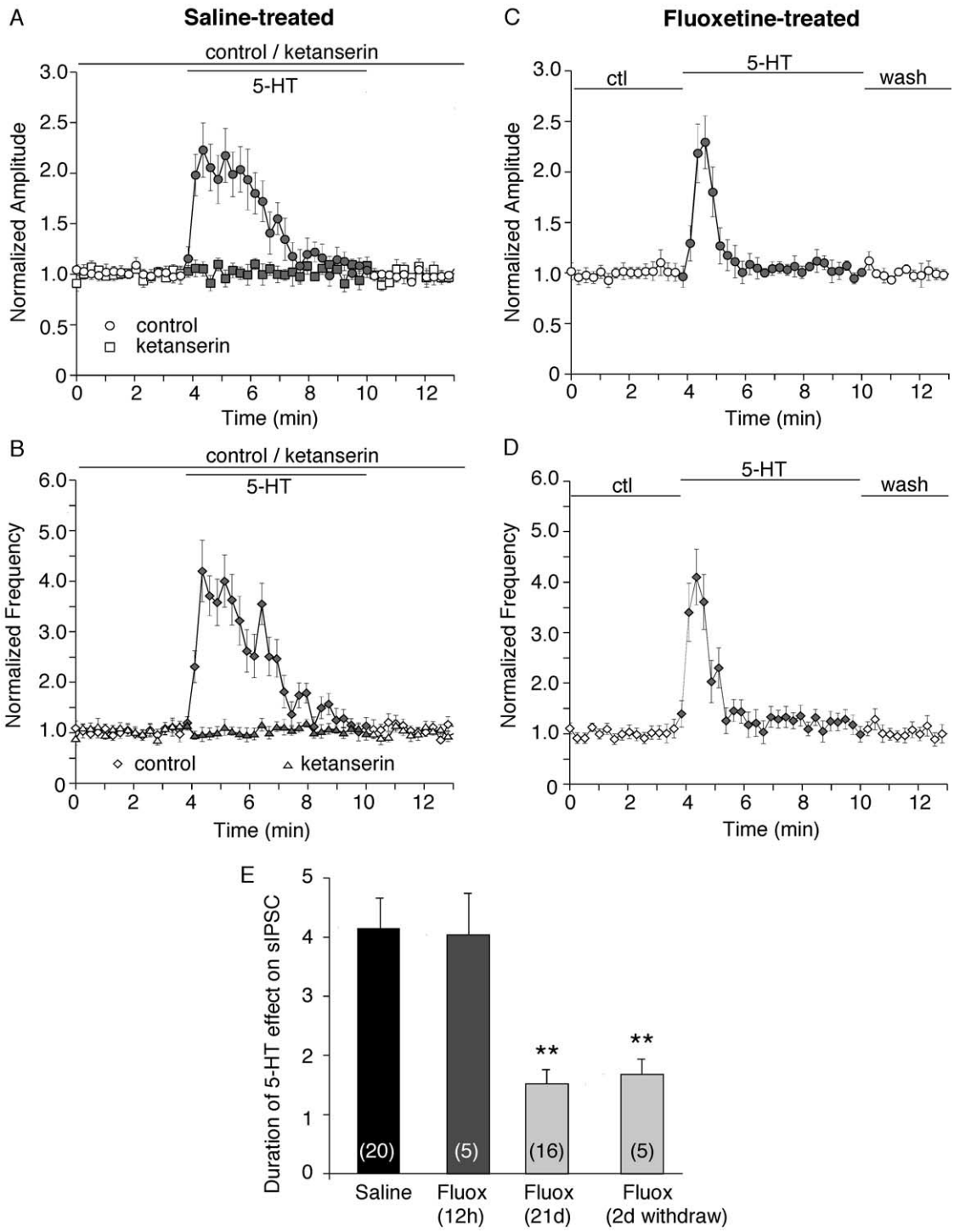

Fig. 2. Summary data showing the effect of 5-HT on sIPSC in neurons from saline- or fluoxetine-treated rats. (A, B) Plot of sIPSC amplitude (A) and frequency (B) against time and agonist (5-HT, 20 μM) application in a sample of neurons from saline-treated rats treated with or without selective 5-HT_2 antagonist ketanserin (10 μM). (C, D) Plot of sIPSC amplitude (C) and frequency (D) against time and agonist (5-HT, 20 μM) 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±S.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.

2E, $n=16$, 10 rats), which was significantly ($P<0.001$, ANOVA) shorter than that in control cells (Fig. 2E, 4.2±0.5 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±13.4%, $n=20$; fluoxetine-treated:

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=8) in neurons from saline-

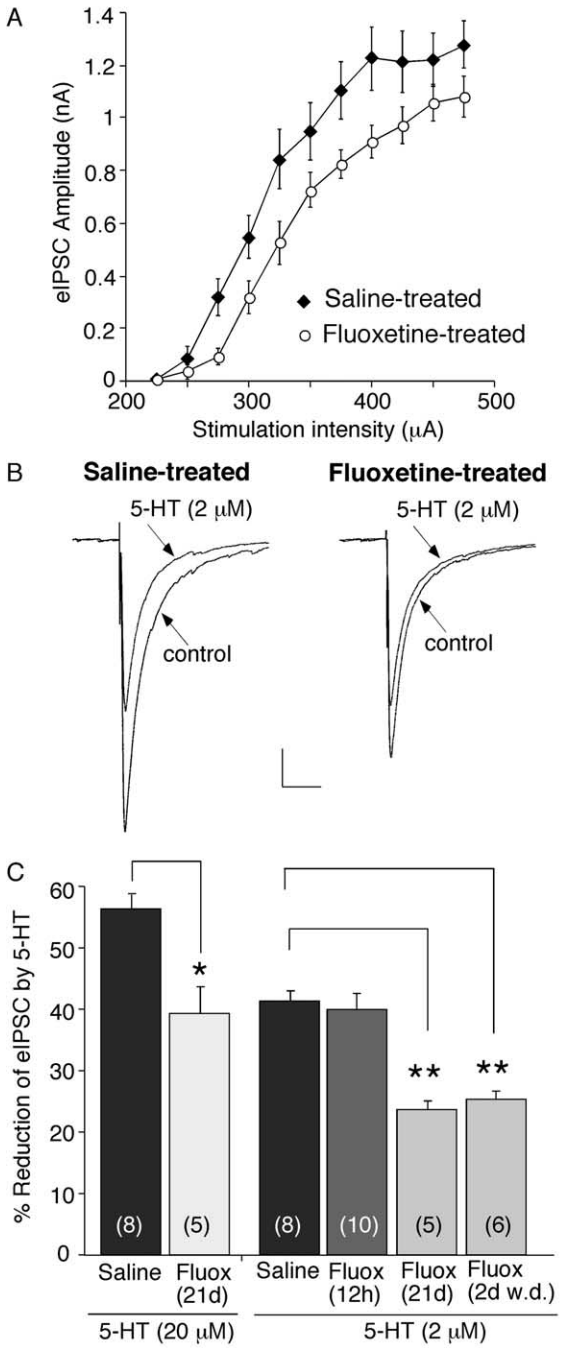

Fig. 3. Chronic fluoxetine treatment reduced eIPSC amplitudes and the 5-HT effect on eIPSC. (A) Plot of eIPSC amplitudes at different stimulus strengths in neurons from saline- or fluoxetine-treated rats. (B) Representative eIPSC traces recorded on a neuron from a saline-treated rat (left) and a neuron from a fluoxetine-treated rat (right) under control condition and during application of 5-HT (2 μM). Scale bars=100 pA, 50 ms. (C) Histograms (mean±S.E.M.) showing the percentage reduction of eIPSC amplitudes by 5-HT (20 μM) in neurons from saline- or fluoxetine (21-day)-treated rats, and by 5-HT (2 μM) in 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<0.01, ** P<0.001, ANOVA.

treated rats (n=4), and this 5-HT effect in neurons from fluoxetine-treated rats (n=3) was significantly smaller (39.5±4.1%, n=5, P<0.01, ANOVA). More substantial difference was found with a low concentration of 5-HT (2 μM), which reduced eIPSC amplitude by 41.2±1.9% (n=8) in neurons from saline-treated rats (n=4), but produced a significantly smaller reduction (23.8±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 μM) on eIPSC was also significantly smaller (Fig. 3C, 25.6±1.1%, n=6, P<0.001, ANOVA). The 5-HT (2 μM)-induced reduction of eIPSC amplitude was unaltered (Fig. 3C, 40.0±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 α-methyl-5-HT (α-Me-5-HT; 20 μM), a selective 5-$HT_{2A/2C}$ agonist, produced a large, desensitizing enhancement of sIPSC amplitude and frequency in approximately 60% (21 of 35) of PFC pyramidal neurons from saline-treated rats (n=12). A representative example is shown in Fig. 4A and B. However, α-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 α-Me-5-HT effect desensitized much faster after fluoxetine treatment (an example shown in Fig. 4C and D). As summarized in Fig. 4E–H, after long-term exposure to fluoxetine, the duration of α-Me-5-HT effects on sIPSC was 1.4±0.2 min (n=7, six rats), which was significantly (P<0.001, ANOVA) shorter than that in control cells (4.1±0.6 min, n=21, 12 rats). Chronic fluoxetine treatment did not significantly alter the enhancing effect of α-Me-5-HT on sIPSC amplitudes (saline-treated: 70.2±10.3%, n=21; fluoxetine-treated: 55.3±9.2%, n=7; P>0.01, ANOVA) or sIPSC frequencies (saline-treated: 175.6±23.4%, n=21; fluoxetine-treated: 132.7±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 α-Me-5-HT in PFC neurons from saline- or fluoxetine-treated rats. Similar to what was found with 5-HT (2 μM, Fig. 3B), α-Me-5-HT (20 μM) 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 (Fig. 5A, B). As summarized in Fig. 5C, in neurons from fluoxetine-treated rats (n=4), α-Me-5-HT (20 μM) reduced eIPSC amplitude by 19.2±1.1% (n=7), which was significantly (P<0.001, ANOVA) smaller than the effect of α-Me-5-HT in neurons (39.8±1.3%, n=12) from saline-treated rats (n=7). After acute treatment with fluoxetine, the effect of α-Me-5-HT on eIPSC was not attenuated (40.5±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 μM) and the selective 5-HT_2 antagonist ketanserin (20 μM) on eIPSC. We found that both methysergide and ketanserin had little effect on eIPSC in PFC neurons (6.5±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±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_{1A} 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_1 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, this study). 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 α-Me-5-HT usually declines within 4 min in the continued presence of the agonist. However, in PFC slices from fluoxetine-treated

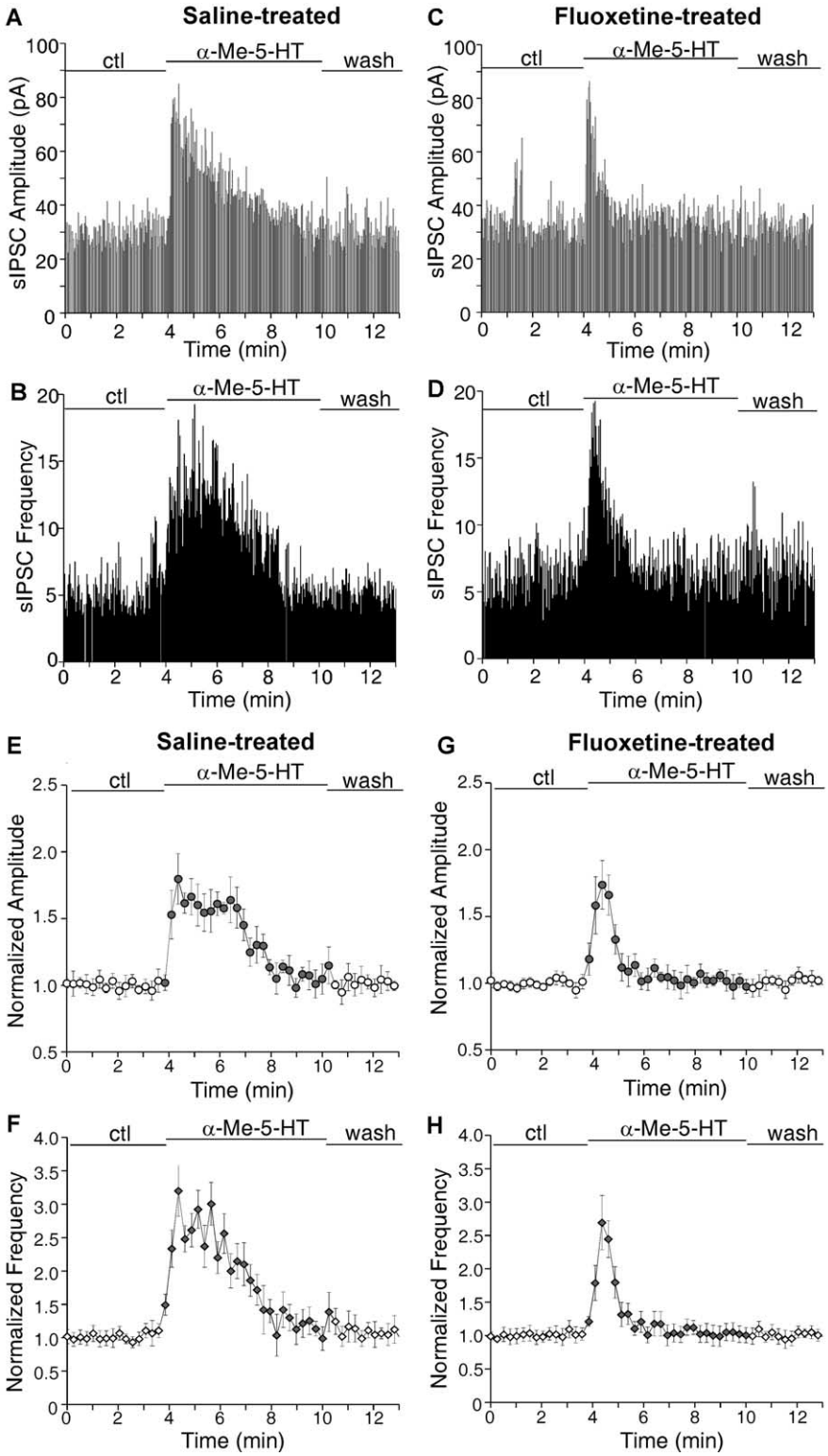

Fig. 4. The enhancing effect of a 5-HT_2 receptor agonist on sIPSC desensitized faster after chronic fluoxetine treatment. (A–D) Plot of sIPSC amplitude (A, C) and frequency (B, D) against time and agonist (α-Me-5-HT, 20 μM) application in a neuron from a saline-treated rat (A, B) and a neuron from a fluoxetine-treated rat (C, D). Bin width: 2 s. Frequency: events/second (Hz). (E–H) Plot of sIPSC 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.

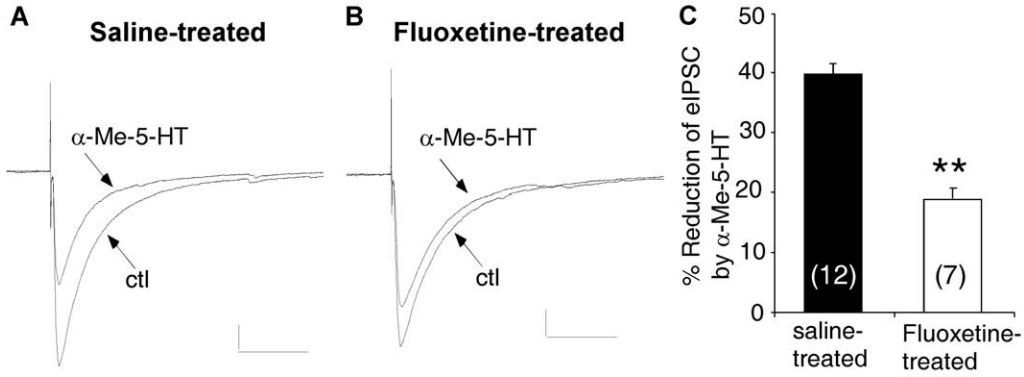

Fig. 5. Chronic fluoxetine treatment reduced the effect of 5-HT_2 receptors on eIPSC. (A) Representative eIPSC traces recorded on a neuron from a saline-treated rat (left) and a neuron from a fluoxetine-treated rat (right) under control condition and during application of α-Me-5-HT (20 μM). Scale bars=100 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. ** $P<0.001$, ANOVA.

animals, the serotonergic regulation of sIPSC lasts much shorter (1.5 min). It suggests that 5-HT_2 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-HT_2 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-HT_2 receptor agonist. This desensitization could result from the sustained activation of synaptic 5-HT_2 receptors due to increased levels of 5-HT after prolonged SSRI treatment. In addition to the potential change in 5-HT_2 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-HT_2-mediated regulation of inhibitory transmission, it is possible that fluoxetine treatment may have also affected 5-HT_{2A}-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-HT_2 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-HT_2 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-HT_2 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-HT_{2A} receptor or receptor-G protein interaction (Damjanoska et al., 2003). Our functional studies suggest that chronic fluoxetine treatment causes 5-HT_2 receptors to desensitize faster (as seen with the regulation of sIPSC by exogenous application of 5-HT or 5-HT_2 agonists) or partially desensitized (as seen with the regulation of eIPSC by exogenous application of 5-HT or 5-HT_2 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 dysregulation 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 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.

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