J Pharmacol Sci **92**, 100 – 107 (2003)

*Journal of Pharmacological Sciences*
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*Full Paper*

# Changes in Synaptic Properties in Cortical-Limbic Communications Induced by Repeated Treatments With Fluvoxamine in Rats

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*Received January 9, 2003; Accepted March 5, 2003*

**Abstract.** There is evidence indicating that dysregulation of coordinated interactions of the cortical-limbic circuitry is associated with anxiety and mood disorders. Our previous study has reported that an enhancement of long-term plasticity in the "limbic-cortical" pathway produced by repeated treatments with fluvoxamine may be involved in the clinical effects of a selective serotonin (5-HT) reuptake inhibitor (SSRI). Here we assessed the effects of single and repeated treatments with fluvoxamine on the synaptic transmission and plasticity in the "cortical-limbic" pathway in vivo. The evoked potentials in the basolateral amygdaloid complex (BLA) by stimulation of the medial prefrontal cortex (mPFC) in halothane-anesthetized rats were recorded. Single administration of fluvoxamine (10 and 30 mg/kg, i.p.) enhanced the efficacy of synaptic transmission at the mPFC-BLA synapses dose-dependently. The enhanced synaptic efficacy induced by 30 mg/kg fluvoxamine was suppressed after long-term administration of fluvoxamine (30 mg/kg per day × 21 days, orally). Repeated treatments with fluvoxamine affected short-term, but not long-term, synaptic plasticity in the mPFC-BLA pathway. These findings indicate that the 5-HTergic system contributes to modulation of synaptic changes in this pathway. Our results also suggest that different changes in synaptic properties in cortical-limbic communications induced by repeated treatments with fluvoxamine may be associated with therapeutic effects of SSRI.

***Keywords***: fluvoxamine, serotonin, medial prefrontal cortex-basolateral amygdaloid complex pathway, synaptic transmission, synaptic plasticity

## Introduction

Many studies have suggested that abnormality of serotonin (5-HT) function is involved in the pathophysiology of psychiatric disorders. Selective 5-HT reuptake inhibitors (SSRIs) are effective in the treatment of anxiety and mood disorders in clinical studies (1, 2) as well as their animal models in preclinical experiments (3, 4). Although SSRIs inhibit 5-HT transporters on the neural membrane, resulting in a rapid increase in concentration of 5-HT within the synaptic clefts in terminal regions and cell bodies, the clinical effects of SSRIs appear after a few weeks. Converging evidence indicates that chronic SSRI treatment has therapeutic effects on anxiety and mood disorders via adaptive neurochemical changes including pre- and post-synaptic regulatory desensitization, up- and down-regulation of various receptors, and receptor-mediated second messenger system and neurotrophic effects (5 – 7).

Recent neuroimaging studies have focused on the networks of various regions in the brain, suggesting that psychiatric patients use different patterns of functional connectivity in the cortical-limbic circuitry (8, 9). Reciprocal pathways linking limbic structures, such as the amygdala and hippocampus, with widely distributed brain stem, striatal, and cortical sites are now well defined; and they are clearly associated with specific cognitive, affective, and emotional behaviors in animals (10, 11). Dysregulation of the coordinated interactions of cortical-limbic communications is implicated in psychiatric illness (12). In particular, neuroimaging studies have found increased activation in the amygdala in anxiety and mood disorders (13 – 18). Furthermore,

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the prefrontal cortex (PFC) has abnormally decreased blood flow, metabolism, and volume in depressive patients (12, 19 – 21). From these findings, hyperfunction in the amygdala and hypofunction in the prefrontal cortical areas may reflect dysregulation in emotional and cognitive processing systems associated with psychiatric disorders. However, the precise interactions between the limbic structures and prefrontal cortical regions are unclear in affective and cognitive dysfunctions of anxiety and mood disorders. We previously showed that repeated treatments with fluvoxamine, an SSRI, produce an enhancement of long-term potentiation (LTP) in the "limbic-cortical" pathway, i.e., from the hippocampus to the medial PFC (mPFC) pathway, suggesting that an SSRI-induced enhancement of the limbic-cortical LTP could contribute to a therapeutic effect with cognitive recovery (22). In the present study, we focused on the "cortical-limbic" connection, namely the inputs of the amygdala received from the prefrontal cortical region. The basolateral complex of amygdala (BLA), which is composed of the lateral, basolateral, and basomedial nucleus, receives a projection from the mPFC. Anatomical and electrophysiological studies indicate that inputs from the infralimbic and prelimbic prefrontal areas terminate in the BLA (23 – 26).

Here we investigated the effects of single and repeated treatments with fluvoxamine on the synaptic transmission and plasticity in the rat mPFC-BLA pathway by using in vivo electrophysiological methods.

## Materials and Methods

Male Wistar rats (230 – 290 g) were purchased from Shizuoka Laboratory Animal Center (Hamamatsu) and were housed at an ambient temperature of 22°C under a 12 h – 12 h light-dark cycle (lights on, 19:00 h) with free access to food and water. All procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of the Hokkaido University School of Medicine.

Single and repeated treatments with fluvoxamine were given according to our previous study (22). Doses of 10 and 30 mg/kg of fluvoxamine dissolved in saline were given intraperitoneally in the single-injection group. In the repeated-treatment group, fluvoxamine (30 mg/kg), dissolved in deionized water when used, was administered orally once a day for 21 days. All administrations were performed between 09:00 and 11:00 h. On the 22nd day, rats subjected to repeat treatments were systemically injected at a dose of 30 mg/kg of fluvoxamine dissolved in saline.

Rats were anesthetized with 1% halothane in 21% $O_2$ and 79% $N_2$ mixture, which was administered through a tracheal cannula, and they were fixed in a stereotaxic frame with the bregma and lambda in the same horizontal plane. Halothane used as an anesthesia enabled us to compare this study with our previous study (22). Throughout the experiment, the blood pressure and the heart rate were monitored and body temperature was maintained at 37°C by using a thermoregulated heating pad. A stimulating electrode with a tip separation of 500 $\mu$m was used to stimulate the prelimbic and infralimbic regions of the PFC (coordinates: 2.7 mm anterior to the bregma, 0.7 mm lateral, 3.6 – 4.3 mm ventral from the cortical surface) according to the atlas of Paxinos and Watson (27). A recording electrode (100 $\mu$m diameter, stainless steel) was placed in the ipsilateral BLA (coordinates: 3.3 mm posterior to the bregma, 5.2 mm lateral, 6.0 – 7.3 mm ventral from the cortical surface). The field potentials evoked by mPFC stimulation were amplified and were monitored with an oscilloscope (VC-10; Nihon Koden, Tokyo); they could be divided into four primary components: two positive and two negative components, named P1, P2, N1, and N2, respectively (26) (Fig. 2A). We focused on the N1 and P2 components in the present study. These components were used as an index of changes in the mPFC-BLA afferent drive (26). The integrated responses were averaged by using a data analyzing system (MASSCOMP; Concurrent, Tokyo) and the amplitude of the N1 and P2 field potentials was measured.

After inserting the electrode, the input/output characterization of each individual rat was determined for the mPFC-BLA pathway by varying the stimulus intensity from 200 $\mu$A to 1200 $\mu$A stepwise. A test stimulus was then chosen to give an approximately 60% maximal response.

We used a paired-pulse ratio (PPR) protocol to investigate the effects of repeated treatments with fluvoxamine on short-term synaptic plasticity in some saline- and repeated-treatment rats after a test stimulus had been chosen. For the paired-pulse experiments, two stimuli were applied at interstimulus intervals (ISIs) ranging from 50 to 150 ms, in steps of 50 ms. The PPR was expressed as the value of S2/S1, where S2 was the amplitude of the second response and S1 was the amplitude of the first one. Here we assessed the negative component N1 for PPR.

The evoked potentials were expressed as percentages of the baseline level determined immediately before the administration of the drug to the rats. Throughout the experiment, test stimuli were delivered every 30 s and nine successive responses were averaged and collected every 5 min. To produce the LTP in the BLA by stimulation of the prelimbic and infralimbic regions of the

prefrontal areas, two series of 10 high-frequency stimulations (250 Hz, 250-$\mu$s duration, 50 trains) at 0.1 Hz were applied 40 min after intraperitoneal injection of the drug or saline.

At the last of the electrophysiological recording sessions, a positive current (70 $\mu$A, 10 s) was passed through a recording electrode from which the evoked potentials were recorded to deposit iron ions. Immediately after the experiments, the rats were perfused through the left ventricle with 300 ml of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.2) containing 5% potassium ferrocyanide to produce a Prussian blue reaction at the iron deposits. The brains were removed and were postfixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.2) for at least two days. Sections (50 $\mu$m) were cut through the recording and stimulating sites on a microslicer, and they were then mounted on gelatin-coated slides. After drying, the slides were stained with Neutral Red, and the recording and stimulating sites were evaluated under a microscope.

All results are given as the mean ± S.E.M. Data analysis used analysis of variance (ANOVA) followed by the two-tailed post-hoc Dunnett's multiple comparison procedure or Student's *t*-test as appropriate. The statistical significance was $P<0.05$.

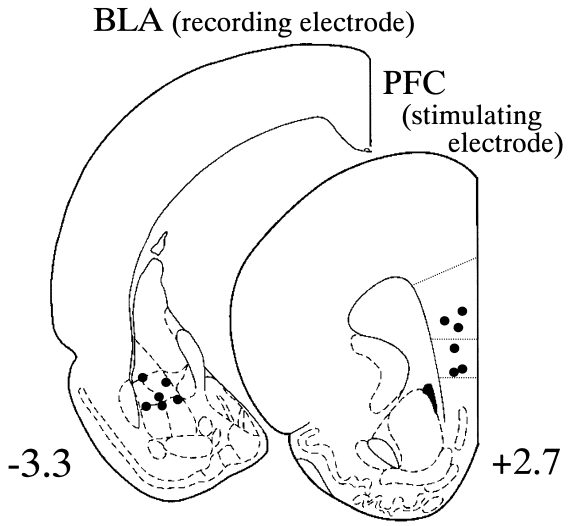


**Fig. 1.** Histology of the BLA and mPFC after electolophysiological experiments. Black dots show the representative locations of the recording electrode in the BLA (–3.3 mm from the bregma) and the stimulating electrode in the prelimbic and infralimbic areas of the PFC (+2.7 mm from the bregma).

## Results

*Effects of single and repeated treatments with fluvoxamine on synaptic transmission in the mPFC-BLA pathway*

As shown in Fig. 1, the recording electrodes were positioned in the lateral and basolateral regions of the BLA. The stimulating electrodes were located in the prelimbic and infralimbic regions of the mPFC.

We determined input/output curves for each group. The increase in evoked potentials depended on the stimulation intensity. The test stimulation intensities of the saline-injected group (466.7 ± 120.2 $\mu$A), the single-treatment group (523.3 ± 89.6 $\mu$A), and the repeated-treatment group (540.0 ± 42.3 $\mu$A) showed no significant difference.

We examined the effect of a single administration of fluvoxamine on the efficacy of synaptic transmission in the mPFC-BLA pathway. The synaptic efficacy was evaluated by monitoring the potentials evoked in the BLA by stimulating the prelimbic and infralimbic regions of the prefrontal cortical areas. After the systemic administration of fluvoxamine (10 and 30 mg/kg, n = 5 and 6, respectively), field potentials (both N1 and P2 peaks) evoked by test stimulation of the mPFC were enhanced in a dose-dependent manner (Fig. 2: A, C). As shown in Fig. 2B, the enhancement of the synaptic efficacy induced by a high dose of fluvoxamine (30 mg/kg) was statistically significant by the area under the curve (AUC) analysis for 80 min after the drug injection when compared with the saline-injected group (n = 7, N1, Dunnett's test, $P<0.001$). AUC analysis for P2 showed a similar significant enhancement of the synaptic efficacy in the mPFC-BLA pathway (Fig. 2D, Dunnett's test, $P<0.05$ at 10 mg/kg and $P<0.0005$ at 30 mg/kg).

We next evaluated the effect of repeated treatments with fluvoxamine on the synaptic efficacy in this pathway. Fluvoxamine (30 mg/kg per day) dissolved in distilled water were repeatedly administered orally for 21 days. On the following day, repeated-treatment rats were intraperitoneally given the same dose of drug. Repeated treatments with fluvoxamine (n = 7) significantly suppressed the enhancement of synaptic efficacy induced by a high dose of single administration (Fig. 3: A, C). The suppression of synaptic efficacy produced by long-term administration of fluvoxamine (30 mg/kg) was statistically significant by AUC analysis for 80 min after the drug injection when compared with the single-treatment group (Fig. 3: B, D, n = 6, Student's *t*-test, $P<0.01$ for N1 and $P<0.05$ for P2).

*Effects of single and repeated treatments with fluvoxamine on mPFC-BLA synaptic plasticity*

We tested the repeated fluvoxamine treatment effect on short- and long-term synaptic plasticity in the mPFC-BLA pathway. First, paired-pulse experiments were

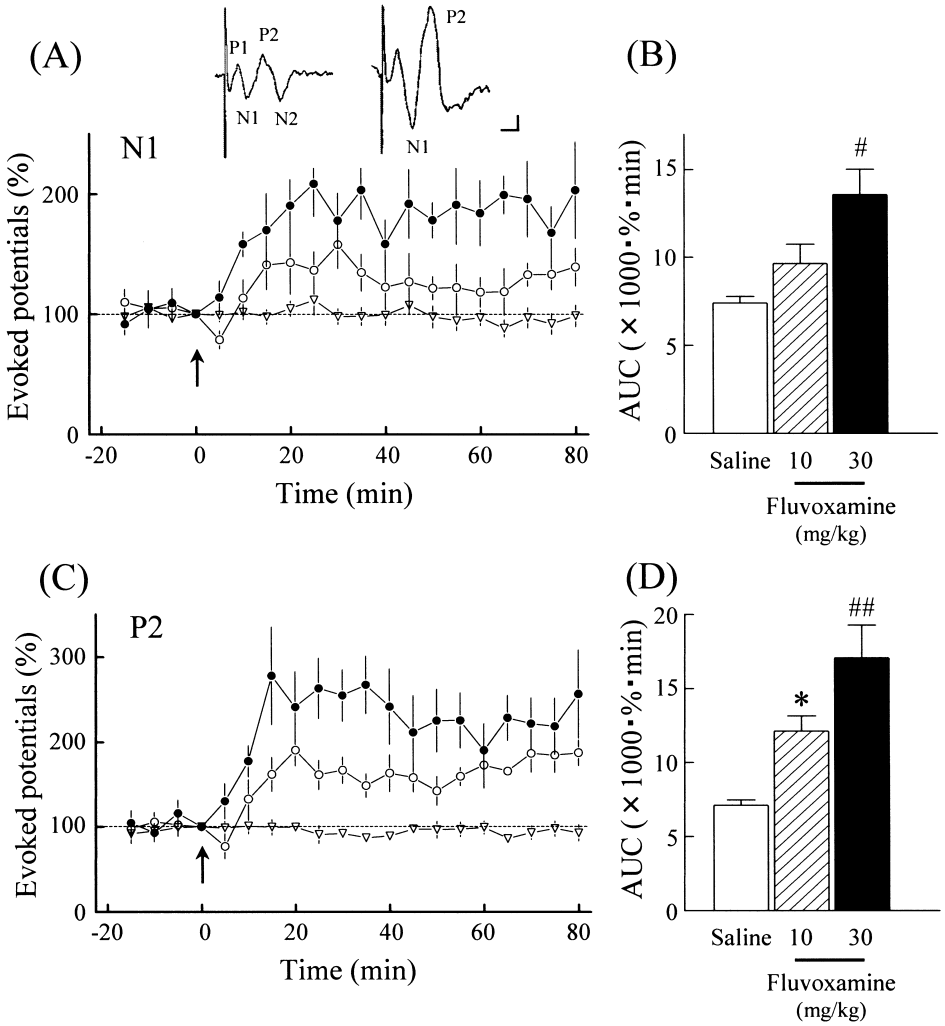


**Fig. 2.** Effects of a single administration of fluvoxamine on the potentials evoked in the BLA by stimulation of the prelimbic and infralimbic regions of the PFC in anesthetized rats. A, C: Time course responses of evoked potentials after single administration of fluvoxamine (10 and 30 mg/kg, n = 5 and 7, respectively) and saline (n = 6). Averaged waveforms at the top of the graph show the evoked potentials immediately before (left) and 80 min after (right) fluvoxamine (30 mg/kg) injection. The evoked potentials are expressed as the percentage of the baseline value before saline or fluvoxamine administration. Calibration: 50 $\mu$V, 20 ms. B, D: AUC of the facilitation of synaptic efficacy in the mPFC-BLA pathway induced by fluvoxamine (10 and 30 mg/kg) dose-dependently. The arrow indicates the injection of the drug or saline. Values are the mean ± S.E.M. Open triangle, saline-injected group; open circle, 10 mg/kg fluvoxamine group; closed circle, 30 mg/kg fluvoxamine group. *$P$<0.05, $^{\#}P$<0.001, and $^{\#\#}P$<0.0005, compared with the saline-injected group.

conducted to examine the effects of repeated treatments with fluvoxamine on short-term plasticity. ISIs ranged from 50 to 150 ms, in steps of 50 ms. The rats treated repeatedly with fluvoxamine showed a significant suppression of the PPRs at each ISI compared with the saline-injected group (Fig. 4). The PPRs were 1.53 ± 0.17 and 1.04 ± 0.09 at 50 ms ($P$<0.05), 2.25 ± 0.34, and 1.32 ± 0.11 at 100 ms ($P$<0.05) and 1.71 ± 0.09 and 1.27 ± 0.06 at 150 ms ($P$<0.005, Student's $t$-test, the saline-injected group and the repeated-treatment group, n = 4 and 6, respectively). Second, two series of high-frequency stimulations of the mPFC region 40 min after the administration of fluvoxamine (30 mg/kg) induced long-lasting increases in evoked potentials; i.e., LTP establishment in the saline-, single-, and repeated-treatment groups. Single and repeated treatments with fluvoxamine had no effect on the LTP, measured by AUC for 60 min after high-frequency stimulation of the mPFC region, in the BLA (data not shown).

## Discussion

The results of this study show that single and repeated treatments with an SSRI can change synaptic properties

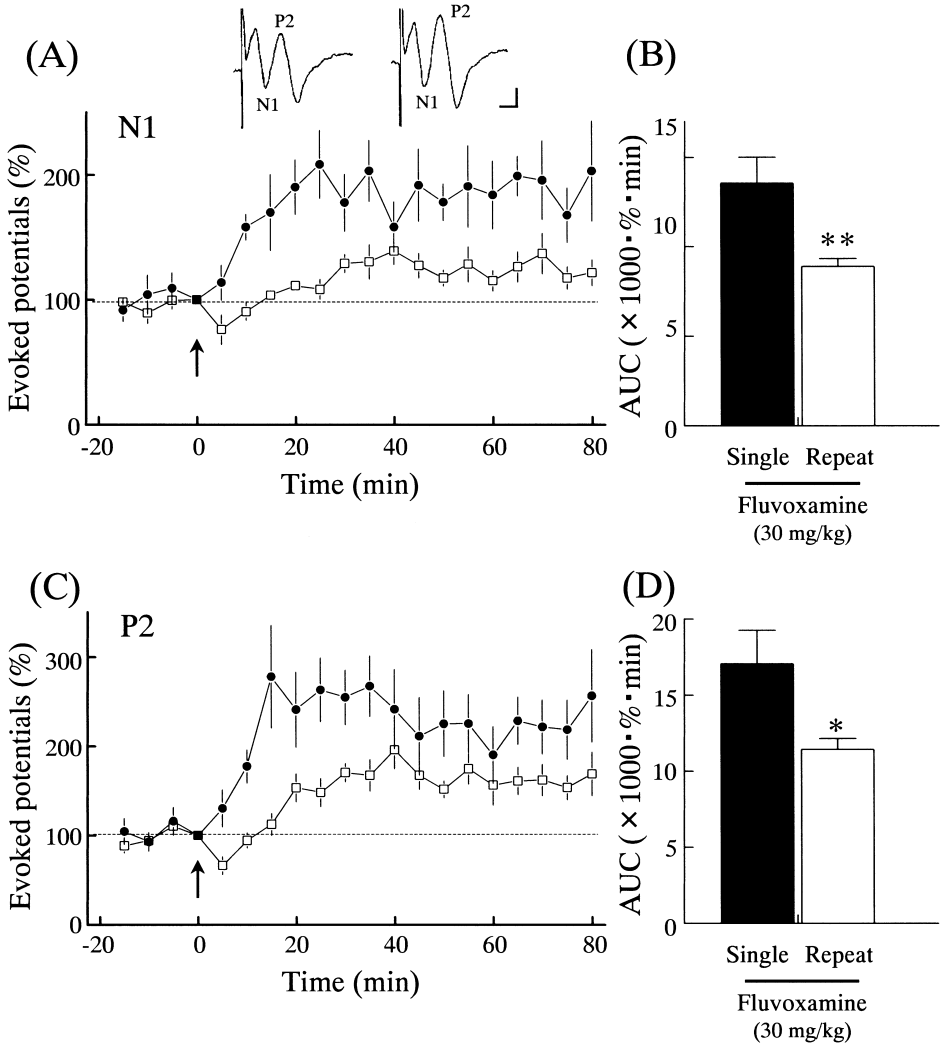


**Fig. 3.** Effects of single and repeated treatments with fluvoxamine (30 mg/kg) on the efficacy of synaptic transmission in the mPFC-BLA pathway. A, C: Time course responses of the evoked potentials after single treatment (n = 6) and repeated treatments with fluvoxamine (n = 7). Averaged waveforms at the top of the graph show the evoked potentials immediately before (left) and 80 min after (right) of the repeated-treatment rat. The evoked potentials are expressed as the percentage of the baseline value before fluvoxamine administration. Calibration: 50 $\mu$V, 20 ms. B, D: AUC of repeated treatments with fluvoxamine depressed the synaptic efficacy in the mPFC-BLA pathway. The arrow indicates the injection of the drug. Values are the mean ± S.E.M. Closed circle, single fluvoxamine group; open square, repeated fluvoxamine group. *$P$<0.05 and **$P$<0.01, compared with the single-treatment group.

in the cortical-limbic pathway. First, single treatment with fluvoxamine produced an enhancement of the efficacy of synaptic transmission at the mPFC-BLA synapses dose-dependently. Second, an enhancement of synaptic efficacy by a high dose of a single administration was suppressed after long-term treatment with fluvoxamine. Finally, repeated treatments with fluvoxamine modulated the short-term, but not long-term, synaptic plasticity in the mPFC-BLA pathway. These findings suggest that cortical-limbic synaptic changes are induced by long-term administration of fluvoxamine in the therapeutic effects of SSRI.

Single administration of fluvoxamine enhanced the efficacy of synaptic transmission in the mPFC-BLA pathway dose-dependently, suggesting that endogenous 5-HT can modulate the synaptic transmission via certain 5-HT receptors on the postsynaptic membrane. Administration of fluvoxamine, an SSRI, increases 5-HT levels within the synaptic clefts by inhibiting 5-HT transporters on the neuronal membrane. Our experiment showed that a single systemic administration of fluvoxamine increased the extracellular levels of 5-HT in the amygdala by using a microdialysis technique (28). Thus, the present findings suggest that increases in endogenous

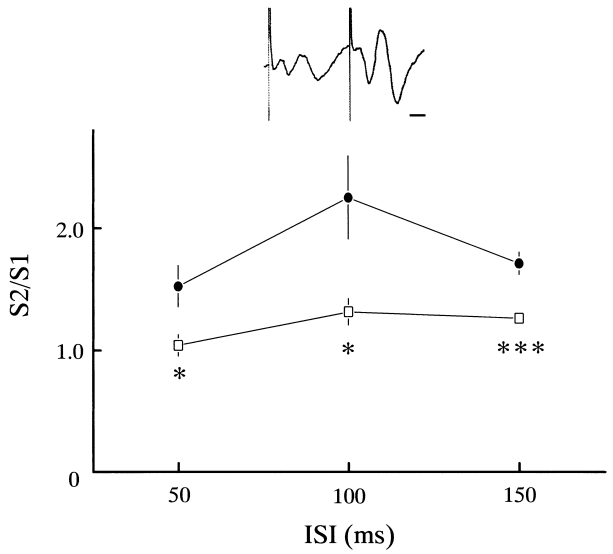


**Fig. 4.** Paired-pulse facilitation in saline- and repeated-treatment rats as a function of the ISI (n = 4 and 6, respectively). Suppression of paired-pulse facilitation in the BLA was induced by long-term administration of fluvoxamine. Averaged waveforms at the top of the graph show the evoked potentials at 100 ms ISI of the saline-injected rat. Calibration: 20 ms. The second response (S2) is expressed as a ratio the first response (S1). Values are the mean ± S.E.M. Closed circle, saline-injected group; open square, repeated fluvoxamine group. **P*<0.05 and ****P*<0.005 represent significant differences.

5-HT concentration produced by fluvoxamine may participate in the enhancement of synaptic efficacy in the mPFC-BLA pathway. Systemic administration of 30 mg/kg of fluvoxamine enhanced the synaptic efficacy in this "cortical-limbic" pathway to 212% and 303% of basal synaptic transmission (N1 and P2, respectively). These enhancements are greater than in the "limbic-cortical" pathway. Our previous experiments in the hippocampo-mPFC pathway showed that the same dose of 30 mg/kg of fluvoxamine enhanced the synaptic efficacy to 158% of basal transmission (22), which may reflect that increases in 5-HT levels in the frontal cortical area induced by SSRIs are smaller than in other brain regions (29). The data shown here provide evidence that the efficacy of synaptic transmission in the mPFC-BLA pathway can be modulated by the 5-HTergic system as well as the dopaminergic system (26). Although the critical role of the mPFC-BLA pathway in emotional and cognitive processes remains elusive, we obtained intriguing results that synaptic transmission in the mPFC-BLA pathway decreased during contextual fear conditioning, as an index of anxiety, in freely moving rats. Our experiments also showed that the anxiolytic agent, tandospirone (a 5-$HT_{1A}$-receptor partial agonist) produced an increase in synaptic transmission in this pathway (unpublished data). These findings suggest that changes in the synaptic efficacy in the mPFC-BLA pathway have an important role in anxiety-related behavior. It has, furthermore, suggested that the plastic change within the amygdala contributes to the formation of fear-related memory (30 – 32).

The enhancement of synaptic transmission in the mPFC-BLA pathway induced by a single treatment with fluvoxamine (30 mg/kg) was significantly suppressed by repeated treatments for 3 weeks. Indeed, clinical studies have shown that fully developed therapeutic efficacy of SSRIs requires 2 to 4 weeks. We also observed that repeated administrations of fluvoxamine suppressed the short-term plasticity (i.e., paired-pulse facilitation) in the mPFC-BLA pathway. This may reflect changes in transmitter release probability at the mPFC-BLA synapses, since paired-pulse facilitation has primarily been attributed to short-term glutamate release and has been used as a tool to implicate presynaptic participation (33). These changes in short-term plasticity also suggest that decreases in glutamate release at the presynapse in the mPFC-BLA pathway were caused by repeated treatments with fluvoxamine. Antidepressant drugs appear to reduce glutamatergic activity or glutamate receptor-related signal transduction (34). Thus, the suppression of paired-pulse facilitation by repeated treatments with fluvoxamine in the present study should decrease the excitability into the amygdala. This decreased excitability may contribute to the suppression of synaptic efficacy compared with a single treatment. Short-term synaptic plasticity, i.e., paired-pulse facilitation, has an important role in behavioral information processing in the hippocampal structures (35, 36). A more recent study also showed that short-term synaptic plasticity might link with the same as behavioral information processing in the "limbic-cortical" pathway; i.e., the hippocampo-mPFC pathway (37). Although further studies are needed to clarify the physiological functions of synaptic properties in the mPFC-BLA pathway, the findings in the present study suggest that repeated treatments with fluvoxamine suppressed an increased flow of excitatory activity into the amygdala, resulting in the therapeutic effects of SSRIs on anxiety and mood disorders. The results obtained here, furthermore, indicate that not only synaptic transmission, but also synaptic plasticity, could be influenced by repeated treatments with SSRI.

Several lines of evidence demonstrated that chronic antidepressive treatments could change the various synaptic properties in the rat hippocampal formation (38 – 41). We previously reported that long-term treatment with fluvoxamine enhanced the establishment of LTP in the hippocampo-mPFC pathway; i.e., the "limbic-cortical" projection (22). This pathway participates in asso-

ciative learning in animal behavioral tests (42, 43). Hypofunction of the prefrontal regions occurs in depressive patients with cognitive dysfunction. The enhancement of LTP seen at the hippocampal-mPFC synapses (i.e., the "limbic-cortical" pathway) may be associated with improvement in cognitive function, resulting in recovery from depressive states. However, repeated treatments with fluvoxamine produced suppression of synaptic transmission and short-term synaptic plasiticity in the mPFC-BLA pathway (i.e., the "cortical-limbic" projection) in the present study, suggesting that decreases in information flows from the cortical areas to the limbic regions may be implicated in improvement of anxiety and mood disorders. These findings suggest that the different changes in synaptic properties in cortical-limbic communications induced by long-term administration of fluvoxamine may be involved in the therapeutic effects of SSRI in psychiatric disorders. In other words, our data that enhanced "limbic-cortical" LTP and suppressed "cortical-limbic" synaptic properties induced by repeated treatments with fluvoxamine are consistent with evidence by clinical studies of the effects of chronic antidepressants on both frontal hypofunction and limbic hyperfunction (12, 21, 44 – 47).

## Acknowledgments

The authors would like to thank Meiji Seika Kaisha (Tokyo) for the gift of fluvoxamine. We are grateful to Ms. Asako Kaku for her technical support.

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