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Cortical 5-hydroxytryptamine_{2A}-receptor mediated excitatory synaptic currents in the rat following repeated daily fluoxetine administration

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Abstract

Down-regulation of 5-hydroxytryptamine_{2A} (5-HT_{2A}) receptors has been a consistent effect induced by most antidepressant drugs. The evidence for down-regulation of 5-HT_{2A} receptor binding following subchronic treatment with fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) is mixed. The question of 5-HT_{2A} receptor sensitivity during chronic administration of antidepressants is important since activation of 5-HT_{2A} receptors is associated with impulsivity. Continued activation of 5-HT_{2A} receptors may functionally oppose activation of other non-5-HT_{2A} receptors in the prefrontal cortex associated with the clinical efficacy of SSRI treatment. Therefore, the effects of repeated daily administration of fluoxetine (10 mg/kg, i.p. × 3 weeks) on pharmacologically characterized electrophysiological response mediated by 5-HT_{2A} receptor activation, 5-HT-induced excitatory postsynaptic currents (EPSCs), in rat prefrontal cortical slices was examined. The concentration-response curve for 5-HT-induced EPSCs was unchanged following subchronic fluoxetine treatment. This subchronic fluoxetine treatment failed to modify electrophysiological responses to AMPA in layer V pyramidal cells as well. These findings would be consistent with the hypothesis that blockade of 5-HT_{2A} receptors may enhance the effects of SSRIs or serotonin/norepinephrine reuptake inhibitors (SNRIs).

Keywords

antidepressant drugs; excitatory postsynaptic currents; pyramidal cells; prefrontal cortex; fluoxetine; 5-HT_{2A} receptors; selective serotonin reuptake inhibitors

One of the fundamentally puzzling questions regarding the antidepressant mechanism of action of selective serotonin reuptake inhibitors (SSRIs) and serotonin/norepinephrine reuptake inhibitors (SNRIs) is why the therapeutic effects usually require two to six weeks of treatment while the acute effects occur within hours. This has led to a hypothesis that the delayed effects require slowly developing neuroplasticity [1]. Another hypothesis for delayed clinical action of SSRIs and SNRIs relates to the delayed onset of a balance of inhibitory effects of serotonergic neurotransmission in limbic circuits following the slowly developing desensitization of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei [2,3].

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However, another fundamental but equally puzzling question is why there is a rapid relapse of depressive symptoms in patients subjected to acute tryptophan depletion shortly after achieving a clinical remission on SSRIs [4,5]. This relatively rapid relapse of depressive symptoms over a time frame of hours is consistent with the expectation that the acute effects of serotonin transporter (SERT) inhibition by SSRIs, at least in part, is responsible for the therapeutic effects of these medications during the early phase of treatment. This begs two fundamental questions. First, which 5-HT receptor subtypes are involved in mediating the antidepressant effects? Second, which 5-HT receptor subtypes, when activated, may functionally oppose these therapeutic effects?

Activation of 5-HT_{2A} receptors may be counterproductive to the optimal efficacy of SSRIs based on a number of observations. 5-HT_{2A} receptor activation in rodents appears increases motor impulsivity and interferes with arousal and attention [6-9]. At a translational level, activation of 5-HT_{2A} receptors in humans appears to result in a disturbance of attentional processes [10,11]. Conversely, activation of 5-HT_{1A} and 5-HT_{2C} receptors appears to functionally oppose the motor impulsivity induced by activation of 5-HT_{2A} receptors [8,9]. Similar opposing relationships between 5-HT_{2A} vs. 5-HT_{2C} and/or 5-HT_{1A} receptors also appears to be present for an antidepressant drug screen which involves impulsive behavior [12-14].

Pharmacological augmentation of known antidepressants by drugs which block 5-HT_{2A} receptors, pharmacogenetic studies, and the down-regulation of 5-HT_{2A} receptors by most antidepressant drug classes support an important role for this 5-HT receptor in the therapeutic action of most antidepressant drugs. Clinical studies have found that the addition of drugs which potently block 5-HT_{2A} receptors (mirtazapine, mianserin, olanzapine, quetiapine) to ongoing treatment with SSRIs improves antidepressant efficacy [15-17]. At a genetic level, different 5-HT_{2A} receptor polymorphisms have been associated with either poor paroxetine tolerability in elderly depressed patients or a good treatment response to SSRIs in the Star-D depression trial [2,3,18,19]. Finally, most antidepressant drugs either acutely block 5-HT_{2A} receptors or decrease the density of 5-HT_{2A} receptors following chronic drug administration [20-25]. This preclinical finding for the tricyclic antidepressant desipramine has been confirmed in human PET imaging studies measuring cortical 5-HT_{2A} receptor binding [26]. Thus, a wide range of studies at both the preclinical and clinical level support the hypothesis that modulation of 5-HT_{2A} receptors may be related to depression and therapeutic responses to antidepressant drugs.

However, evidence for 5-HT_{2A} receptor down-regulation with the most studied SSRI, fluoxetine, has been generally negative. Of the 13 studies examining the effects of daily systemic administration of fluoxetine (most with 10 mg/kg/day; 14-28 days), only two studies were consistent with fluoxetine-induced down-regulation of 5-HT_{2A} receptor binding sites [27,28], while three studies suggested up-regulation of 5-HT_{2A} receptor binding sites. One of the two studies demonstrating fluoxetine-induced down-regulation of 5-HT_{2A} receptors by fluoxetine, did confirm this effect with the TCA chlorimipramine, but failed to show a positive effect with two other TCAs, imipramine or amitriptyline [27]. Only one of the three studies finding evidence for up-regulation of 5-HT_{2A} receptor binding sites confirmed the widely replicated result for imipramine-induced down-regulation of 5-HT_{2A} receptors [29-32]. Six of the eight studies finding no change in 5-HT_{2A} receptor binding did find positive effects with a tricyclic antidepressant comparator drug [22,33-37]. One of the two negative studies which did not examine other positive comparator antidepressant drugs, however, did measure both [¹²⁵I]DOI binding to the agonist site and [³H]ketanserin binding to the antagonist site [38]. Thus, relatively little evidence exists that the SSRI fluoxetine regulates 5-HT_{2A} receptors in the cortex similarly to other antidepressant drugs. Nevertheless, the SSRI sertraline was found to desensitize the phosphatidyl inositol transduction pathway mediated by 5-HT_{2A} receptor

activation without changing 5-HT_{2A} receptor binding [25]. Additionally, one major gap in the literature of 5-HT_{2A} receptor regulation is a demonstration of the effects of fluoxetine on 5-HT_{2A} receptor-mediated responses from identified prefrontal cortical and neocortical cells given the likely importance of the prefrontal cortex in both the pathophysiology and treatment of depression.

Therefore, the purpose of the present experiments was to examine the regulation by subchronic fluoxetine treatment of 5-HT_{2A} responses on glutamatergic thalamocortical afferents [39-41] when recording intracellularly from layer V pyramidal cells in the mPFC. Previous experiments with 30 nM concentrations of the selective 5-HT_{2A} receptor antagonist M100907 (pA₂ 8.8 and 8.7 for this response in two different cells) completely blocked the 5-HT-induced increase in excitatory synaptic potentials recorded from layer V pyramidal cells [42]. Schild analyses with a set of antagonists with varying selectivity for 5-HT_{2A} vs. 5-HT_{2C} receptors provided strong support that activation of 5-HT_{2A} receptors predominantly, if not exclusively, mediates the 5-HT-induced excitatory synaptic currents [43]. Secondly, the rate of desensitization of 5-HT-induced excitatory synaptic currents was also studied in rats treated with subchronic fluoxetine.

Male Sprague-Dawley rats (n=26; Camm, Wayne, NJ) were 120-200 g at the beginning of chronic antidepressant treatment. All subjects were allowed a 7 day adaptation period following arrival from the supplier. They were housed in suspended stainless steel wire cages (18 × 36 × 20 cm) with two rats occupying each cage. The colony room was maintained at 20°C and relative humidity (60%). The room was illuminated 12 h/day (0700-1900 hr). All rats had free access to laboratory chow (Teklad 4% Rat Diet) and water. The principles of laboratory animal care (NIH publication No. 80-23, revised 1996) were followed. All procedures were approved by the Yale University Animal Care and Use Committee. All rats were treated with either vehicle or fluoxetine (10 mg/kg, i.p.) for 21 days except for a portion of the animals in the 5-HT_{2A} receptor desensitization experiment which were naïve to treatment.

Brain slices were prepared as described previously [44]. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) approximately 24 h following the last treatment and decapitated. Coronal slices (500 μm) were cut with an oscillating-blade tissue slicer at a level corresponding to approximately 2.5 mm anterior to bregma for recording from the mPFC. A slice containing the mPFC was then transferred to the stage of a fluid-gas interface chamber, which had a constant flow of humidified 95% O₂, 5% CO₂. The slices were perfused in a chamber heated to 34 °C with normal ACSF, which consisted of 126 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 2 mM MgSO₄, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, and 10 mM D-glucose.

Intracellular recording and single-electrode voltage clamping were conducted in prefrontal cortical layer V pyramidal cells by using an Axoclamp-2A (Axon Instruments, Inc., Foster City, CA) as previously described [42]. Stubby electrodes (~8 mm, shank to tip) with relatively low capacitance and resistance (30-60 MOhms) were filled with 1 M potassium acetate. The cells were voltage clamped at -70 mV. Phase lag was used to prevent oscillations; false clamping was avoided by utilizing optimal capacitance neutralization and by allowing settling to a horizontal baseline, as verified by monitoring input voltage continuously. Layer V pyramidal cells were recorded in a zone one-half to two-thirds the distance between the pial surface and the white matter in the mPFC (anterior cingulate and prelimbic area; Cg1, Cg2, Cg3).

The excitatory postsynaptic currents (EPSCs) recorded under these conditions do not appear to be contaminated by reversed inhibitory postsynaptic currents [42]. The voltage-clamp signals were low-pass filtered (1000 Hz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments, Inc., Foster City, CA).

For the intracellular recordings, EPSC frequencies were obtained from 10 consecutive episodes (1 s duration) during the baseline and drug treatment periods. EPSC frequencies were determined with Axograph peak detect software; signals <10 pA were excluded from the measurements. The determination of EC₅₀ values for the suppression of 5-HT-induced increases in EPSC frequency or of evoked EPSCs were calculated by nonlinear curve fitting (Delta Graph). A two-factor (5-HT concentration and drug treatment) repeated measures ANOVA was performed. Post-hoc followup of significant results utilized the Newman-Keuls test. Comparisons for treatment effects on AMPA-mediated responses utilized a paired t-test. For the desensitization experiment, a two-factor (drug treatment and time) repeated measures ANOVA was performed. Significance levels was set at $p < 0.05$.

All of the layer V pyramidal cells recorded from in the mPFC were regularly spiking pyramidal cells, similar to those previously described (see Fig. 1). In current clamp, 5-HT did not induce action potentials in any of these cells consistent with past results recording from the rat PFC using slices from rats at least 21 days old [45]. 5-HT did induce EPSCs when recording in voltage clamp mode (Fig. 1). Consistent with previous work [42,43], the 5-HT-induced EPSCs increased in a concentration-dependent manner from 3 to 300 μM with a near maximal effect at 100 μM , independent of treatment condition ($F(4,48)=18.70$, $p < 0.001$, Fig. 1, 2A). The EC₅₀'s for the vehicle- and fluoxetine-treated groups were 15.9 and 15.0 μM , respectively. Significant increases over baseline EPSC frequency were observed for both the vehicle-treated group and the fluoxetine-treated group at all 5-HT concentrations above 10 μM ($p < 0.05$ at 30 μM and $p < 0.001$ at 100 and 300 μM , Newman-Keuls test; Fig. 2A). The subchronic drug treatment condition (fluoxetine vs. vehicle) was not significant ($F(1,12)=0.02$, $p > 0.1$). Similarly, the interaction between 5-HT concentration and subchronic drug treatment was not significant ($F(4,48)=0.08$, $p > 0.1$).

Similar to a failure to modulate the effects of bath application of 5-HT (60 s exposures), the electrophysiological responses of layer V pyramidal cells to bath application of AMPA (5 μM , 60 s) was also not altered by subchronic fluoxetine treatment (not shown). The frequency of AMPA-induced EPSCs was unchanged in the cells recorded from fluoxetine-treated rats (37.3 ± 9.1 EPSCs/s, $n=6$) compared to the cells from vehicle-treated rats (46.6 ± 9.7 EPSCs/s, $n=7$; $t(11)=0.69$, $p < 0.1$). Furthermore, the AMPA-induced inward current observed in the fluoxetine-treated group (305 ± 72.9 nA, $n=6$) was not different from the vehicle-treated group (390 ± 90.6 nA, $n=7$; $t(11)=0.71$, $p=0.49$).

In preliminary experiments, the desensitization of 5-HT-induced EPSCs was followed for 10 min of continuous 100 μM 5-HT. While the drug treatment condition was not significant ($F(1,10)=1.57$, $p > 0.1$), both the time factor ($F(9,90)=82.11$, $p < 0.001$) and the drug treatment $\times$ time interaction factor ($F(9,90)=3.00$, $p < 0.01$) were significant (Fig. 2B). A trend for significantly lower 5-HT responses were observed for the fluoxetine-treated group at the 7 and 9 min. time points ($p < 0.1$, unpaired t-test). The frequency of 5-HT-induced EPSCs at the end of the 10 min application were 31.8 ± 7.1 and 8.6 ± 6.1 % of the respective control responses following the first min of 5-HT. Following washout of 5-HT, the frequency of 5-HT-induced EPSCs tended to remain significantly lower in the fluoxetine-treated group at the two time points examined (5 min, $p=0.057$; 30 min, $p=0.11$).

The present results are the first intracellular recordings from prefrontal cortical pyramidal cells that measure a 5-HT_{2A} receptor-mediated response following daily treatment with the SSRI fluoxetine. Subchronic treatment with fluoxetine, unlike imipramine and electroconvulsive shock [46], failed to attenuate 5-HT-induced EPSCs. Fluoxetine also failed to alter excitatory effects of bath AMPA application. While chronic antidepressant treatment can regulate the AMPA receptor subunit GluR1 in the hippocampus [47-49], the regulation of membrane-bound GluR1 by either the SSRI paroxetine or the tricyclic antidepressant desipramine does not appear

to generalize to the frontal cortex [49]. The interpretation of the negative effects for AMPA bath application is tempered by the fact that AMPA receptors would be expected to be desensitized under these conditions. However, the 5-HT-induced EPSCs, are mediated by a release of excitatory amino acids and subsequent activation of AMPA receptors [50]. Thus, the failure of a three week period of fluoxetine treatment to alter excitatory synaptic currents induced by 5-HT_{2A} receptor activation is consistent with both the general lack of effect of the SSRI fluoxetine on 5-HT_{2A} receptor binding in the rat prefrontal cortex and the lack of effect of SSRIs on regulating the membrane-bound levels of AMPA receptors in the PFC.

Further work will be required to determine if 5-HT-induced EPSCs do desensitize at a faster rate in cells from rats treated with fluoxetine or another SSRI as suggested by the preliminary findings here. If confirmed, this could be a potential explanation for a previous study where extracellularly recorded field potentials in the rat frontal cortex induced by the partial 5-HT_{2A/2C} agonist DOI (10 min exposure to cortical slices) were attenuated with repeated daily citalopram treatment for two weeks [51]. Desensitization of 5-HT_{2A} receptors could also explain the earlier observation that subchronic treatment with the SSRI sertraline down-regulated the downstream phosphatidyl inositol transduction pathway despite failing to alter 5-HT_{2A} receptor binding [25]. Furthermore, 5-HT_{2A} receptors from different cellular compartments in the mPFC could show differential regulation. For example, following repeated daily treatment of rats with electroconvulsive shock, 5-HT_{2A} responses in the mPFC (5-HT-induced EPSCs) are attenuated while the 5-HT-induced activation of GABAergic interneurons in the piriform cortex is enhanced [46].

Using similar conditions, chronic treatment with imipramine was shown to down-regulate the induction of excitatory synaptic currents in the mPFC induced by 5-HT_{2A} receptor activation [46]. This result is in good agreement with a plethora of receptor binding studies studying the effects of chronic treatment with imipramine and other tricyclic antidepressants since the initial studies by Peroutka and Snyder [22]. The dissociation between imipramine and fluoxetine on cortical 5-HT_{2A} receptor regulation is of interest as the selective 5-HT_{2A} receptor antagonist M100907 synergistically enhanced the antidepressant action of fluoxetine using a behavioral antidepressant drug screen which is also modulated by impulsivity [12]. The known relationships linking activation of 5-HT_{2A} receptors and certain types of impulsive behavior and perceptual disturbances are consistent with the hypothesis that addition of 5-HT_{2A} receptor antagonists to SSRIs and/or SNRIs might provide improved antidepressant efficacy, especially with respect to attention, arousal, and sleep [16].

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Figure 1.

Serotonin concentration-response relationships in layer V pyramidal neurons of rat PFC from two different rats treated with each vehicle (left) or fluoxetine (right; 10 mg/kg, ip $\times$ 21 days). 5-HT (3-300 μ M) was applied for one min periods and the induction of EPSCs was recorded. The tracings at the bottom show the current-voltage relationship for the respective regularly spiking neuron recorded under current clamp conditions (spike heights $>$ 80 mV and 0.2 nA current steps), typical for layer V pyramidal cells throughout the mPFC.

A : Fluoxetine (10 mg/kg, ip x 3 wks) vs. vehicle

Figure 2.

Repeated daily treatment with fluoxetine fails to alter the frequency of EPSCs in the mPFC induced by 5-HT_{2A} receptor activation. TOP PANEL(A): subchronic fluoxetine administration (10 mg/kg, i.p. for 21 days; n=6) does not modulate the frequency of 5-HT-induced EPSCs recorded from layer V pyramidal cells of the PFC compared to vehicle-treated rats (n=8). The basal frequency of EPSCs recorded from the layer V pyramidal cells was 3.5 ± 1.1 (fluoxetine group, mean $\pm$ SEM) and 2.0 ± 0.34 (vehicle group). BOTTOM PANEL (B): subchronic fluoxetine administration does appear to enhance the rate and amplitude of desensitization to a 10 min continuous 5-HT (100 μM) exposure to the slice when recording EPSCs from mPFC layer V pyramidal cells.