Published in final edited form as:

Mol Cell Neurosci. 2008 June ; 38(2): 290–299.

Modulation of Neuronal Excitability by Serotonin-NMDA Interactions in Prefrontal Cortex

Ping Zhong, Eunice Y. Yuen, and Zhen Yan*

Department of Physiology & Biophysics and New York State Center of Excellence in Bioinformatics & Life Sciences, State University of New York, Buffalo, NY

Abstract

Both serotonin and NMDA signaling in prefrontal cortex (PFC) are implicated in mental disorders, including depression and anxiety. To understand their potential contributions to PFC neuronal excitability, we examined the effect of co-activation of 5-HT and NMDA receptors on action potential firing elicited by depolarizing current injection in PFC pyramidal neurons. In the presence of NMDA, a low concentration of the 5-HT_{1A} agonist 8-OH-DPAT substantially reduced the number of spikes, and a low concentration of the 5-HT_{2A/C} agonist α -Me-5HT significantly enhanced it, while both agonists were ineffective when applied alone. The 8-OH-DPAT effect on firing was mediated by inhibition of protein kinase A (PKA), whereas the α -Me-5HT effect was mediated by activation of protein kinase C (PKC). Moreover, the extracellular signal-regulated kinase (ERK), a signaling molecule downstream of PKA and PKC, was involved in both 5-HT_{1A} and 5-HT_{2A/C} modulation of neuronal excitability. Biochemical evidence showed that 5-HT_{1A} decreased, whereas 5-HT_{2A/C} increased the activation of ERK in an NMDA-dependent manner. In animals exposed to acutely stress, the enhancing effect of 5-HT_{2A/C} on firing was lost, while the decreasing effect of 5-HT_{1A} on firing was intact. Concomitantly, the effect of 5-HT_{2A/C}, but not 5-HT_{1A}, on ERK activation was abolished in stressed animals. Taken together, our results demonstrate that distinct 5-HT receptor subtypes, by interacting with NMDA receptors, differentially regulate PFC neuronal firing, and the complex effects of 5-HT receptors on excitability are selectively altered under stressful conditions, which are often associated with mental disorders.

Keywords

5-HT_{1A} receptor; 5-HT₂ receptor; NMDA receptor; action potential firing; ERK; stress; prefrontal cortex

The prefrontal cortex (PFC) receives a dense serotonergic innervation from the raphe nuclei (Smiley and Goldman-Rakic, 1996). The serotonergic system plays an important role in regulating PFC functions, including emotional control, cognitive behaviors and working memory (Williams et al., 1996; Buhot 1997; Williams et al., 2002). Aberrant serotonin system

* Correspondence should be addressed to Zhen Yan, Ph.D., Department of Physiology and Biophysics, State University of New York at Buffalo, 124 Sherman Hall, Buffalo, NY, 14214, USA. Email: zhenyan@buffalo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

This PDF receipt will only be used as the basis for generating PubMed Central (PMC) documents. PMC documents will be made available for review after conversion (approx. 2-3 weeks time). Any corrections that need to be made will be done at that time. No materials will be released to PMC without the approval of an author. Only the PMC documents will appear on PubMed Central -- this PDF Receipt will not appear on PubMed Central.

has been implicated in the pathogenesis of mental disorders associated with PFC dysfunction, such as depression, anxiety and schizophrenia (Doris et al., 1999; Gross et al., 2002; Lemonde et al., 2003). The serotonin system is the major target of antidepressants, anxiolytics and atypical antipsychotics (Deakin, 1988; Griebel, 1995; Meltzer et al., 2003).

The pleiotropic functions of serotonin are afforded by the concerted actions of multiple serotonin receptor subtypes (Martin et al., 1998; Andrade, 1998). PFC pyramidal neurons contain several serotonin receptor subtypes, with a particularly high density of 5-HT_{1A} and 5-HT_{2A} receptors (Jakab and Goldman-Rakic, 1998; Feng et al., 2001). Electrophysiological studies have found that these receptors have different effects on membrane excitability depending on cell types and development stages (Andrade, 1998; Beique et al., 2004). 5-HT_{1A} receptor activation can induce a membrane hyperpolarization in hippocampal neurons (Andrade et al., 1986), probably by opening inward rectifying potassium channels (Colino and Halliwell, 1987), inhibiting voltage-dependent calcium channels (Penington and Kelly, 1990), or activating the TWIK-1 type of two-pore domain potassium channels (Deng et al., 2007). In contrast, 5-HT₂ receptor activation can induce a membrane depolarization in cortical neurons (Araneda and Andrade, 1991; Aghajanian and Marek 1997), probably by inhibiting an inwardly rectifying potassium conductance (North and Uchimura, 1989) or activating a cation nonselective current (Haj-Dahmane and Andrade, 1996).

Since the activity of PFC pyramidal neurons encodes information storage in working memory (Goldman-Rakic, 1995), we would like to understand the role of 5-HT_{1A} and 5-HT_{2A} receptors on the excitability of these cells indicated by action potential (AP) firing. Studies on neuronal excitability usually use somatic injection of current pulses to directly depolarize membrane potential and elicit spikes. This method tends to dismiss the role of synaptic transmission, which is mediated by ligand-gated channels (e.g. glutamate and GABA_A receptor channels), in controlling the membrane potential and action potential firing. Therefore, inducing the activation of ligand-gated channels by exposing cells to corresponding ligands is important for unmasking the role of these channels in influencing cell excitability. Our previous studies have shown that 5-HT, by activating different receptors, differentially regulate these ligand-gated channels in PFC (Feng et al., 2001; Cai et al., 2002a, b; Yuen et al., 2005), suggesting that 5-HT receptors may also affect neuronal excitability by targeting ligand-gated channels. To test this, we examined the effect of co-activation of 5-HT and glutamate receptors on action potential firing. We found that activation of NMDA receptor channels facilitates the regulation of spikes by 5-HT_{1A} and 5-HT_{2A} receptors in PFC pyramidal neurons. It provides a framework for understanding the role of 5-HT-NMDA interactions in regulating PFC functions that are controlled by the activity of pyramidal projection neurons.

Methods

Patch-clamp recording in PFC slices

PFC slices were prepared from Sprague-Dawley rats (3-4 weeks old) as described previously (Zhong et al. 2003). All experiments were conducted with the approval of State University of New York at Buffalo Animal Care Committee. In brief, animals were anesthetized by inhaling 2-bromo-2-chloro-1,1,1-trifluoroethane (1 ml/100 g, Sigma) and decapitated. Brains were then quickly removed, iced and sliced with a Leica (Nussloch, Germany) VP1000S Vibrotome. Slices were incubated for 1-5 hr 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, 26 NaHCO₃, 1.25 NaH₂PO₄, 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 measure neuronal excitability, the whole-cell current-clamp technique (Zhong et al., 2003) was used to record spikes evoked by a 500ms depolarizing current pulse (in the range

of 140-200 pA). PFC slice (300 μ m) was positioned in a heated (30 °C) perfusion chamber attached to the fixed-stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial cerebrospinal fluid (ACSF). The internal solution contains (in mM): 60 K₂SO₄, 60 N-methyl-glucamine, 40 HEPES, 4 MgCl₂, 0.5 BAPTA, 12 phosphocreatine, 2 Na₂ATP, 0.2 Na₃GTP, and 0.1 leupeptin, pH 7.2-7.3, 265-270 mosM. Pyramidal neurons located in layer V of the rat PFC, which were identified by their large soma sizes and apical dendrites, were visualized with a 40 $\times$ water-immersion lens and illuminated with near infrared (IR) light, and the image was detected with an IR-sensitive CCD camera. Recordings were made with Multiclamp 700A amplifier (Axon Instruments) that was controlled and monitored with a computer running pClamp (v. 8) with a DigiData 1322A interface (Axon Instruments). Tight seals (2-5 G Ω) from visualized neurons were obtained by inducing negative pressure. The membrane was disrupted with additional suction, and the whole-cell configuration was obtained. The access resistances ranged from 10-15 M Ω . Neuronal input resistance was determined by dividing the difference in membrane voltage in responses to a hyperpolarizing current pulse that caused a voltage deflection $\sim$ 10 mV (Yang et al., 1996). All data are shown as mean $\pm$ SEM. ANOVA tests were performed to compare the differential degrees of modulation between groups subjected to different treatments.

Serotonin receptor ligands including 8-OH-DPAT, α -Me-5HT, NAN-190, Ketanserin (Sigma, St. Louis, MO), as well as second messenger reagents PKI₆₋₂₂, U73122, Calphostin, and U0126 (Calbiochem, La Jolla, CA), were made up as concentrated stocks in water or DMSO and stored at -20°C. Stocks were thawed and diluted immediately before use. The concentration of DMSO in recording solutions was less than 1:1000, which had no effect by itself on membrane properties or action potential firing.

Stress paradigm

The forced swim stress protocol was similar as what we described previously (Tan et al 2004). In brief, rats were positioned in a cylindrical glass tank (24.5 cm high $\times$ 18.5 cm diameter) filled with water to a depth of 20 cm. This depth allowed the rats to reach the bottom with their tails only. Rats were forced to swim in warm water (24 -26°C) for 30 min, and then were anesthetized and sacrificed.

Western blot analysis

PFC slices from normal or stressed animals were prepared as described previously (Gu et al., 2003). After incubation with various 5-HT receptor agonists, slices were homogenized in boiling 1% SDS, followed by centrifugation (13,000 $\times$ g, 10 min) to remove insoluble materials. Equal amounts of supernatant fractions were resolved in SDS-PAGE and probed with primary antibodies. Total and Thr202/Tyr 204 phosphorylated-ERK antibodies were used (Cell Signaling, 1:1000). After incubation with horseradish peroxidase-conjugated anti-rabbit antibodies (Amersham, 1:2000), positive bands were detected with an enhanced chemiluminescence detection system (Amersham Biosciences). Quantification was obtained from densitometric measurements of immunoreactive bands on films.

Results

5-HT_{1A} and 5-HT_{2A/C} receptors, by interacting with NMDA receptors, exert opposing actions on PFC neuronal excitability

To test the effect of 5-HT_{1A} and 5-HT_{2A} receptors, which are enriched in PFC pyramidal neurons (Feng et al., 2001), on neuronal excitability, we examined their effects on action potentials (AP) elicited by injection of current pulses. Application of a saturating concentration of the 5-HT_{1A} agonist 8-OH-DPAT (20 μ M) produced a significant reduction of the firing rate ($32.4 \pm 4.7\%$, $n = 7$), while the saturating concentration of 5-HT_{2A/C} agonist α -Me-5HT (20

μM) significantly increased the firing rate ($35.8 \pm 5.2\%$, $n = 7$), consistent with previous results (Araneda and Andrade, 1991). However, at low concentrations, both 8-OH-DPAT ($1 \mu\text{M}$) and $\alpha\text{-Me-5HT}$ ($0.8 \mu\text{M}$) failed to induce significant changes in AP firing (8-OH-DPAT: 4-5% reduction; $\alpha\text{-Me-5HT}$: 2-7% increase, Figure 1E).

To unmask the role of glutamatergic transmission, which is mainly mediated by NMDA and AMPA receptor channels, in influencing cell excitability, we exposed cells to low concentrations of NMDA or AMPA. As shown in Figure 1A-D, bath application of NMDA (4 or $10 \mu\text{M}$) substantially enhanced the rate of firing elicited by small depolarizing current pulses ($4 \mu\text{M}$ NMDA: $72.7 \pm 16.4\%$ increase, compared to ACSF control, $n = 6$). Without current injection, these low concentrations of NMDA alone did not evoke spikes. In the presence of NMDA (4 or $10 \mu\text{M}$), the firing rate was significantly decreased by a low concentration of 8-OH-DPAT ($1 \mu\text{M}$, Figure 1A, 1B), and was significantly enhanced by a low concentration of $\alpha\text{-Me-5HT}$ ($0.8 \mu\text{M}$, Figure 1C, 1D), while both 5-HT receptor agonists were ineffective when applied alone at these concentrations. As summarized in Figure 1E, when different currents were injected to generate 3-5, 6-8 or 9-11 of spikes in the NMDA alone condition (baseline), 8-OH-DPAT reduced the number of spikes by 20-40% or 25-45% (with 4 or $10 \mu\text{M}$ NMDA present, $p < 0.01$, ANOVA), while $\alpha\text{-Me-5HT}$ increased the number of spikes by 24-40% or 26-42% (with 4 or $10 \mu\text{M}$ NMDA present, $p < 0.01$, ANOVA). As shown in Table 1, compared to NMDA alone (baseline), the latency to the first spike and rheobase (determined as the smallest current injection that elicited a single spike) were significantly ($p < 0.05$, ANOVA) increased by 8-OH-DPAT and decreased by $\alpha\text{-Me-5HT}$. Other parameters, including membrane potential, input resistance, afterhyperpolarization (AHP), action potential threshold, amplitude and halfwidth, were not significantly altered. Moreover, low concentrations of 8-OH-DPAT or $\alpha\text{-Me-5HT}$ (in the presence of NMDA) did not change the passive membrane properties, as indicated by the I-V relationship shown in Figure 1F and 1G.

In contrast to the strong effects of 8-OH-DPAT and $\alpha\text{-Me-5HT}$ on AP firing in the presence of NMDA, both agonists at their low concentrations failed to alter the neuronal excitability in the presence of a low concentration ($0.2 \mu\text{M}$) of AMPA (Supplemental Figure 1, 8-OH-DPAT: $4.3 \pm 2.5\%$ reduction, $n = 4$; $\alpha\text{-Me-5HT}$: $4.8 \pm 1.9\%$ increase, $n = 4$). Compared to control (ACSF), AMPA ($0.2 \mu\text{M}$) alone significantly increased the firing rate ($53.6 \pm 9.4\%$, $n = 5$). These results indicate that NMDA, but not AMPA, specifically facilitated the regulation of AP firing by low concentrations of 5-HT receptor agonists in PFC pyramidal neurons.

We next examined the receptors that mediate the effects of 8-OH-DPAT or $\alpha\text{-Me-5HT}$ on AP firing in the presence of NMDA. The selective 5-HT_{1A} antagonist NAN-190 ($1 \mu\text{M}$) abolished the 8-OH-DPAT-induced decrease in firing rate ($-4.1 \pm 1.3\%$, $n = 6$, Figure 2A, 2C), indicating that 5-HT_{1A} receptors mediate the effect of 8-OH-DPAT. Likewise, the selective 5-HT_{2A/C} receptor antagonist ketanserin ($5 \mu\text{M}$) blocked the $\alpha\text{-Me-5HT}$ -induced increase in firing rate ($6.3 \pm 2.4\%$, $n = 6$, Figure 2D, 2F), indicating that 5-HT_{2A/C} receptors mediate the effect of $\alpha\text{-Me-5HT}$. In the presence of APV (25 or $50 \mu\text{M}$), a selective NMDAR antagonist, the inhibitory action of 8-OH-DPAT or the enhancing action of $\alpha\text{-Me-5HT}$ on excitability was blocked (Figure 2B, 2E, 8-OH-DPAT: $-4.6 \pm 3.2\%$, $n = 5$, Figure 2C; $\alpha\text{-Me-5HT}$: $6.1 \pm 3.7\%$, $n = 5$, Figure 2F), confirming the NMDAR-dependence of the effects of both 5-HT receptor agonists on AP firing.

To demonstrate which action, i.e. a 5-HT₁-mediated decrease or a 5-HT₂-mediated increase of the firing rate, is predominant in response to 5-HT, we examined the effect of two doses of 5-HT ($1 \mu\text{M}$, $10 \mu\text{M}$) in the presence of NMDA ($4 \mu\text{M}$). As shown in Figure 3A-C, 5-HT ($1 \mu\text{M}$) produced a $\sim 20\%$ reduction and 5-HT ($10 \mu\text{M}$) produced a $\sim 40\%$ reduction of the firing rate. Since 5-HT₁ has higher affinity (nanomolar) than 5-HT₂ (micromolar) to 5-HT, the action

of 5-HT₁ is usually predominant (especially with low doses of 5-HT), which is consistent with our results.

The 5-HT_{1A} and 5-HT_{2A/C} regulation of PFC neuronal excitability involve divergent and convergent signaling molecules

We then examined the cellular mechanism involved in the regulation of excitability by 5-HT-NMDA interactions. A classic pathway for 5-HT_{1A} receptors is to couple to G_i/G_o proteins to inhibit adenylate cyclase and cAMP formation (Raymond et al., 1999). This led us to speculate that the 5-HT_{1A} reduction of AP firing is through the inhibition of PKA. If that is the case, then the effect of 5-HT_{1A} should be occluded by inhibiting PKA. As shown in Figure 4A and 4B, the reducing effect of 8-OH-DPAT (with NMDA present) on spikes was prevented in cells dialyzed with the PKA inhibitor PKI₆₋₂₂ (20 μM, $-6.9 \pm 3.0\%$, $n = 5$, Figure 4E), while it was not affected by the PKC inhibitor calphostin (1 μM, Figure 4C, $30.6 \pm 3.5\%$, $n = 4$, Figure 4E), suggesting the specific involvement of PKA. Since inhibition of PKA could result in the inhibition of ERK via the Rap1/B-Raf/MEK cascade (Vossler et al., 1997; Roberson et al., 1999), we also examined the involvement of ERK in the 5-HT_{1A} regulation of AP firing. As shown in Figure 4D, injecting cells with the ERK kinase inhibitor U0126 (20 μM) prevented 8-OH-DPAT from reducing the firing rate ($-5.0 \pm 2.3\%$, $n = 12$, Figure 4E). Taken together, these results suggest that the suppression of neuronal excitability by 5-HT_{1A}-NMDA interaction involves the inhibition of PKA and ERK in PFC pyramidal neurons.

In studies using cell lines, it has been found that activation of 5-HT₂ receptors stimulates phospholipase C β isoform (PLCβ), leading to the release of inositol-1,4,5-triphosphate (IP₃) and diacylglycerol (DAG) through the hydrolysis of membrane phosphoinositol lipids. Thus, we tested whether the signaling molecules in the PLCβ-mediated pathway was involved in the regulation of PFC neuronal excitability by 5-HT_{2A/C}-NMDA interactions. As shown in Figure 5A-D, α-Me-5HT (with NMDA present) lost its capability to increase the firing rate in neurons loaded with the PLC inhibitor U73122 (5 μM, $8.8 \pm 2.0\%$, $n = 6$, Figure 5F) or the PKC inhibitor calphostin (1 μM, $7.9 \pm 2.4\%$, $n = 5$, Figure 5F), but not the PKA inhibitor PKI₆₋₂₂ (20 μM, $29.15 \pm 3.3\%$, $n = 4$, Figure 5F), suggesting the specific involvement of PKC. Since activation of PKC could result in the activation of ERK via the Ras/Raf-1/MEK cascade (Roberson et al., 1999), we also examined the involvement of ERK in the 5-HT_{2A/C} regulation of AP firing. As shown in Figure 5E, dialysis with the ERK kinase inhibitor U0126 (20 μM) prevented α-Me-5HT from increasing the firing rate ($8.4 \pm 3.2\%$, $n = 8$, Figure 5F). These results suggest that the enhancement of neuronal excitability by 5-HT_{2A/C}-NMDA interaction involves the activation of PKC and ERK in PFC pyramidal neurons.

The 5-HT_{1A} and 5-HT_{2A/C} effects on PFC neuronal excitability are differentially altered in animals exposed to acute stress

Since the serotonin system is involved in mediating anxiety behaviors and stress responses (Deakin 1988; Griebel, 1995; Shakesby et al., 2002), we subsequently examined whether acute stress influenced the serotonin effect on PFC neuronal excitability. The stress procedures we used entailed forcing the rat to swim in deep water for 30 min (Price et al., 2002; Roche et al., 2003). As shown in Figure 6A, 8-OH-DPAT (with NMDA present) reduced AP firing to a similar extent in control vs. stressed animals (control: $-32.3 \pm 2.2\%$, $n = 27$; stressed: $-30.5 \pm 5.0\%$, $n = 5$, Figure 6C). However, α-Me-5HT (with NMDA present) failed to enhance AP firing in stressed animals (control: $31.4 \pm 2.0\%$, $n = 15$; stressed: $9.7 \pm 2.5\%$, $n = 9$, Figure 6B and 6C). It indicates that the effect of 5-HT_{2A/C} on PFC neuronal excitability is selectively sensitive to stressful conditions.

To find out the potential mechanism underlying the different sensitivities to stressful conditions of the 5-HT_{1A} vs. 5-HT_{2A/C} regulation of PFC neuronal excitability, we examined their

common target ERK. Western blot analyses were conducted to detect the effect of 5-HT_{1A} or 5-HT_{2A} agonist on ERK activation in PFC slices from control vs. stressed animals. As shown in Figure 7A, in control animals, in the presence of NMDA (4 μ M), the level of p-ERK was decreased by 8-OH-DPAT (1 μ M, $-71.1 \pm 5.9\%$, $n = 3$), and was increased by α -Me-5HT (0.8 μ M, $118.8 \pm 17.2\%$, $n = 3$, Figure 7B). However, in stressed animals, the α -Me-5HT-induced increase of p-ERK was completely abolished ($-16.8 \pm 2.2\%$, $n = 4$, Figure 7B), whereas the 8-OH-DPAT-induced decrease of p-ERK was not altered ($-64.0 \pm 6.5\%$, $n = 4$, Figure 7B). Both agonists at the low concentrations had little effect on p-EPK in the absence of NMDA (Figure 7C). These results suggest that 5-HT_{1A} and 5-HT_{2A/C} receptors, by interacting with NMDA receptors, regulate ERK activation in an opposing manner. The selective alteration of the 5-HT_{2A/C} effect on PFC neuronal excitability in stressful conditions is likely attributable to the loss of 5-HT_{2A/C} regulation of ERK activation.

Discussion

Serotonin, by activating different receptor subtypes, regulates membrane excitability in the central nervous system in a complex manner (Andrade, 1998). The involvement of various voltage-gated channels in this action of serotonin receptors has been revealed (Colino and Halliwell, 1987; Penington and Kelly, 1990; Haj-Dahmane and Andrade, 1996; Carr et al., 2002). However, application of a low concentration of the 5-HT_{1A} or 5-HT_{2A/C} agonist alone fails to alter the AP firing elicited by somatic current injection in PFC pyramidal neurons (Fig. 1E), suggesting that the low level of 5-HT_{1A} or 5-HT_{2A/C} activation is not sufficient to trigger the change of voltage-gated channels that affects neuronal excitability.

It is largely unknown whether serotonin could affect neuronal firing activity by interacting with ligand-gated channels. Previous studies have shown that the NMDAR channel is an important target of 5-HT₂ and 5-HT_{1A} receptors (Blank et al., 1996; Arvanov et al., 1999; Yuen et al., 2005). Thus, we examined the effect of serotonin receptors on the excitability of PFC pyramidal neurons when NMDA receptors are co-activated. In the presence of NMDA, application of a low concentration of the 5-HT_{1A} or 5-HT_{2A/C} agonist exerts a reducing or enhancing effect on the AP firing respectively, suggesting that NMDAR activation provides a “gate” to facilitate the opposing regulation of neuronal excitability by the low level of 5-HT_{1A} or 5-HT_{2A/C} activation. Interestingly, dopamine D1 and D2 receptors can also modulate PFC pyramidal cell excitability, by interacting with different glutamate receptor subtypes (Kuei and O'Donnell, 2004).

How does 5-HT_{1A} or 5-HT_{2A/C} receptor regulate AP firing in the presence of NMDA? One possible mechanism is through the direct action on NMDAR channels. However, we found that the low concentration of 5-HT_{1A} or 5-HT_{2A/C} agonist (e.g. 1 μ M of 8-OH-DPAT or 0.8 μ M of α -Me-5HT) failed to cause a significant change in the NMDAR-mediated ionic current (data not shown). Thus, the reason why NMDA facilitates serotonergic regulation of neuronal excitability is likely to be the change of intracellular signaling molecule(s) downstream of the Ca²⁺ flow through NMDAR channels.

Our electrophysiological and biochemical evidence show that the opposing actions of 5-HT_{1A} or 5-HT_{2A/C} on neuronal excitability are mediated by the differential regulation of a converging target, ERK. ERK can be regulated by the PKA or PKC cascade (Roberson et al., 1999) downstream of 5-HT_{1A} or 5-HT_{2A/C} receptors. However, application of the low concentration of 5-HT_{1A} or 5-HT_{2A/C} agonist (e.g. 1 μ M of 8-OH-DPAT or 0.8 μ M of α -Me-5HT) alone was insufficient to significantly change the ERK activity (Fig. 6C). Again, NMDAR activation provides a “gate” to facilitate the opposing regulation of ERK activity by 5-HT_{1A} or 5-HT_{2A/C} receptors (Fig. 6A and 6B), probably via facilitating their coupling to the PKA or PKC cascade. Since ERK activation can increase the amplitude of backpropagating

action potentials by phosphorylating dendritic A-type K⁺ channel Kv4.2 subunits (Yuan et al., 2002), we speculate that one possible mechanism underlying the regulation of neuronal excitability by 5-HT-NMDA interactions is through the ERK modification of dendritic K⁺ channels.

To understand the potential implication of the regulation of neuronal excitability by 5-HT-NMDA interactions in cognitive and emotional processes, we examined animals exposed to acute stress, since many mental illnesses are exacerbated by stress conditions (Mazure, 1995; Arnsten 1998). Several lines of evidence have shown that stress interferes with serotonin neurotransmission by changing serotonin release or serotonin receptor functions (Adell et al., 1997; Maswood et al., 1998; Lowry et al., 2000; Tan et al., 2004). The forced swim test, a behavioral paradigm often used to evaluate antidepressant/anxiolytic efficacy, was used as a stressor in our studies. The effect of 5-HT_{2A/C}, but not 5-HT_{1A}, on AP firing is lost in stressed animals, which is associated with the selective loss of 5-HT_{2A/C} increase of ERK activity. It suggests that 5-HT_{2A/C} receptors are probably desensitized and inactivated by elevated levels of serotonin in response to stress stimulation. The persevered 5-HT_{1A} decrease of AP firing will help to dampen the excitability of PFC pyramidal neurons, which will lead to the suppression of PFC output under stress conditions.

Serotonin-glutamate interaction has been proposed to be a new target for antipsychotic drugs (Aghajanian and Marek, 1999). In this study, we demonstrate that different serotonin receptors, by interacting with NMDA receptors, influence the excitability of PFC pyramidal neurons via converging on ERK. It provides a potential mechanism that underlies the functional role of both serotonin system and NMDA receptors in normal cognition and mental disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by grants from National Institute of Health to Z.Y. We would like to thank Xiaoqing Chen for her technical support.

References

- Adell A, Casanovas JM, Artigas F. Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. *Neuropharmacology* 1997;36:735–41. [PubMed: 9225300]
- Aghajanian GK, Marek GJ. Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* 1997;36:589–599. [PubMed: 9225284]
- Aghajanian GK, Marek GJ. Serotonin–glutamate interactions: a new target for antipsychotic drugs. *Neuropsychopharmacology* 1999;21:S122–S133.
- Andrade R. Regulation of membrane excitability in the central nervous system by serotonin receptor subtypes. *Ann N Y Acad Sci* 1998;861:190–203. [PubMed: 9928257]
- Andrade R, Malenka RC, Nicoll RA. A G protein couples serotonin and GABAB receptors to the same channels in hippocampus. *Science* 1986;234:1261–5. [PubMed: 2430334]
- Araneda R, Andrade R. 5-Hydroxytryptamine-2 and 5-hydroxytryptamine-1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* 1991;40:399–412. [PubMed: 1851255]
- Arnsten AF. The biology of being frazzled. *Science* 1998;280:1711–2. [PubMed: 9660710]
- Arvanov VL, Liang X, Magro P, Roberts R, Wang RY. A pre- and postsynaptic modulatory action of 5-HT and the 5-HT_{2A, 2C} receptor agonist DOB on NMDA-evoked responses in the rat medial prefrontal cortex. *Eur J Neuroscience* 1999;11:2917–2934.

- Beique JC, Campbell B, Perring P, Hamblin MW, Walker P, Mladenovic L, Andrade R. Serotonergic regulation of membrane potential in developing rat prefrontal cortex: coordinated expression of 5-hydroxytryptamine (5-HT)_{1A}, 5-HT_{2A}, and 5-HT₇ receptors. *J Neurosci* 2004;24:4807–4817. [PubMed: 15152041]
- Blank T, Zwart R, Nijholt I, Spiess J. Serotonin 5-HT₂ receptor activation potentiates NMDA receptor-mediated ion currents by protein kinase C-dependent mechanism. *J Neurosci Res* 1996;45:153–160. [PubMed: 8843032]
- Buhot MC. Serotonin receptors in cognitive behaviors. *Curr Opin Neurobiol* 1997;7:243–54. [PubMed: 9142756]
- Cai X, Flores-Hernandez J, Feng J, Yan Z. Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. *J Physiol* 2002;540(Pt 3):743–59. [PubMed: 11986365]
- Cai X, Gu Z, Zhong P, Ren Y, Yan Z. Serotonin 5-HT_{1A} receptors regulate AMPA receptor channels through inhibiting Ca²⁺/calmodulin-dependent kinase II in prefrontal cortical pyramidal neurons. *J Biol Chem* 2002;277:36553–36562. [PubMed: 12149253]
- Carr DB, Cooper DC, Ulrich SL, Spruston N, Surmeier DJ. Serotonin receptor activation inhibits sodium current and dendritic excitability in prefrontal cortex via a protein kinase C-dependent mechanism. *J Neurosci* 2002;22:6846–55. [PubMed: 12177182]
- Colino A, Halliwell JV. Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* 1987;328:73–7. [PubMed: 3600775]
- Deakin JF. 5HT₂ receptors, depression and anxiety. *Pharmacol Biochem Behav* 1988;29:819–820. [PubMed: 3413204]
- Deng PY, Poudel SK, Rojanathammanee L, Porter JE, Lei S. Serotonin inhibits neuronal excitability by activating two-pore domain k⁺ channels in the entorhinal cortex. *Mol Pharmacol* 2007;72:208–18. [PubMed: 17452494]
- Doris A, Ebmeier K, Shajahan P. Depressive illness. *Lancet* 1999;354:1369–75. [PubMed: 10533878]
- Feng J, Cai X, Zhao J, Yan Z. Serotonin receptors modulate GABA_A receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. *J Neurosci* 2001;21:6502–6511. [PubMed: 11517239]
- Goldman-Rakic PS. Cellular basis of working memory. *Neuron* 1995;14:477–485. [PubMed: 7695894]
- Griebel G. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther* 1995;65:319–395. [PubMed: 7644567]
- Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R. Serotonin_{1A} receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 2002;416:396–400. [PubMed: 11919622]
- Gu Z, Zhong P, Yan Z. Activation of muscarinic receptors inhibits β -Amyloid peptide - induced signaling in cortical slices. *J Biol Chem* 2003;278:17546–56. [PubMed: 12606559]
- Haj-Dahmane S, Andrade R. Muscarinic activation of a voltage-dependent cation nonselective current in rat association cortex. *J Neurosci* 1996;16:3848–61. [PubMed: 8656279]
- Jakab RL, Goldman-Rakic PS. 5-Hydroxytryptamine_{2A} serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc Natl Acad Sci U S A* 1998;95:735–40. [PubMed: 9435262]
- Tseng, Kuei Y.; O'Donnell, Patricio. Dopamine–Glutamate Interactions Controlling Prefrontal Cortical Pyramidal Cell Excitability Involve Multiple Signaling Mechanisms. *J Neurosci* 2004;24:5131–9. [PubMed: 15175382]
- Lemond S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, Sequeira A, Kushwaha N, Morris SJ, Basak A, Ou X, Albert PR. Impaired Repression at a 5-Hydroxytryptamine 1A Receptor Gene Polymorphism Associated with Major Depression and Suicide. *J Neurosci* 2003;23:8788. [PubMed: 14507979]
- Lowry CA, Rodda JE, Lightman SL, Ingram CD. Corticotropin-releasing factor increases in vitro firing rates of serotonergic neurons in the rat dorsal raphe nucleus: evidence for activation of a topographically organized mesolimbocortical serotonergic system. *J Neurosci* 2000;20:7728–36. [PubMed: 11027235]

- Martin GR, Eglen RM, Hamblin MW, Hoyer D, Yocca F. The structure and signalling properties of 5-HT receptors: an endless diversity? *Trends Pharmacol Sci* 1998;19:2–4. [PubMed: 9509891]
- Maswood S, Barter JE, Watkins LR, Maier SF. Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. *Brain Res* 1998;783:115–20. [PubMed: 9479059]
- Mazure, CM. Does stress cause psychiatric illness?. American Psychiatric Press; 1995.
- Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:1159–72. [PubMed: 14642974]
- North RA, Uchimura N. 5-Hydroxytryptamine acts at 5-HT₂ receptors to decrease potassium conductance in rat nucleus accumbens neurones. *J Physiol* 1989;417:1–12. [PubMed: 2621587]
- Penington NJ, Kelly JS. Serotonin receptor activation reduces calcium current in an acutely dissociated adult central neuron. *Neuron* 1990;4:751–8. [PubMed: 2140514]
- Price ML, Kirby LG, Valentino RJ, Lucki I. Evidence for corticotropin-releasing factor regulation of serotonin in the lateral septum during acute swim stress: adaptation produced by repeated swimming. *Psychopharmacology (Berl)* 2002;162:406–14. [PubMed: 12172694]
- Raymond JR, Mukhin YV, Gettys TW, Garnovskaya MN. The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br J Pharmacol* 1999;127:1751–1764. [PubMed: 10482904]
- Roberson ED, English JD, Adams JP, Selcher JC, Kondratieff C, Sweatt JD. The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. *J Neurosci* 1999;19:4337–4348. [PubMed: 10341237]
- Roche M, Commons KG, Peoples A, Valentino RJ. Circuitry underlying regulation of the serotonergic system by swim stress. *J Neurosci* 2003;23:970–977. [PubMed: 12574426]
- Shakesby AC, Anwyl R, Rowan MJ. Overcoming the effects of stress on synaptic plasticity in the intact hippocampus: rapid actions of serotonergic and antidepressant agents. *J Neurosci* 2002;22:3638–3644. [PubMed: 11978839]
- Smiley JF, Goldman-Rakic PS. Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. *J Comp Neurol* 1996;367:431–43. [PubMed: 8698902]
- Tan H, Zhong P, Yan Z. Corticotropin-releasing factor and acute stress prolongs serotonergic regulation of GABA transmission in prefrontal cortical pyramidal neurons. *J Neurosci* 2004;24:5000–5008. [PubMed: 15163692]
- Vossler MR, Yao H, York RD, Pan MG, Rim CS, Stork PJ. cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* 1997;89:73–82. [PubMed: 9094716]
- Williams GV, Rao SG, Goldman-Rakic PS. The physiological role of 5-HT_{2A} receptors in working memory. *J Neurosci* 2002;22:2843–2854. [PubMed: 11923449]
- Williams J, Spurlock G, McGuffin P, Mallet J, Nothen MM, Gill M, Aschauer H, Nylander PO, Macciardi F, Owen MJ. Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptamine type 2a-receptor gene. European Multicentre Association Study of Schizophrenia (EMASS) Group. *Lancet* 1996;347:1294–6. [PubMed: 8622505]
- Yang CR, Seamans JK, Gorelova N. Electrophysiological and morphological properties of layers V-VI principal pyramidal cells in rat prefrontal cortex in vitro. *J Neurosci* 1996;16:1904–21. [PubMed: 8774458]
- Yuan LL, Adams JP, Swank M, Sweatt JD, Johnston D. Protein kinase modulation of dendritic K⁺ channels in hippocampus involves a mitogen-activated protein kinase pathway. *J Neurosci* 2002;22:4860–4868. [PubMed: 12077183]
- Yuen EY, Jiang Q, Chen P, Gu Z, Feng J, Yan Z. Serotonin 5-HT_{1A} receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. *J Neurosci* 2005;25:5488–5501. [PubMed: 15944377]
- Zhong P, Gu Z, Wang X, Jiang H, Feng J, Yan Z. Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. *J Biol Chem* 2003;278:26888–26896. [PubMed: 12746438]

Figure 1.

In the presence of NMDA, the 5-HT_{1A} or 5-HT_{2A/C} agonist reduces or enhances PFC pyramidal cell excitability, respectively. **A-D**, Representative traces of action potential firing evoked by depolarizing current pulses illustrating the effect of 8-OH-DPAT (1 μM, A, B) or α-Me-5HT (0.8 μM, C, D) applied in the presence of NMDA (4 or 10 μM). Scale bars: 20 mV, 100 ms. **E**, Cumulative data (mean ± SEM) showing the percentage change of the firing rate by 8-OH-DPAT or α-Me-5HT in the absence (compared to ACSF) or presence of NMDA (compared to NMDA alone). Different currents were injected to generate 3-5, 6-8 or 9-11 spikes under the control condition (ACSF or NMDA alone). The number of cells tested in each condition is shown in each bar. *: p<0.01, ANOVA, compared to the agonist effects in the absence of

NMDA. **F, G**, Summary I-V graphs (voltages in response to a series of current injections) depicting passive membrane properties in cells before and after 8-OH-DPAT (1 μ M, F) or α -Me-5HT (0.8 μ M, G) application in the presence of NMDA (4 μ M).

Figure 2.

5-HT_{1A} or 5-HT_{2A/C} receptors mediate the agonist effects on AP firing, which is dependent on co-activation of NMDA receptors. **A, B, D, E**, Representative AP firing traces showing the effect of 8-OH-DPAT (1 μM) or α-Me-5HT (0.8 μM) (with 4 μM of NMDA present) in the presence of the 5-HT_{1A} antagonist NAN-190 (1 μM, **A**), 5-HT_{2A/C} antagonist ketanserin (5 μM, **D**), or NMDA receptor antagonist APV (50 μM, **B, E**). Scale bars: 20 mV, 100 ms. Currents were injected to generate 6-8 spikes under the control condition (NMDA alone). **C, F**, Cumulative data (mean ± SEM) showing the percentage change of the firing rate by 8-OH-DPAT (**C**) or α-Me-5HT (**F**) (with NMDA present) in the absence or presence of various antagonists. *: p < 0.01, ANOVA, compared to the effect in the absence of antagonists (-).

Figure 3.

In the presence of NMDA, low doses of 5-HT reduce PFC pyramidal cell excitability. **A, B,** Representative AP traces showing the effect of 5-HT (1 μ M, A) or 5-HT (10 μ M, B) on the firing rate (with 4 μ M of NMDA present). Scale bars: 20 mV, 100 ms. **C,** Cumulative data (mean $\pm$ SEM) showing the percentage change of the firing rate by different doses of 5-HT (with NMDA present).

Figure 4.

The regulation of AP firing by 5-HT_{1A}-NMDA interaction involves PKA and ERK. **A-D**, Representative AP firing traces showing the effect of 8-OH-DPAT (1 μ M) in the presence of NMDA (4 μ M) in neurons dialyzed without (control, A) or with the PKA inhibitor PKI₆₋₂₂ (20 μ M, B), PKC inhibitor calphostin (1 μ M, C), or ERK inhibitor U0126 (20 μ M, D). Scale bars: 20 mV, 100 ms. Currents were injected to generate 6-8 spikes under the control condition (NMDA alone). **E**, Cumulative data (mean $\pm$ SEM) showing the percentage decrease of the firing rate by 8-OH-DPAT (with NMDA present) in the absence or presence of various inhibitors. *: $p < 0.01$, ANOVA, compared to the effect in the absence of kinase inhibitors (-).

Figure 5.

The regulation of AP firing by 5-HT_{2A/C}-NMDA interaction involves PKC and ERK. **A-E**, Representative AP firing traces showing the effect of α -Me-5HT (0.8 μ M) in the presence of NMDA (4 μ M) in neurons dialyzed without (control, A) or with the PLC inhibitor U73122 (5 μ M, B), PKC inhibitor calphostin (1 μ M, C), PKA inhibitor PKI₆₋₂₂ (20 μ M, D), or ERK inhibitor U0126 (20 μ M, E). Scale bars: 20 mV, 100 ms. Currents were injected to generate 6-8 spikes under the control condition (NMDA alone). **F**, Cumulative data (mean $\pm$ SEM) showing the percentage increase of the firing rate by α -Me-5HT (with NMDA present) in the absence or presence of various inhibitors. *: p < 0.01, ANOVA, compared to the effect in the absence of kinase inhibitors (-).

Figure 6.

Acute stress attenuates the effect of 5-HT_{2A/C}, but not 5-HT_{1A}, on AP firing. **A-B**, Representative AP firing traces showing the effect of 8-OH-DPAT (1 μ M, A) or α -Me-5HT (0.8 μ M, B) in the presence of NMDA (4 μ M) in PFC pyramidal neurons from non-stressed (control) vs. stressed animals. Scale bars: 20 mV, 100 ms. Currents were injected to generate 6-8 spikes under the control condition (NMDA alone). **C**, Cumulative data (mean $\pm$ SEM) showing the percentage change of the firing rate by 8-OH-DPAT or α -Me-5HT (with NMDA present) in PFC neurons from control vs. stressed rats. *: p < 0.01, ANOVA, compared to the effect in control animals.

Figure 7.

The 5-HT_{2A/C}, but not 5-HT_{1A}, effect on ERK activation is lost in stressed animals. **A**, Western blot analysis of Thr202/Tyr204phos-ERK and total ERK in PFC slices treated without or with 8-OH-DPAT (1 μ M, 10 min) or α -Me-5HT (0.8 μ M, 10 min) in the presence of NMDA (4 μ M, 10 min pre-incubated) from control vs. stressed animals. **B**, Bar graphs showing the percentage change of p-ERK by 8-OH-DPAT or α -Me-5HT (with NMDA present) in PFC slices from control vs. stressed animals. **C**, Western blot analysis of p-ERK and total ERK in PFC slices from control animals treated without or with 8-OH-DPAT (1 μ M, 10 min) or α -Me-5HT (0.8 μ M, 10 min) in the absence of NMDA. *: p<0.01, ANOVA, compared to the effect in control animals.

Table 1

Membrane properties of PFC pyramidal neurons treated with different drugs.

	ACSF	NMDA	NMDA+DPAT
Resting Potential (mV)	-71.6 ± 2.1	-68.3 ± 2.5	-70.1 ± 3.5
Spike Threshold (mV)	-52.3 ± 2.6	-51.8 ± 2.2	-52.1 ± 4.1
Input Resistance (MΩ)	133.7 ± 22.4	150.5 ± 34.6	146.4 ± 39.3
Max AHP (mV)	6.5 ± 0.8	5.4 ± 0.6	6.2 ± 0.7
Spike Amplitude (mV)	79.3 ± 3.2	78.8 ± 4.0	78.5 ± 5.2
Spike Halfwidth (ms)	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.3
Latency to 1 st spike (ms)	71.1 ± 3.9	41.8 ± 3.1	60.2 ± 3.4 *
Rheobase (pA)	121.7 ± 5.6	76.6 ± 5.1	105.0 ± 5.8 *
	NMDA		NMDA+α-Me-5HT
Resting Potential (mV)	-68.7 ± 2.7		-66.8 ± 3.2
Spike Threshold (mV)	-51.6 ± 2.1		-51.9 ± 3.3
Input Resistance (MΩ)	148.5 ± 33.5		158.1 ± 37.8
Max AHP (mV)	5.5 ± 1.2		4.1 ± 1.3
Spike Amplitude (mV)	78.7 ± 3.9		78.1 ± 4.7
Spike Halfwidth (ms)	2.1 ± 0.2		2.1 ± 0.3
Latency to 1 st spike (ms)	43.4 ± 3.8		30.4 ± 2.1 *
Rheobase (pA)	74.8 ± 4.8		53.6 ± 5.2 *

Values are expressed as mean ± SEM.

* p < 0.05, ANOVA, compared with control (NMDA alone), n = 5-6 per group.