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

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**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 acute 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-$HT_2$ 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, 2002; Buhot, 1997). Aberrant serotonin system 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-$HT_2$ 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

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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

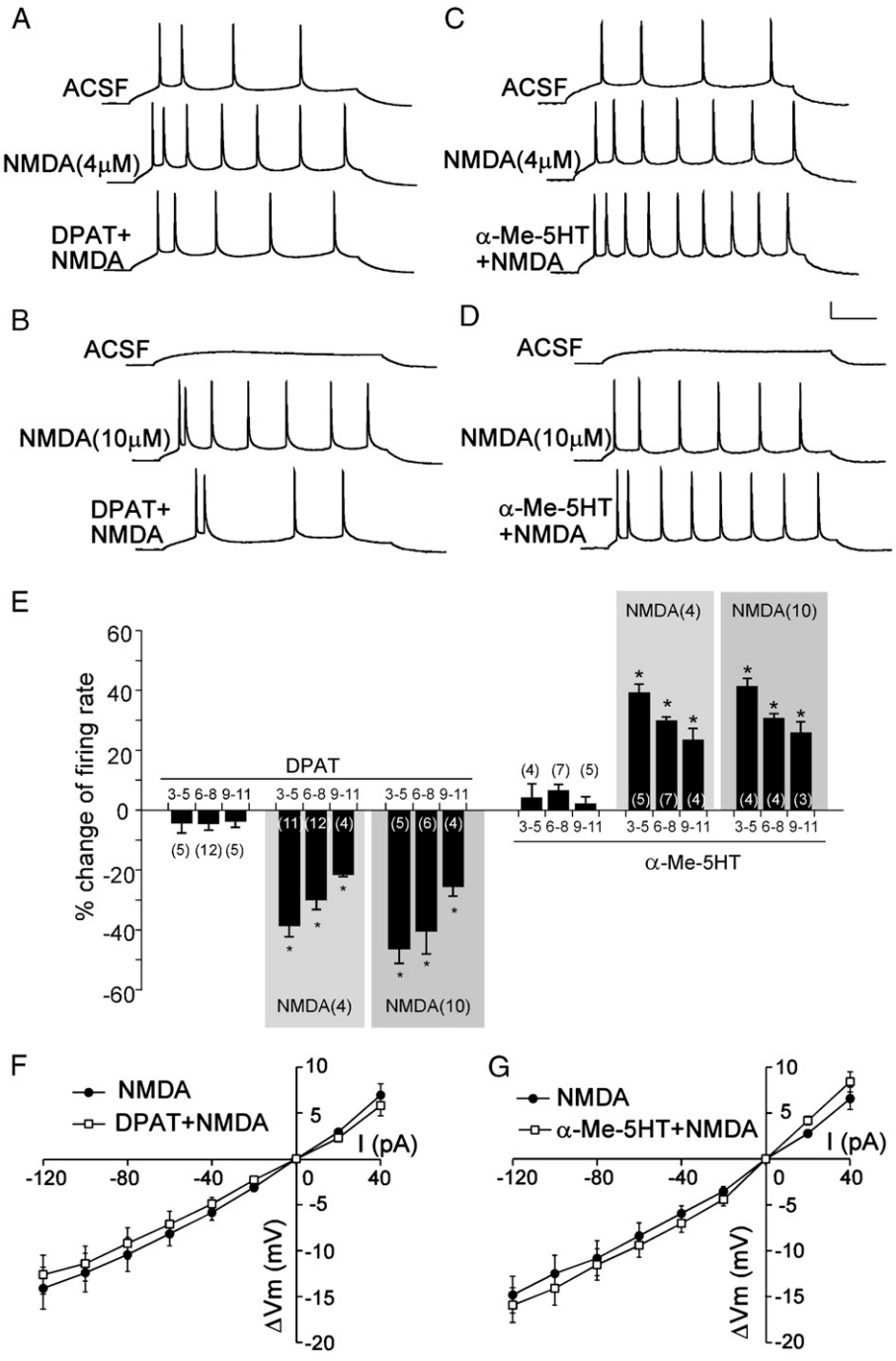


Fig. 1. In the presence of NMDA, the $5\text{-}HT_{1A}$ or $5\text{-}HT_{2A/C}$ agonist reduces or enhances PFC pyramidal cell excitability, respectively. **A**–**D**, Representative traces of action potential firing evoked by depolarizing current pulses illustrating the effect of 8-OH-DPAT (1 μM, A, 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 μM, F) or α-Me-5HT (0.8 μM, G) application in the presence of NMDA (4 μM).

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.

## 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 μM) produced a significant reduction of the firing rate (32.4±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±5.2%, n=7), consistent with previous results (Araneda and Andrade, 1991). However, at low concentrations, both 8-OH-DPAT (1 μM) and α-Me-5HT (0.8 μM) failed to induce significant changes in AP firing (8-OH-DPAT: 4-5% reduction; α-Me-5HT: 2–7% increase, Fig. 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 Figs. 1A–D, bath application of NMDA (4 or 10 μM) substantially enhanced the rate of firing elicited by small depolarizing current pulses (4 μM NMDA: 72.7±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 μM), the firing rate was significantly decreased by a low concentration of 8-OH-DPAT (1 μM, Figs. 1A, 1B), and was significantly enhanced by a low concentration of α-Me-5HT (0.8 μM, Figs. 1C, 1D), while both 5-HT receptor agonists were ineffective when applied alone at these concentrations. As summarized in Fig. 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 μM NMDA present, $p<0.01$, ANOVA), while α-Me-5HT increased the number of spikes by 24–40% or 26–42% (with 4 or 10 μM 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 α-Me-5HT. Other parameters, including membrane potential, input resistance, after-hyperpolarization (AHP), action potential threshold, amplitude and halfwidth, were not significantly altered. Moreover, low concentrations of 8-OH-DPAT or α-Me-5HT (in the presence of NMDA) did not change the passive membrane properties, as indicated by the I–V relationship shown in Figs. 1F and 1G.

In contrast to the strong effects of 8-OH-DPAT and α-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 μM) of AMPA (Supplemental Fig. 1, 8-OH-DPAT: 4.3±2.5% reduction, n=4; α-Me-5HT: 4.8±1.9% increase, n=4). Compared to control (ACSF), AMPA (0.2 μM) alone significantly increased the firing rate (53.6±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.

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 (M?) | 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 1st 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 1st 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.

We next examined the receptors that mediate the effects of 8-OH-DPAT or α-Me-5HT on AP firing in the presence of NMDA. The selective 5-$HT_{1A}$ antagonist NAN-190 (1 μM) abolished the 8-OH-DPAT-induced decrease in firing rate (−4.1±1.3%, n=6, Figs. 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 μM) blocked the α-Me-5HT-induced increase in firing rate (6.3±2.4%, n=6, Figs. 2D, 2F), indicating that 5-$HT_{2A/C}$ receptors mediate the effect of α-Me-5HT. In the presence of APV (25 or 50 μM), a selective NMDAR antagonist, the inhibitory action of 8-OH-DPAT or the enhancing action of α-Me-5HT on excitability was blocked (Figs. 2B, 2E, 8-OH-DPAT: −4.6±3.2%, n=5, Fig. 2C; α-Me-5HT: 6.1±3.7%, n=5, Fig. 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-$HT_1$-mediated decrease or a 5-$HT_2$-mediated increase of the firing rate, is predominant in response to 5-HT, we examined the effect of two doses of 5-HT (1 μM, 10 μM) in the presence of NMDA (4 μM). As shown in Figs. 3A-C, 5-HT (1 μM) produced a ~20% reduction and 5-HT (10 μM) produced a ~40% reduction of the firing rate. Since 5-$HT_1$ has higher affinity (nanomolar) than 5-$HT_2$ (micromolar) to 5-HT, the action of 5-$HT_1$ 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

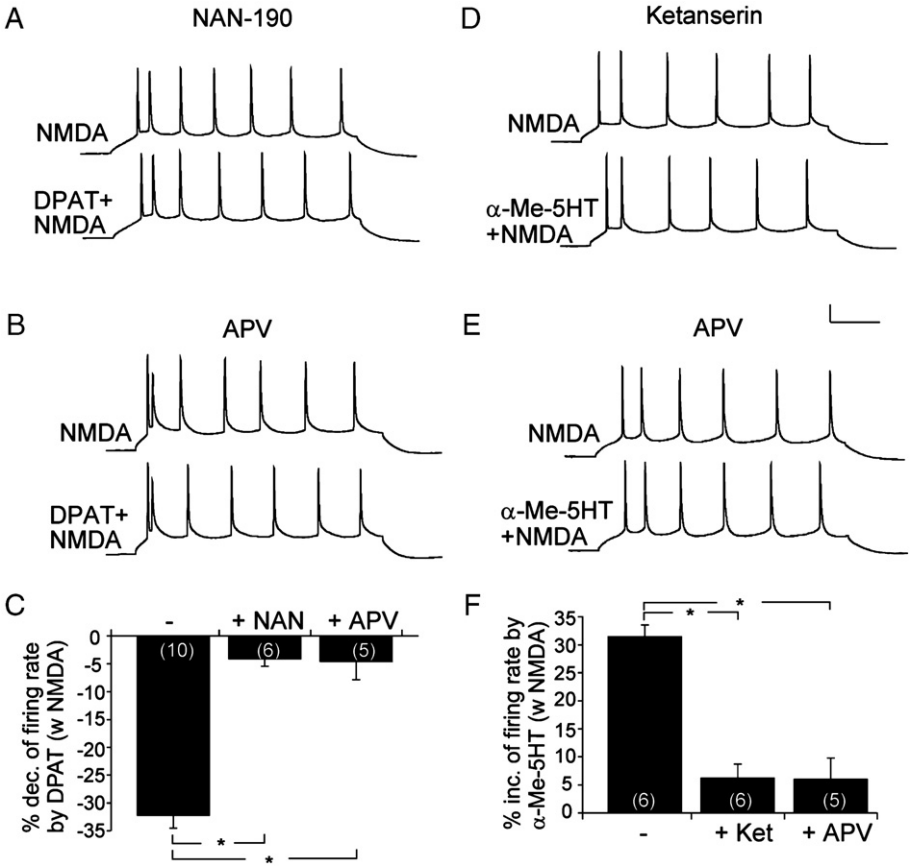


Fig. 2. 5-$HT_{1A}$ or 5-$HT_{2A/C}$ receptors mediate the agonist effects on AP firing, which is dependent on co-activation of NMDA receptors. **A, B, D, E**, Representative AP firing traces showing the effect of 8-OH-DPAT (1 μM) or α-Me-5HT (0.8 μM) (with 4 μM of NMDA present) in the presence of the 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 (−).

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 Figs. 4A and 4B, the reducing effect of 8-OH-DPAT (with NMDA present) on spikes was prevented in cells dialyzed with the PKA inhibitor $PKI_{6-22}$ (20 μM, −6.9±3.0%, n=5, Fig. 4E), while it was not affected by the PKC inhibitor calphostin (1 μM, Fig. 4C, 30.6±3.5%, n=4, Fig. 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 Fig. 4D, injecting cells with the ERK kinase inhibitor U0126 (20 μM) prevented 8-OH-DPAT

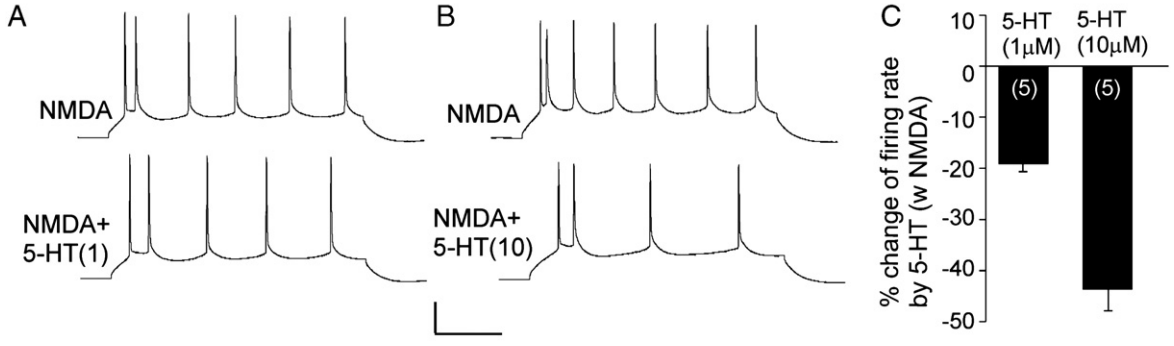


Fig. 3. In the presence of NMDA, low doses of 5-HT reduce PFC pyramidal cell excitability. **A, B**, Representative AP traces showing the effect of 5-HT (1 μM, A) or 5-HT (10 μM, B) on the firing rate (with 4 μM of NMDA present). Scale bars: 20 mV, 100 ms. **C**, Cumulative data (mean±SEM) showing the percentage change of the firing rate by different doses of 5-HT (with NMDA present).

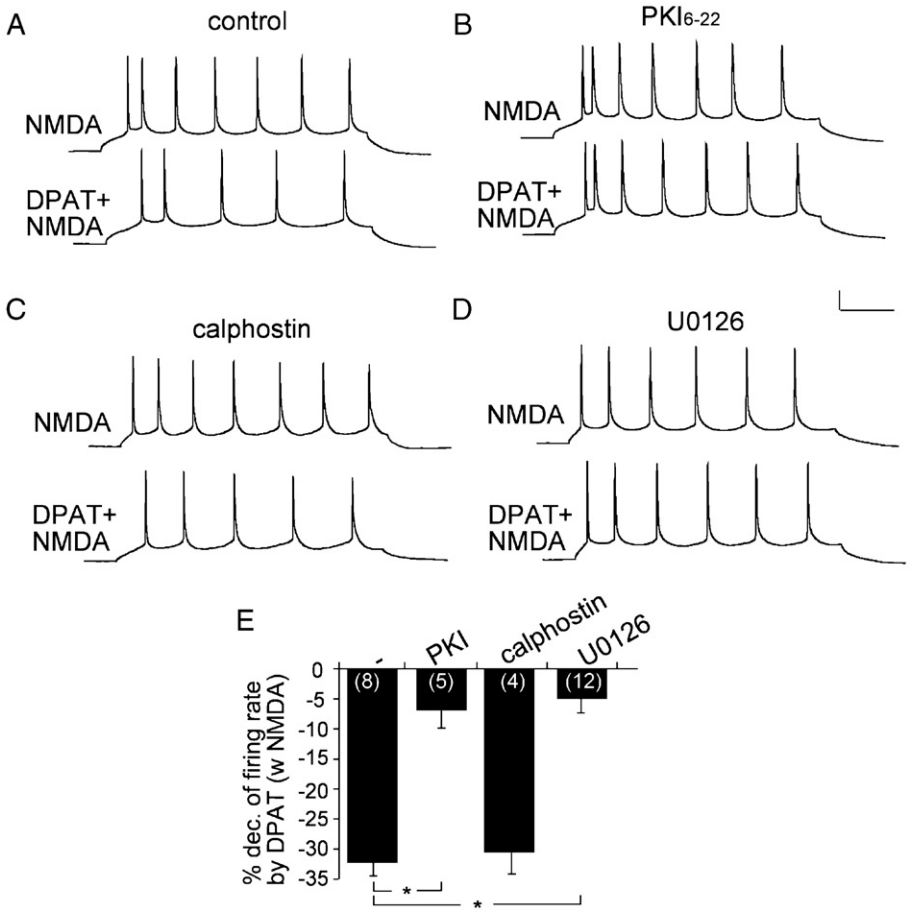


Fig. 4. The regulation of AP firing by 5-$HT_{1A}$-NMDA interaction involves PKA and ERK. **A**–**D**, Representative AP firing traces showing the effect of 8-OH-DPAT (1 μM) in the presence of NMDA (4 μM) in neurons dialyzed without (control, A) or with the PKA inhibitor $PKI_{6–22}$ (20 μM, B), PKC inhibitor calphostin (1 μM, C), or ERK inhibitor U0126 (20 μM, 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 ± 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 (−).

from reducing the firing rate (−5.0 ± 2.3%, n = 12, Fig. 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-$HT_2$ receptors stimulates phospholipase C β isoform (PLCβ), leading to the release of ionsitol-1,4,5-triphosphate ($IP_3$) 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 Figs. 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 ± 2.0%, n = 6, Fig. 5F) or the PKC inhibitor calphostin (1 μM, 7.9 ± 2.4%, n = 5, Fig. 5F), but not the PKA inhibitor $PKI_{6–22}$ (20 μM, 29.15 ± 3.3%, n = 4, Fig. 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 Fig. 5E, dialysis with the ERK kinase inhibitor U0126 (20 μM) prevented α-Me-5HT from increasing the firing rate (8.4 ± 3.2%, n = 8, Fig. 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 Fig. 6A, 8-OH-DPAT (with NMDA present) reduced AP firing to a similar extent in control vs. stressed animals (control: −32.3 ± 2.2%, n = 27; stressed: −30.5 ± 5.0%, n = 5, Fig. 6C). However, α-Me-5HT (with NMDA present) failed to enhance AP firing in stressed animals (control: 31.4 ± 2.0%, n = 15; stressed: 9.7 ± 2.5%, n = 9, Figs. 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-$HT_{2A/C}$

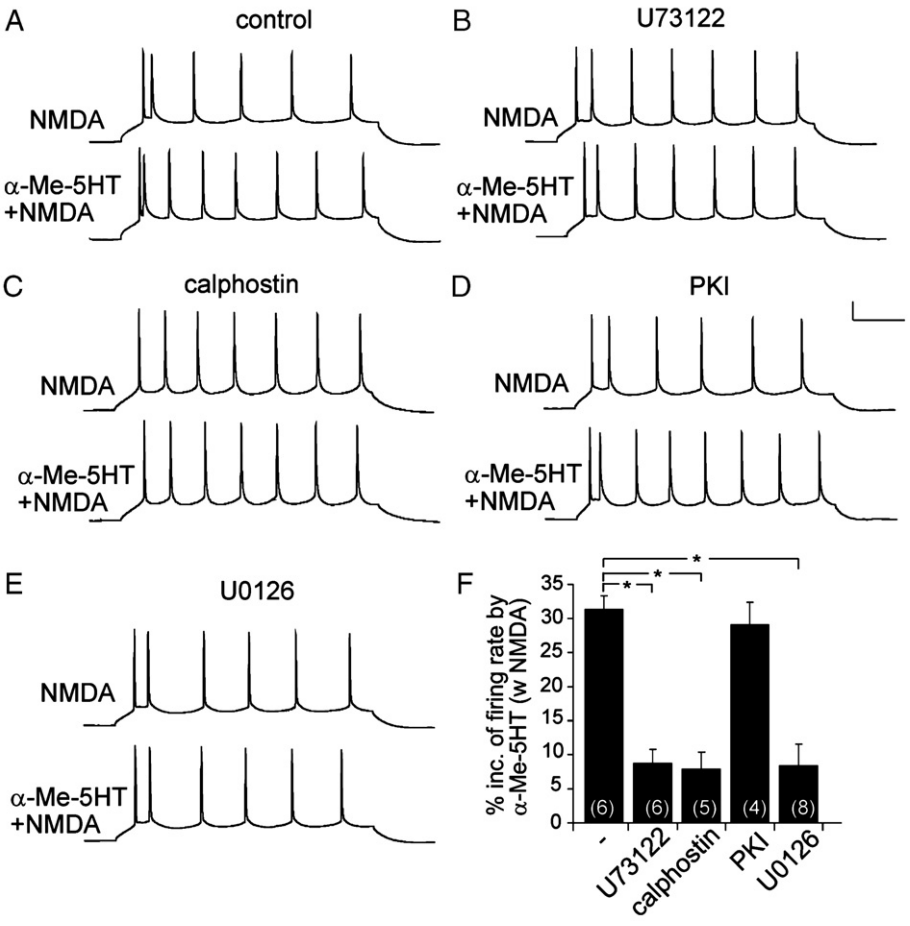


Fig. 5. The regulation of AP firing by 5-$HT_{2A/C}$-NMDA interaction involves PKC and ERK. **A–E**, Representative AP firing traces showing the effect of α-Me-5HT (0.8 μM) in the presence of NMDA (4 μM) in neurons dialyzed without (control, A) or with the PLC inhibitor U73122 (5 μM, B), PKC inhibitor calphostin (1 μM, C), PKA inhibitor $PKI_{6–22}$ (20 μM, D), or ERK inhibitor U0126 (20 μM, 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±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 (−).

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 Fig. 7A, in control animals, in the presence of NMDA (4 μM), the level of p-ERK was decreased by 8-OH-DPAT (1 μM, −71.1±5.9%, n=3), and was increased by α-Me-5HT (0.8 μM, 118.8±17.2%, n=3, Fig. 7B). However, in stressed animals, the α-Me-5HT-induced increase of p-ERK was completely abolished (−16.8±2.2%, n=4, Fig. 7B), whereas the 8-OH-DPAT-induced decrease of p-ERK was not altered (−64.0±6.5%, n=4, Fig. 7B). Both agonists at the low concentrations had little effect on p-EPK in the absence of NMDA (Fig. 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-$HT_2$ 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 (Tseng and O'Donnell, 2004).

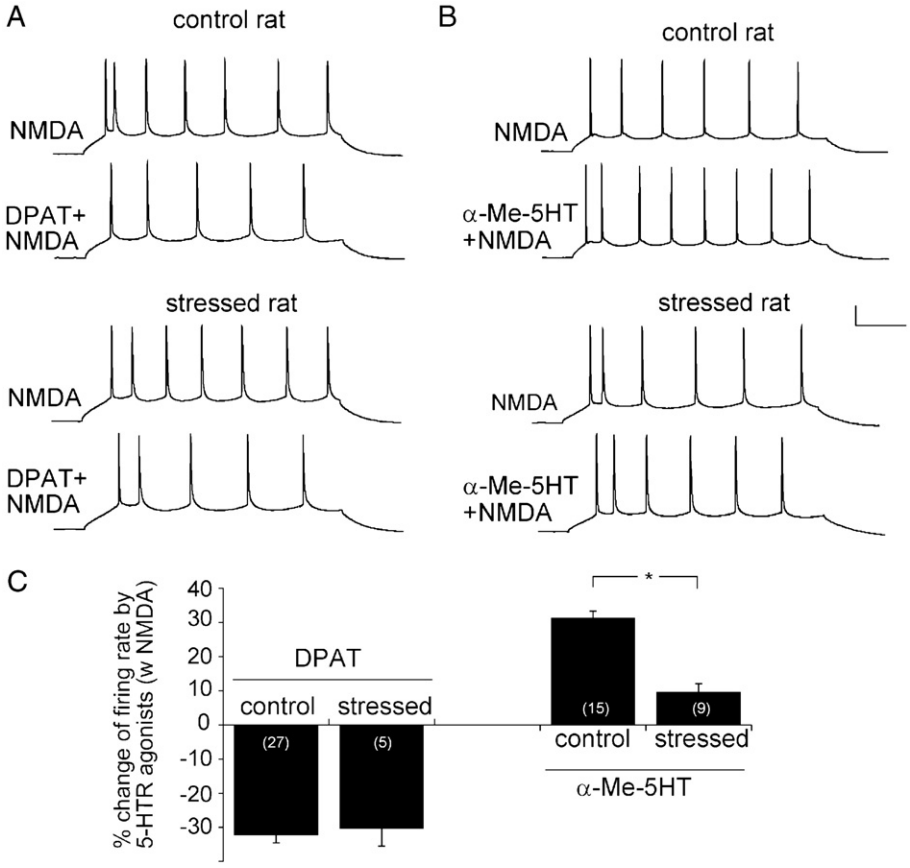


Fig. 6. Acute stress attenuates the effect of 5-$HT_{2A/C}$, but not 5-$HT_{1A}$, on AP firing. **A**–**B**, Representative AP firing traces showing the effect of 8-OH-DPAT (1 μM, A) or α-Me-5HT (0.8 μM, B) in the presence of NMDA (4 μM) in PFC pyramidal neurons from non-stressed (control) vs. stressed 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±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.

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 μM of 8-OH-DPAT or 0.8 μM 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 $Ca^{2+}$ flow through NMDAR channels.

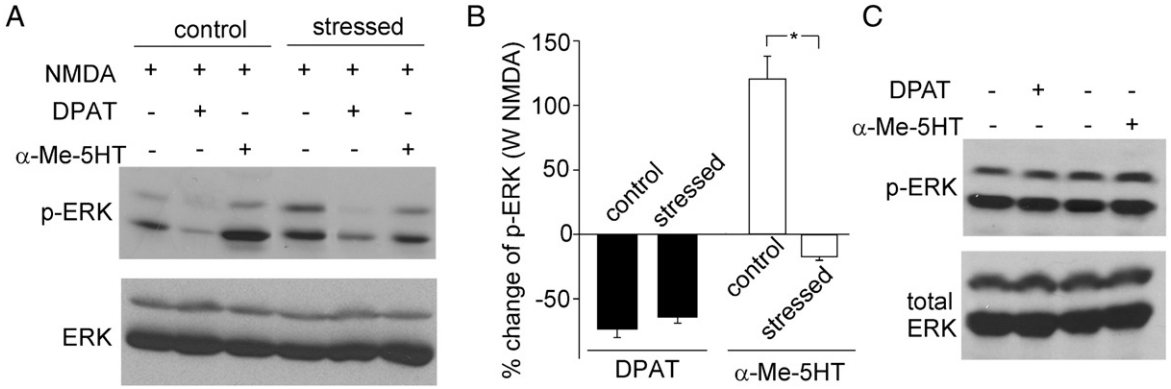


Fig. 7. The 5-$HT_{2A/C}$, but not 5-$HT_{1A}$, effect on ERK activation is lost in stressed animals. **A**, Western blot analysis of $^{Thr202/Tyr204}$phos-ERK and total ERK in PFC slices treated without or with 8-OH-DPAT (1 μM, 10 min) or α-Me-5HT (0.8 μM, 10 min) in the presence of NMDA (4 μM, 10 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 μM, 10 min) or α-Me-5HT (0.8 μM, 10 min) in the absence of NMDA. *: $p<0.01$, ANOVA, compared to the effect in control animals.

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 μM of 8-OH-DPAT or 0.8 μM 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 (Figs. 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-$HT_{2A/C}$-induced 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.

### Experimental 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 $NaHCO_3$-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 measure neuronal excitability, the whole-cell current-clamp technique (Zhong et al., 2003) was used to record spikes evoked by a 500 ms depolarizing current pulse (in the range of 140–200 pA). PFC slice (300 μm) 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 $K_2SO_4$, 60 N-methyl-glucamine, 40 HEPES, 4 $MgCl_2$, 0.5 BAPTA, 12 phosphocreatine, 2 $Na_2ATP$, 0.2 $Na_3GTP$, 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× 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 GΩ) 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 MΩ. Neuronal input resistance was determined by dividing the difference in membrane voltage in responses to a hyperpolarizing current pulse that caused a voltage deflection ~10 mV (Yang et al., 1996). All data are shown as mean ± 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 $PKI_{6–22}$, 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 × 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×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.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mcn.2008.03.003.

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