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Prefrontal cortical network activity: Opposite effects of psychedelic hallucinogens and D1/D5 dopamine receptor activation

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Abstract

The fine-tuning of network activity provides a modulating influence on how information is processed and interpreted in the brain. Here, we use brain slices of rat prefrontal cortex to study how recurrent network activity is affected by neuromodulators known to alter normal cortical function. We previously determined that glutamate spillover and stimulation of extrasynaptic NMDA receptors are required to support hallucinogen-induced cortical network activity. Since microdialysis studies suggest that psychedelic hallucinogens and dopamine D1/D5 receptor agonists have opposite effects on extracellular glutamate in prefrontal cortex, we hypothesized that these two families of psychoactive drugs would have opposite effects on cortical network activity.

We found that network activity can be enhanced by DOI (a psychedelic hallucinogen that is a partial agonist of serotonin 5-HT_{2A/2C} receptors) and suppressed by the selective D1/D5 agonist SKF 38393. This suppression could be mimicked by direct activation of adenylyl cyclase with forskolin or by addition of a cAMP analog. These findings are consistent with previous work showing that activation of adenylyl cyclase can upregulate neuronal glutamate transporters, thereby decreasing synaptic spillover of glutamate. Consistent with this hypothesis, a low concentration of the glutamate transporter inhibitor TBOA restored electrically-evoked recurrent activity in the presence of a selective D1/D5 agonist, whereas recurrent activity in the presence of a low level of the GABA_A antagonist bicuculline was not resistant to suppression by the D1/D5 agonist. The tempering of network UP states by D1/D5 receptor activation may have implications for the proposed use of D1/D5 agonists in the treatment of schizophrenia.

Keywords

rat; 5-HT_{2A} receptors; adenylyl cyclase; cAMP; neuronal glutamate transporter; EAAT3; EAAC1; caveolins

Introduction

In cortical neurons *in vivo*, recurrent network activity drives periods of depolarization (UP states) (Steriade et al., 1993; Cowan & Wilson, 1994; Lewis and O'Donnell, 2000; Petersen et al., 2003). These states appear to be mediated synaptically, driven by a mixture of excitatory and inhibitory inputs both *in vivo* (Haider et al., 2006) and *in vitro* (Shu et al., 2003). Network

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activity has been suggested to provide a “context” in which information is interpreted and decisions are made (McCormick, 2005). Bi-directional modulation of network activity may work to enhance cortical efficiency by increasing the signal-to-noise ratio (Winterer and Weinberger, 2004). It has been hypothesized that aberrations in the modulation of network activity may account for the perceptual and cognitive abnormalities in schizophrenia (Lewis et al., 2005).

Persistent network activity can be observed in brain slice of prefrontal cortex. Such network activity occurs spontaneously or can be evoked with a small electrical stimulus to the mid-layers of ferret cortex (McCormick et al., 2003; Shu et al., 2003) or multiple stimuli to the thalamus in mouse thalamocortical slice (Beierlein et al., 2002). Such network activity has been shown to rely on NMDA receptors (Shu et al., 2003) and increases the likelihood that a neuron will fire in response to an excitatory synaptic input (Shu et al., 2003; Haider et al., 2006). Similar states (originally termed “asynchronous, late excitatory postsynaptic currents (EPSCs)”) evoked by local electrical stimulation have also been described in rat prefrontal slice (Aghajanian and Marek, 1999). These states could be enhanced by psychedelic hallucinogens (Aghajanian and Marek, 1999) and were also found to be NMDA dependent (Stutzmann et al., 2001). Here, we show that these hallucinogen-enhanced, late “EPSCs” involve both inhibitory as well as excitatory activity, similar to the *in vitro* and *in vivo* network activity recently described by McCormick and colleagues (Shu et al., 2003; Haider et al., 2006).

How neurotransmitters and drugs modulate recurrent network activity is not well understood. We previously found that glutamate spillover and stimulation of extrasynaptic NMDA receptors plays a key role in network activity in the presence of psychedelic hallucinogens (Lambe and Aghajanian, 2006). Since microdialysis has shown that these compounds increase extracellular glutamate in prefrontal cortex (Scruggs et al., 2003; Muschamp et al., 2004), we hypothesized that a modulator that decreases extracellular glutamate in prefrontal cortex would decrease recurrent network activity induced by hallucinogens. Dopamine D1/D5 receptor agonists meet this criteria because they have been found to decrease extracellular glutamate in prefrontal cortex in microdialysis experiments (Abekawa et al., 2000; Harte and O'Connor, 2004). Consistent with this hypothesis, dopamine D1/D5 receptors reduce the reliability of unitary excitatory transmission in ferret slice (Gao et al., 2001) and excite prefrontal interneurons in rat slice (Zhou et al., 1999; Seamans et al., 2001b; Gorelova et al., 2002; Trantham-Davidson et al., 2004). A recent study in prefrontal slice from nonhuman primate showed that D1/D5 receptors selectively excite fast-spiking, non-adapting interneurons (Kroner et al., 2006).

Consistent with the hypothesis that dopamine may reduce noise in prefrontal cortex, imaging studies in humans suggest that dopamine focuses the hemodynamic response of prefrontal cortex during tasks (reviewed in Winterer and Weinberger, 2004; Mattay et al., 1996, 2002, 2003). Prefrontal recordings in nonhuman primates performing working memory tasks have shown that a low and local *blockade* of D1/D5 receptors *enhances* task-related excitability (Williams and Goldman-Rakic, 1995). Conversely, the delay-period excitability of single prefrontal neurons could be reduced to background levels with local application of the D1/D5 receptor agonist SKF38393 (Williams and Goldman-Rakic, 1995). Recent work from Arnsten and colleagues shows that D1 agonists and cAMP-analogs, suppresses delay period firing of prefrontal neurons involved in a working memory task (Arnsten, 2006; Wang et al., 2006).

Yet, there is a paradox. A substantial literature supports an excitatory role for D1/D5 stimulation in prefrontal cortex. The D1/D5 receptors have been shown to enhance NMDA-mediated activity *in vitro* (Wang and O'Donnell, 2001; Seamans et al., 2001a). In the presence of excitatory agents, such as NMDA (6–8 μ M) or high dose bicuculline (10 μ M), high concentrations of D1/D5 agonists can induce avalanches of synaptic activity in acute slice

(Beggs and Plenz, 2003) induce bistable membrane states (Tseng and O'Donnell, 2005), and enhance the spatiotemporal spread of cortical excitation (Bandyopadhyay et al., 2005). Interestingly, a recent study showing an enhancement of recurrent activity by D1/D5 receptors in the presence of bicuculline also showed a *suppression* of recurrent activity by dopamine in the *absence* of bicuculline (Onn et al., 2006); however, the dopamine receptor mediating this suppression was not identified. *In vivo* stimulation of the ventral tegmental area, whose neurons release dopamine and glutamate in cortex, can prolong plateau depolarizations in a D1-dependent manner (Lewis and O'Donnell, 2000). Yet these depolarizations are associated with reduced spiking (Lewis and O'Donnell, 2000), and thus may be different than network driven UP states which are associated with enhanced spiking *in vivo* (Haider et al., 2006) and *in vitro* (Shu et al., 2003).

In prefrontal cortical slice from rat, we found that low concentrations of agonists of D1/D5 receptors potently suppressed recurrent activity in prefrontal cortex. Their effects were opposite to the enhancement of network-driven UP states by psychedelic hallucinogens and could completely overwhelm both basal and DOI-enhanced network activity.

Experimental procedures

Coronal slices (400 μ m thick) of the medial prefrontal cortex were prepared from adolescent/young adult Sprague–Dawley rats (35–60 days old), in accordance with protocols approved by the Yale University Animal Care Committee. The brain was cooled as rapidly as possible with $\sim 4^{\circ}\text{C}$ oxygenated sucrose ACSF (254mM sucrose was substituted for NaCl). The slices were placed in a modified superfusion chamber (Warner Instruments, Hamden, CT) mounted on the stage of an Olympus BX50WI microscope (Olympus; Melville, NY). Great care was taken not to damage the cortical network during slice placement. Regular ACSF (128mM NaCl, 3mM KCl, 1.25mM NaH_2PO_4 , 10mM D-glucose, 26mM NaHCO_3 , 2mM CaCl_2 and 2mM MgSO_4 ; pH 7.35) was bubbled with 95% oxygen and 5% carbon dioxide and flowed over slice at 3–4 ml/min. After 5 minutes of recovery in room temperature ACSF, the bath was warmed to 33°C . After 1–2 hours of recovery, the slice was warmed to $35\text{--}36^{\circ}\text{C}$ for recording. The temperatures during brain removal, recovery, and recording were found to be critical for recurrent network activity. For the supplementary experiment, we used ACSF with more physiological concentrations of calcium and magnesium ions (1 mM each).

Whole-cell patch electrodes (4–6 M Ω) contained 120mM potassium gluconate, 5mM KCl, 2mM MgCl, 4mM $\text{K}_2\text{-ATP}$, 0.4mM $\text{Na}_2\text{-GTP}$, 10mM $\text{Na}_2\text{-phosphocreatine}$, and 10mM HEPES (adjusted to pH 7.33 with KOH). Layer V pyramidal neurons were patched under visual control using IR-DIC microscopy. The mean membrane properties of pyramidal neurons were as follows: 88.1 ± 2.5 mV spike amplitude; 81.0 ± 9.7 M Ω input resistance, and -73.3 ± 1.1 mV resting potential. Neurons were recorded in current clamp or voltage-clamp modes. In current-clamp, neurons were recorded at their resting potentials. In voltage-clamp, neurons were held either near the reversal potential for chloride or at a more depolarized potential to examine inhibitory components. Currents were recorded using continuous single electrode voltage clamp mode with an Axoclamp 2A or 2B, lowpass filtered at 3 kHz, amplified 100x through Cyberamp and digitized at 15 kHz, and acquired using pClamp 9/ Digidata 1322A (Axon Instruments, Union City, CA).

Platinum–iridium bipolar stimulating electrodes with sharpened, 3 μ m tips (FHC, Bowdoinham, ME) were placed midlayer in the medial prefrontal cortex and controlled via a Pulsemaster A300 interval generator (WPI, Sarasota, FL) and a stimulus isolation unit (Axon Instruments). Stimulating currents were 0.2 ms in duration and ranged from 5–30 μ A. During the baseline period in each experiment, stimuli were adjusted to produce a distinct fast EPSC but kept below the level that recruited late outward currents. Fast and late EPSC areas (nA*ms)

were measured using Axograph software (Axon Instruments). We measured the absolute area under the curve from the point of return to baseline after the fast EPSC (or deviation from smooth decay phase in the case of a late EPSC that emerged without a return to baseline) to the point of return to baseline (or end of the trace) after the late EPSC.

Drugs were obtained from Sigma (St Louis, MO) or Tocris (Ellisville, MO) and applied in the fast-flowing bath (3-4 ml/min). Dopamine stock solution was prepared in 20 mM sodium meta-bisulfite to prevent oxidation. For each application of dopamine, new solution was prepared by adding an aliquot of stock solution (stored at -20°C) to pre-oxygenated ACSF containing 20 μM sodium meta-bisulfite. Room lights were kept off, and the dopamine solution was oxygenated during application.

Results

Recurrent network activity can be evoked electrically in brain slice of rat prefrontal cortex

Local cortical stimulation can evoke a mixture of excitatory and inhibitory recurrent activity in rat prefrontal slice, as shown in current clamp in Figure 1 and as previously described in ferret prefrontal slice (Shu et al., 2003). Voltage-clamping a single neuron does not change the activity of the network, and allows the isolation of either the excitatory or inhibitory component of the synaptic input (Figure 1A2). The electrical stimulus evokes both a fast EPSC and a fast IPSC, each of which can be seen at different membrane potentials. The fast IPSC is not seen when the neuron is held near the reversal potential for chloride ($\sim 78\text{mV}$). However, when the neuron is clamped to a depolarized potential, there is reduced driving force on sodium and potassium ions through the AMPA channels and enhanced driving force on the chloride ions through GABA-A channels, which makes the fast and network IPSCs appear more prominent. As suggested by previous work (Aghajanian and Marek, 1999) and as shown in Figure 1B, bath application of the psychedelic hallucinogen DOI increases the probability of recurrent network activity occurring after any given stimulus and increases the duration of this network activity.

Recurrent network activity is enhanced by 5-HT_{2A} yet suppressed by D1/D5 stimulation

In order to quantify the selective effects of neuromodulators on the recurrent network activity underlying electrically-evoked UP states, we compared how treatments changed the areas of the evoked excitatory postsynaptic current (EPSC) and the excitatory component of the network activity (late EPSCs). Averaged traces in Figure 2 show the ability of the psychedelic hallucinogen DOI (3 μM ; 5-15 minutes; $N = 10$) to extend the duration of recurrent network activity evoked with a small electrical stimulus. Each trace shown is the average of 10 responses in voltage clamp to the same electrical stimulation delivered at 0.1 Hz. Although averages are most reliable for detecting the effects of pharmacological manipulations, they tend to mask the gamma frequency (30 – 80 Hz) of the oscillations. These oscillations in both basal and DOI-enhanced network activity can be better seen in the individual traces shown in Figure 1.

A low concentration of the D1/D5 partial agonist SKF 38393 (100 nM; 4 minutes) almost completely suppressed both basal ($N = 5$) and DOI-enhanced network activity ($N = 10$). This suppression occurred without consistent effects on resting potential or area of the fast EPSC. The data for the effects of SKF 38393 on the fast EPSC and network activity are summarized in Figure 2. In Figure 2C, the time course of the effects of DOI and SKF 38393 on network activity are shown. It must be noted that the effects of DOI are very slow to washout and remain maximal for several hours (Lambe and Aghajanian, 2006). The dose response relationship for SKF 38393 suppression of DOI-enhanced network activity gave an IC_{50} of 31.2 nM. This suppression was similar to the SKF 38393 suppression of basal network activity *in the absence of DOI* (IC_{50} of 34.9; illustrated in Figure 2D, E). The IC_{50} figures are consistent with the

efficacy and potency previously shown for SKF38393 in an adenylyl cyclase assay (Arnt et al., 1992). There was also no significant change in the fast EPSC for the group of neurons in Figure 1E (Area under basal conditions, 3.0 ± 0.7 nA*ms; area during maximum SKF, 2.7 ± 0.6 ; N = 6, Paired *t* test; *P* = NS).

Recurrent network activity occurs spontaneously with greater frequency in ACSF with lower concentrations of magnesium and calcium ions, as has been previously described in brain slice of ferret (Shu et al., 2003). In slices pretreated with DOI, spontaneous network activity was elicited by lowering the calcium and magnesium from 2 mM to 1 mM (Supplemental Data). Like electrically-evoked network activity, this spontaneous network activity could be completely suppressed with SKF 38393 (100 nM; 3-5 minutes; N=3).

Suppression of recurrent network activity appears specific to the D1/D5 dopamine receptors

Dopamine (1-10 μ M) also suppressed recurrent network activity evoked electrically, as illustrated in Figure 3A and B. This suppression was unchanged by pre-treatment and co-application of raclopride (100 nM – 1 μ M; N = 4), an antagonist of the D2 family of dopamine receptors. However, the suppression of network activity by dopamine was reduced dramatically by prior application of SCH 23390 (300 nM; N = 5), a dopamine D1/D5 family antagonist.

In agreement with this observation, the D2 family agonist quinpirole (1 μ M; 10 minutes; N = 4) did not significantly suppress recurrent network activity (Figure 3C). By contrast, the SKF 38393 suppression of network activity could be completely blocked by the D1/D5 receptor antagonist SCH 23390 (300 nM; 20 minutes; N = 6). Since SCH 23390 can bind to 5-HT_{2A} receptors, we also tested a different D1/D5 antagonist, SCH 39166 (1 μ M; 15-20 minutes; N = 5). The latter antagonist is somewhat less potent but has low affinity for 5-HT_{2A} receptors (Alburges et al., 1992). Like SCH 23390, it strongly blocks SKF 38393 suppression of network activity (Fig. 3C).

D1/D5 receptor suppression can be mimicked with 8-Br-cAMP or forskolin

Since it has been suggested that D1/D5 receptors may couple to more than one transduction pathway, we examined the signaling pathway involved in the suppression of recurrent network activity. As shown in Figure 4, directly activating adenylyl cyclase with forskolin (10 μ M; 5 minutes; N= 4) mimicked D1/D5 activation in that it was able to suppress recurrent network activity completely. By contrast, 1, 9-dideoxyforskolin – a negative control that does not stimulate adenylyl cyclase – failed to suppress network activity (data not shown; N = 3). To test the effects of a second messenger further along the Gs pathway, we applied a phosphodiesterase-resistant cAMP analog, 8-Br-cAMP (0.5 mM; 5-10 minutes; N= 5). Like forskolin, 8-Br-cAMP potently suppressed network activity, as shown in Figure 4, without significantly altering the fast EPSC (area of fast EPSC before forskolin, 1.8 ± 0.2 nA*ms; after forskolin, 1.9 ± 0.1 nA*ms; paired *t* test, *P* = NS; N= 4. Area of fast EPSC before 8-Br-cAMP, 2.5 ± 0.3 , after 8-Br-cAMP, 2.1 ± 0.1 ; paired *t* test, *P* = NS; N= 5;). Cyclic AMP can act through the protein kinase A pathway, but also has direct effects in cortex, including enhancement of hyperpolarization activated currents (Rosenkrantz and Johnston, 2006).

Mechanism of D1/D5 suppression of recurrent network activity

Since many studies have shown D1 excitation of cortical GABAergic activity (Zhou et al., 1999; Seamans et al., 2001b; Gorelova et al., 2002; Trantham-Davidson et al., 2004; Kroner et al., 2006), we examined whether network activity enhanced by a low dose of bicuculline methiodide would persist in the presence of SKF 38393. These experiments confirmed earlier work showing that a low concentration of bicuculline (1 μ M) itself enhances electrically-evoked network activity (Luhmann and Prince, 1990) without triggering epileptiform activity (Aghajanian and Marek, 1999). However, Figure 5 shows that network activity in the presence

of bicuculline was readily suppressed by same low concentration of SKF 38393 that suppresses basal or DOI enhanced network activity (100 nM; N = 6).

Earlier work from our laboratory has shown that glutamate spillover is critical for recurrent network activity (Lambe and Aghajanian, 2006). Neuronal glutamate transporters have been suggested to be critically important in preventing glutamate from escaping from synapses (Takayasu et al., 2005). Interestingly, surface expression of the neuronal glutamate transporter present in cerebral cortex, EAAT3, is upregulated rapidly through the Gs pathway (Pisano et al. 1996; Guillet et al., 2001). To address the hypothesis that D1/D5 receptors suppress network activity by upregulating glutamate transport and decreasing glutamate spillover, we added a low level of the relatively nonspecific glutamate transporter inhibitor TBOA (6 μ M; 5-10 minutes; N=5) to a concentration of D1/D5 agonist able to maximally suppress recurrent network activity (100 nM SKF 38393). In the presence of TBOA, network activity reappeared, despite the continued application of SKF 38393 (Figure 6A). Prior application of TBOA (3-6 μ M; 10 minutes), shifted the dose response curve for SKF 38393 to the right by almost two orders of magnitude. TBOA has high affinity for both the neuronal (EAAT3; K_i = 6 μ M; Jabaudon et al., 1999) and glial (EAAT2; K_i = 6 μ M; Jabaudon et al., 1999) glutamate transporters. By contrast, an inhibitor of glial glutamate transporters, trans-PDC (6-30 μ M; 10-15 minutes; N = 6) – which has similar affinity for EAAT2 as TBOA (K_i = 16 μ M; Koch et al., 1999) but very low affinity for EAAT3 (K_i > 300 μ M; Koch et al., 1999) – did not alter the SKF38393 suppression of network activity, as shown in Figure 6. While these results are suggestive that D1 dopamine stimulation may alter glutamate uptake, further investigation is needed to prove this relationship.

Discussion

D1/D5 dopamine receptors were found to be remarkably potent modulators of network activity in brain slice of rat prefrontal cortex. While the psychedelic hallucinogen DOI could intensify electrically-evoked recurrent network activity in frequency and duration, DOI-enhanced network activity was still susceptible to D1/D5 suppression. Consistent with the known coupling of D1/D5 receptors through the cyclic AMP pathway, the suppression of recurrent network activity by SKF 38393 was mimicked by forskolin, a direct activator of adenylyl cyclase, and by 8-Br-cAMP, a phosphodiesterase-resistant of cAMP. These findings were surprising since D1/D5 receptors have been suggested to enhance network activity in slice (Beggs and Plenz, 2003; Tseng and O'Donnell, 2005; Bandyopadhyay et al., 2005; Onn et al., 2006). However, these studies show this D1/D5 enhancement in the presence of excitatory agents (such as NMDA or high doses of bicuculline) that *override* endogenous mechanisms of glutamate uptake. In Tseng and O'Donnell (2005), exogenous NMDA is shown to put the neurons into a constant state of depolarization and spiking (essentially a continuous UP state) and the co-application of SKF 38393 brings in the hyperpolarized, nonresponsive periods (Tseng and O'Donnell, 2005). To our knowledge, this is the first study *in vitro* to examine the effects of selective D1/D5 receptor activation on recurrent network activity under basal conditions and in the presence of a psychedelic hallucinogen.

Network activity depends on glutamate spillover (Lambe and Aghajanian, 2006), and activation of adenylyl cyclase can decrease synaptic glutamate spillover by rapidly increasing surface expression the neuronal glutamate transporter EAAT3 (Pisano et al., 1996; Guillet et al., 2005). Consistent with this idea, we found that inhibiting neuronal glutamate transporters overcame D1/D5 suppression of network activity in prefrontal cortical slice. This experiment is similar in some ways to the studies that examined D1 effects on network activity after manipulations with high NMDA or bicuculline that override endogenous mechanisms of glutamate uptake (see above). The neuronal glutamate transporter EAAT3 is abundantly present in rat prefrontal cortex (Shashidharan et al., 1997), a region with high levels of 5-

HT_{2A} receptors (Willins et al., 1997; Miner et al., 2003) and D1/D5 dopamine receptors (Ciliax et al., 2000; Khan et al., 2000). Recent work has shown that all three of these proteins bind to caveolins (Bhatnagar et al., 2004; Yu et al., 2004; Gonzalez et al., SFN abstract, 2005). Although there is limited evidence that caveolins are present in cortical pyramidal cells, the closely-related flotillins are found in prefrontal cortex (Kokubo et al., 2000). These associations suggest that a macromolecular signaling complex could be involved in the modulation of recurrent network activity.

Several candidate mechanisms

D1/D5 receptors have been shown to have myriad, often contradictory, effects on cortical neurons. Recent work suggests that D1/D5 activation in cerebral cortex can suppress certain aspects of pyramidal neuron excitability by increasing hyperpolarization-activated currents (Rosenkranz and Johnston, 2006) or through decreasing transient sodium currents (Maurice et al., 2001; Peterson et al., 2006). These mechanisms may be more critical for the modulation of network activity than previously suspected. These findings are also consistent with the D1/D5 reduction of efficacy of unitary excitatory neurotransmission shown with dual whole cell recordings (Gao et al., 2001). In addition, D1/D5 dopamine receptors excite prefrontal interneurons in rat (Zhou et al., 1999; Seamans et al., 2001b; Gorelova et al., 2002; Trantham-Davidson et al., 2004) and selectively activate fast-spiking interneurons in monkey (Kroner et al., 2006). Recent work has demonstrated the critical role of interneuron synchrony in generating recurrent activity (Hasenstaub et al., 2005). This work suggests that changing the balance of excitatory and inhibitory synaptic activity would alter network activity. Since glutamate is a precursor for GABA synthesis, another aspect of the explanation could lie in the presence of EAAT3 in interneurons (Sepkuty et al., 2002; Weller et al., SFN abstract, 2005). If EAAT3 upregulation through the G(s) pathway were sufficient to increase GABA production, greater tonic release of GABA by a subpopulation of interneurons could be involved in suppressing network activity. Overall, multiple mechanisms may contribute to the D1/D5 suppression of basal and DOI-enhanced cortical recurrent activity. While our results suggest that D1 dopamine stimulation may alter glutamate uptake, further, further studies are necessary to examine this potential mechanism directly.

Clinical implications

The potency of the dopamine D1/D5 suppression of network activity raises the question of its role at the peak of the inverted “U” relationship – where too little dopamine and too much dopamine each decrease prefrontal cognitive function (Arnsten et al., 1994). Our results suggest that dopamine would modulate the timing and duration of recurrent network activity, perhaps keeping them within a range of normal by balancing other influences that would prolong them (such as 5-HT_{2A} stimulation). This view of dopamine is consistent with the recent proposal that D1 receptor stimulation promotes efficient cortical activation (Winterer and Weinberger, 2004; Mattay et al., 2002), as well as with clinical literature showing that D1/D5 antagonists exacerbate psychosis in people with schizophrenia (see Abi-Dhargham and Laurelle, 2005). Promoting D1/D5 receptor activation in prefrontal cortex has been suggested as a potential adjunct in treatment of schizophrenia (Abi-Dhargham and Laurelle, 2005; Marcus et al., 2005). However, these relationships are far from straightforward. In the rat, infusion of low levels of SKF 38393 and other D1/D5 agonists into medial prefrontal cortex of rat disrupts performance on certain prefrontal tasks (Zahrt et al., 1997) and enhances others (Granon et al., 2000). Understanding the mechanism through which D1/D5 receptors affect signal and noise aspects of network activity will help fine-tune a strategy to improve cognitive deficits in schizophrenia without worsening psychosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

5-HT, 5-hydroxytryptamine (serotonin); DOI, 2,5-dimethoxy-4-iodoamphetamine; NMDA, *N*-methyl-D-aspartic acid; EPSCs, excitatory postsynaptic currents; IPSCs, inhibitory postsynaptic currents; ACSF, artificial cerebral spinal fluid; IR-DIC, infrared differential interference contrast; EAAT, excitatory amino acid transporter; TBOA, threo- β -benzoylaspartic acid; trans-PDC, trans-pyrrolidine-2,4-dicarboxylate; BIC, bicuculline methiodide.

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Figure 1.

Recurrent network activity can be evoked electrically in brain slice of rat prefrontal cortex.

(A) Current and voltage clamp recordings from a neuron under basal conditions. (1) A stimulus (10 μ A; gray arrow) evokes network activity in the slice that depolarizes the neuron and increases its synaptic noise and spiking. (2) Voltage-clamping this neuron at different potentials allows the visualization of the inhibitory and excitatory aspects of the fast PSC and electrically-evoked network activity in the slice (V_H = holding potential). No channel inhibitors are applied. (B) The oscillations of the basal and DOI-enhanced network activity show characteristic gamma wavelength activity (30 – 80 Hz). Single sweeps in voltage clamp (V_H = -75 mV) show electrically-evoked (0.1 Hz) fast EPSCs and the excitatory component of the evoked network activity under basal conditions and following application of the psychedelic hallucinogen DOI (3 μ M; 15 minutes).

Figure 2.

A psychedelic hallucinogen and a D1/D5 agonist have opposite effects on recurrent network activity in brain slice of rat prefrontal cortex. (A) Average of 10 sweeps (0.1 Hz) in voltage-clamp ($V_H = -78$ mV) showing the electrically-evoked fast EPSC and the excitatory component of evoked network activity (late EPSCs): (1) under basal conditions, (2) after application of DOI (3 μ M; 5 minutes), (3) during application of SKF 38393 (100 nM; 4 minutes), and (4) after 15 minutes washout from SKF 38393. Note the delay in evoked network activity during washout of SKF 38393. (B) Bar graph shows the areas (mean $\pm$ S.E.) of the electrically-evoked fast EPSC and the late EPSC or network activity in the above conditions (N = 10 neurons). DOI significantly enhanced the area of the late EPSC, and SKF 38393 significantly suppressed the late EPSC. * denotes $P < 0.01$; ** denotes $P < 0.00001$. (C) Graph shows the time course of network activity and the evolution of the drug effects on a representative neuron. Each circle is the average of the 6 electrically-evoked network events in voltage-clamp that are stimulated during each one minute period (0.1 Hz stimulus frequency). DOI (3 μ M; 5 min) and SKF 38393 (100 nM, 4 min) were applied as indicated. It

must be noted that the DOI-mediated enhancement of network activity is slow to wash out and remains at a peak for hours. (D) Superimposed averaged sweeps in voltage clamp during sequential application of increasing doses of SKF 38393 (0 nM to 300 nM; 4 minute applications) for one neuron. As shown, the fast EPSC has not changed, but the network activity is dose-dependently suppressed. (D) Dose response curve from 6 neurons for the SKF 38393 suppression of DOI-enhanced network activity.

Figure 3.

Evoked network activity is suppressed selectively through D1/D5 activation. (A) Averaged sweeps in voltage clamp showing electrically-evoked fast EPSC and the excitatory component of the evoked network activity (late EPSCs): (1) under basal conditions, (2) after application of DOI, (3) during application of dopamine (10 μ M); (4) after washout of dopamine; (5) during pre-application of raclopride (300 nM; 10 minutes); (6) during co-application of raclopride (300 nM) and dopamine (10 μ M); (7) after pre-application of SCH 23390 (300 nM; 15 minutes); and (8) during application of dopamine (10 μ M). (B) Bar chart showing the percentage by which dopamine (3-10 μ M) suppresses the fast and the late EPSCs under basal conditions and in the presence of the D2 antagonist, raclopride (300 nM – 1 μ M; N = 4), or after application

of the D1/D5 antagonist SCH23390 (300 nM; 10 minutes; N = 5). Dopamine significantly suppressed the late EPSC under basal conditions and in the presence of raclopride; * denotes $P < 0.01$. Except for illustration in part (A) of this figure, raclopride and SCH23390 were not used in the same slices. (C) Bar chart shows the percentage by which the respective D2 and D1/D5 agonists, quinpirole (quin; 1 μ M; 10 minutes; N = 4) and SKF 38393 (SKF; 100 nM; 4 minutes; N = 10) suppress the fast and the late EPSCs under basal conditions and in the presence of antagonists. Only SKF 38393 suppressed evoked network activity or late EPSCs (* denotes $P < 0.001$). This suppression was blocked by pre-application of the selective D1/D5 receptor antagonists SCH 23390 (300 nM; 20 minutes; N = 6) or SCH 36199 (1 μ M; 15-20 minutes; N = 5).

Figure 4.

Second messengers in the G_s pathway suppress evoked network activity. (A) Bar chart shows the mean $\pm$ S.E. data for the effects of forskolin (direct activator of adenylyl cyclase; 10 μ M; 4 minutes; N = 4) and 8-Br-cAMP (0.5 mM; 5-10 minutes; N = 4) on the fast EPSC and evoked network activity (late EPSCs). Both treatments selectively suppressed network activity (* denotes $P < 0.01$). (B) Averaged sweeps in voltage clamp showing that 8-Br-cAMP (0.5 mM; 5 minutes) suppresses the evoked network activity without changing the fast EPSC.

Figure 5.

SKF 38393 suppresses network activity in the presence of a low dose of the GABA-A receptor antagonist, bicuculline methiodide. (A1) Averaged traces show the electrically-evoked fast EPSC and network activity in the presence of a low dose of bicuculline (BIC; 1 μ M; 3 minutes). (2) In continued presence of bicuculline (1 μ M), SKF 38393 (100 nM; 5 min) suppresses network activity but does not alter the fast EPSC in this neuron. (B) The bar chart shows the mean $\pm$ S.E. for the area of the fast EPSC and the excitatory component of evoked network activity (late EPSCs) during application of bicuculline alone (1 μ M; 3-5 min) and then bicuculline (1 μ M) co-applied with SKF 38393 (100 nM; 3-5 min). SKF 38393 dramatically reduced network activity (N = 6; paired *t* test; ** denotes $P < 0.01$). The fast EPSC was also slightly suppressed by SKF 38393 (N = 6; paired *t* test; * denotes $P = 0.04$).

Figure 6.

Blocking the neuronal, but not the glial, glutamate transporter can restore evoked network activity in the presence of the D1/D5 stimulation. (A) The superimposed traces on the left show that the glial glutamate transporter inhibitor trans-PDC (6 μ M; 10 minutes) cannot restore evoked network activity in the presence of SKF 38393 (100 nM). The traces on the right show that TBOA (6 μ M; 5 minutes), which also blocks the neuronal glutamate transporter, can restore evoked network activity in the presence of a maximally-suppressing level of SKF 38393. (B) Bar chart shows the mean $\pm$ S.E. for the area of the fast EPSC and the excitatory component of evoked network activity (late EPSCs) during application of SKF 38393 (100 nM) in the presence of trans-PDC (6-30 μ M; 10 minutes; N = 6) or TBOA (3-6 μ M; 3-10 minutes; N = 5). * denotes $P < 0.01$.