

Post-pubertal Emergence of Prefrontal Cortical Up States Induced by D₁-NMDA Co-activation

Kuei Y. Tseng and Patricio O'Donnell

Center for Neuropharmacology and Neuroscience, 47 New Scotland Avenue, Albany Medical College (MC-136), Albany, New York 12208, USA

Dopamine–glutamate interactions may contribute to persistent electrical activity in the prefrontal cortex (PFC). We tested whether a D₁ modulation of NMDA function can result in persistent depolarization *in vitro*. D₁-NMDA co-activation yielded depolarizing plateaus resembling *in vivo* up states in PFC pyramidal neurons recorded in slices from adult (PD 45–65), but not pre-pubertal (PD 29–38) rats. These plateaus required intracellular Ca²⁺, activation of L-type Ca²⁺ channels and protein kinase A. These events were eliminated by intracellular administration of the voltage-gated Na⁺ channel blocker QX-314 or by interrupting synaptic activity with bath application of tetrodotoxin or the AMPA antagonist CNQX, suggesting that they require both intrinsic and synaptic mechanisms. These recurrent depolarizations could constitute important elements in cortical information processing, allowing synaptic plasticity and memory functions. Acquiring these PFC D₁-NMDA interactions after puberty may be a critical element for developing mature cognitive abilities.

Keywords: D₁ receptors, electrophysiology, NMDA, persistent activity, prefrontal cortex, puberty

Introduction

It is well known that dopamine (DA) can modulate glutamate function in the brain. The interactions between these neurotransmitters are diverse and depend on the receptor subtypes involved. A consistent finding in this regard has been a D₁ enhancement of NMDA-mediated responses (Cepeda and Levine, 1998; Cepeda *et al.*, 1998; Wang and O'Donnell, 2001). It may not be coincidence that cells in which this interaction has been observed (e.g. pyramidal cortical neurons and striatal medium spiny neurons) exhibit spontaneous glutamate-driven plateau depolarizations when recorded *in vivo* (O'Donnell and Grace, 1995; Wilson and Kawaguchi, 1996; Lewis and O'Donnell, 2000; Goto and O'Donnell, 2001). Indeed, *in vivo* intracellular recordings from prefrontal cortical (PFC) pyramidal neurons reveal a very negative resting membrane potential (down state) interrupted by plateau depolarizations (up states; Branchereau *et al.*, 1996; Lewis and O'Donnell, 2000). Up states are driven by synchronous excitatory afferents and are periods of active information processing during which synaptic plasticity mechanisms may be enabled (O'Donnell, 2003). Although up-down transitions have been extensively characterized in anesthetized animals and sleep (Mahon *et al.*, 2001; Steriade *et al.*, 2001), a recent study demonstrated their presence in awake animals and a role of sensory inputs in up states (Petersen *et al.*, 2003). There is some indication that DA may control PFC up states. For example, electrical and chemical stimulation of the ventral tegmental area (VTA) evokes plateau depolarizations resembling up states, which are shortened by a D₁ antagonist (Lewis

and O'Donnell, 2000). Complete DA receptor blockade fails to abolish the onset of the evoked depolarization, suggesting that the transition to the up state is mediated by a non-DA mechanism, with DA acting to sustain the depolarization. Because D₁ receptors enhance NMDA function in the PFC (Zheng *et al.*, 1999; Wang and O'Donnell, 2001; Seamans *et al.*, 2001a; Gonzalez-Islas and Hablitz, 2003), it is conceivable that a sufficient level of D₁-NMDA co-activation could elicit plateau depolarizations resembling up states in a slice preparation. To address this issue, the effects of a D₁ agonist and NMDA on spontaneous activity in PFC pyramidal neurons were tested using whole-cell current clamp recordings.

The age of animals used to prepare slices is an important consideration in whole cell recordings. It is well known that postnatal developmental transformations in the brain, especially in the PFC and limbic system, are prominent during adolescence across a variety of species (for a review, see Spear, 2000). Most rat studies have been conducted to date in slices from pre-pubertal animals. It is rare to find reports of PFC recordings, for example, in rats >40 days old. Because puberty in rats occurs between postnatal day (PD) 40 and PD 45 and cortical circuits may mature at that age (Spear, 2000; Zhu, 2000), it is important to determine DA–glutamate interactions in adult animals. This becomes essential when studying persistent activity that may be relevant to *in vivo* up states. *In vivo* intracellular recordings from pre-pubertal animals (PD 28–35) have shown up states of smaller amplitude than similar recordings obtained from adult animals (O'Donnell *et al.*, 2002). Therefore, the experiments aimed at elucidating the cellular signaling pathways involved in D₁-NMDA interactions were conducted in slices obtained from animals at least 45 days old. Additional recordings were conducted in slices from younger animals to determine the age at which these interactions emerge.

Materials and Methods

Slice Preparation

Slices containing the medial PFC were obtained from adult (PD 45–65) or pre-pubertal (PD 29–38) rats. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) before being decapitated. Brains were rapidly removed into ice-cold artificial CSF [aCSF (in mM): 125 NaCl, 25 NaHCO₃, 10 glucose, 3.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 3 MgCl₂; pH 7.45, osmolarity 295 ± 5 mOsm]. Coronal slices (300 μm) were cut on a Vibratome in ice-cold aCSF, transferred and incubated in warm (33–35°C) aCSF solution constantly oxygenated with 95% O₂-5% CO₂ for at least 90 min before recording.

Electrophysiology

Whole-cell current clamp recordings were performed in pyramidal neurons located in deep layers of the medial PFC, identified under visual guidance using infrared-differential interference contrast (IR-DIC) video microscopy with a 40× water-immersion objective. The image

was detected with an IR-sensitive CCD camera and displayed on a monitor. All experiments were conducted at 33–35°C. In the recording aCSF (perfused at 2 ml/min), CaCl₂ was increased to 2 mM and MgCl₂ was decreased to 1 mM. Patch pipettes (5–8 MΩ) were filled with (in mM): 115 K-gluconate, 10 HEPES, 2 MgCl₂, 20 KCl, 2 MgATP, 2 Na₂-ATP and 0.3 GTP (pH = 7.3, 280 ± 5 mOsm). Signals were acquired using a Multiclamp 700A amplifier, digitized with a Digidata 1322A (Axon) and fed to a computer for off-line analysis. In each cell, membrane potential and input resistance were analyzed before (baseline) and after drug treatment. Only cells with a stable resting membrane potential of at least -65 mV (baseline) and overshooting action potentials were included in the study. Passive membrane properties (membrane potential and input resistance) and action potential characteristics (amplitude, duration and number evoked) were measured during a baseline period and during drug delivery. Whole-cell pipette series resistance was <20 MΩ, and was bridge compensated by ~80% (with the computer-controlled amplifier). Series resistance was monitored throughout the experiments and if it changed by >15%, the data were excluded for analysis. The D₁ agonist SKF38393 (Sigma, St Louis, MO), NMDA (RBI) and the D₁ antagonist SCH23390 (RBI) were dissolved in the external solution. For intracellular Ca²⁺ chelation, BAPTA (Sigma) was included in the recording micropipette at a concentration of 2 mM. L-type Ca²⁺ channels were blocked by bath application of nifedipine (Sigma, 10 μM). In some experiments, excitatory inputs arriving from other activated neurons contained in the slice were blocked by bath application of tetrodotoxin (TTX; Sigma, 0.05 μM) or CNQX (Sigma, 10 μM). To block protein kinase A activity, KT-5720 (Calbiochem, La Jolla, CA) was included in the bath solution. Both control and drug-containing aCSF were continuously oxygenated throughout the experiments. Typically, baseline recordings were conducted for ~15 min before perfusing with a solution containing SKF38393 (2 μM) or NMDA (8 μM) for 5 min. When both agonists were combined, baseline recordings were followed by SKF (2 μM) for 2 min, then followed by the D₁ + NMDA cocktail for 5 min. The effects of D₁ + NMDA were evident during the first minute of bath application and become stable during the subsequent 2–3 min. All neurons recorded in the present study showed a similar pattern of responses to D₁ + NMDA and required 10–20 min to completely wash out the effects. All data shown were obtained from the stable 2–3 min following drug administration. The doses were selected based on pilot experiments determining dose-response curves for these agents, which revealed that 8 μM NMDA is an effective concentration yielding constant depolarization and action potential firing and 2 μM SKF38393 is a marginally effective concentration that does not have a significant effect on its own, but potentiates NMDA responses (P. O'Donnell and K.Y. Tseng, unpublished observations).

Data Analysis

The Fisher exact probability test (FET) was used to evaluate the relationship between two dichotomous variables. Student's *t*-test was used for two-group comparisons involving a single continuous variable. Drug effects along two or more variables were compared using repeated measures ANOVA. If data were not normally distributed or had unequal variances, Kruskal-Wallis ANOVA by ranks was preferred for multiple comparisons involving interrelated proportions. Normality was assessed with the Kolmogorov-Smirnov test and homogeneity of variances was assessed with Levene's test. Differences between experimental conditions were considered statistically significant when *P* < 0.05.

Results

Whole-cell Recordings from Adult PFC Neurons

Whole-cell current clamp recordings of PFC pyramidal neurons were performed in slices obtained from adult (PD 45–65) rats. These neurons (*n* = 83) displayed a negative resting membrane potential (-69.8 ± 3.3 mV, mean ± SD) and had an input resistance of 120.1 ± 31.1 MΩ. Action potentials could be observed in response to depolarizing current (Fig. 1*a*) or occasionally during spontaneous depolarizations (Fig. 1*b*).

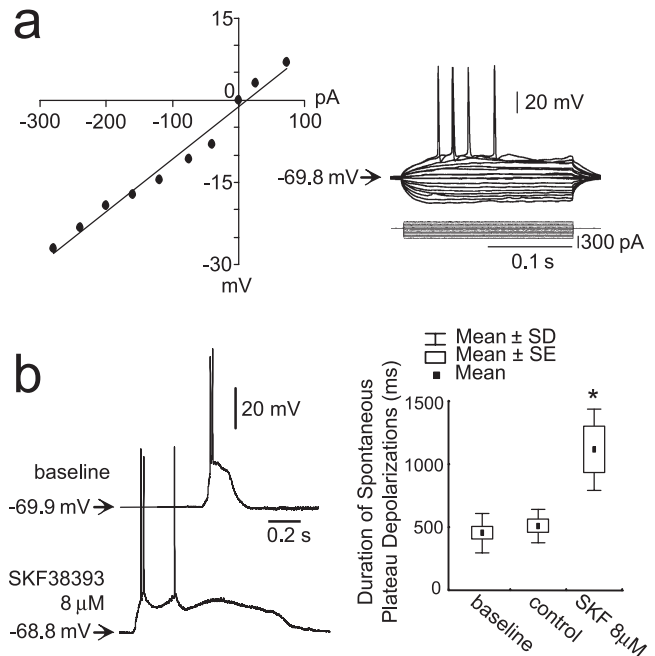


Figure 1. Spontaneous plateau depolarizations in adult PFC slices. (*a*) Representative current-voltage (*I/V*) plot (left) obtained from the responses to 200 ms intracellular current pulses (-300 pA to +100 pA) in a PFC pyramidal neuron recorded from an adult rat brain slice (P52). Current steps and voltage responses are overlaid at right. (*b*) Left: examples of sporadic spontaneous plateau depolarizations recorded in baseline conditions (top) and in the presence of the D₁ agonist SKF38393 (8 μM; bottom). Longer plateaus were observed with D₁ receptor activation [**P* < 0.01, compared with control; *F*(1,8) = 11.28, repeated measures ANOVA].

Spontaneous Plateau Depolarizations in Mature Prefrontal Cortical Slices

Spontaneous plateau depolarizations were observed sporadically during baseline recordings. These events lasted 452.9 ± 122.2 ms and occurred about once every 12 min (0.0014 ± 0.0004 Hz, *n* = 16). Spontaneous depolarizations were not observed in slices from immature animals (PD < 40, *n* = 26). Because neocortical pyramidal neurons undergo several changes in both morphological and physiological properties during postnatal development until PD 42 (Zhu, 2000), we interpreted the depolarizing events as resulting from critical glutamate activation in a mature cortical network. Bath-application of the D₁ agonist SKF38393 (8 μM) significantly increased the duration of plateau depolarizations in all cells tested (*n* = 4, Fig. 1*b*), supporting the idea that D₁ receptors may contribute to sustaining up states in PFC pyramidal neurons (Lewis and O'Donnell, 2000). The frequency of these events, however, remained unchanged in presence of the D₁ agonist (from 0.0013 ± 0.0004 to 0.0012 ± 0.0003; *P* = 0.7).

Given that a D₁ enhancement of NMDA-mediated responses has been observed in PFC pyramidal neurons (Wang and O'Donnell, 2001), we investigated whether combined administration of NMDA with a marginally effective dose of the D₁ agonist enhanced the occurrence of spontaneous plateau depolarizations. Bath-application of SKF38393 (2 μM) did not induce significant changes in resting membrane potential (from -69.2 ± 2.5 mV to -68.8 ± 2.9 mV; *n* = 8; Fig. 2*a*) or in cell excitability measured as the response to intracellular current injection (data not shown; higher agonist doses were required to increase excitability). In contrast, 12 of 14 cells treated with 8 μM NMDA (85%) exhibited a marked membrane depolarization

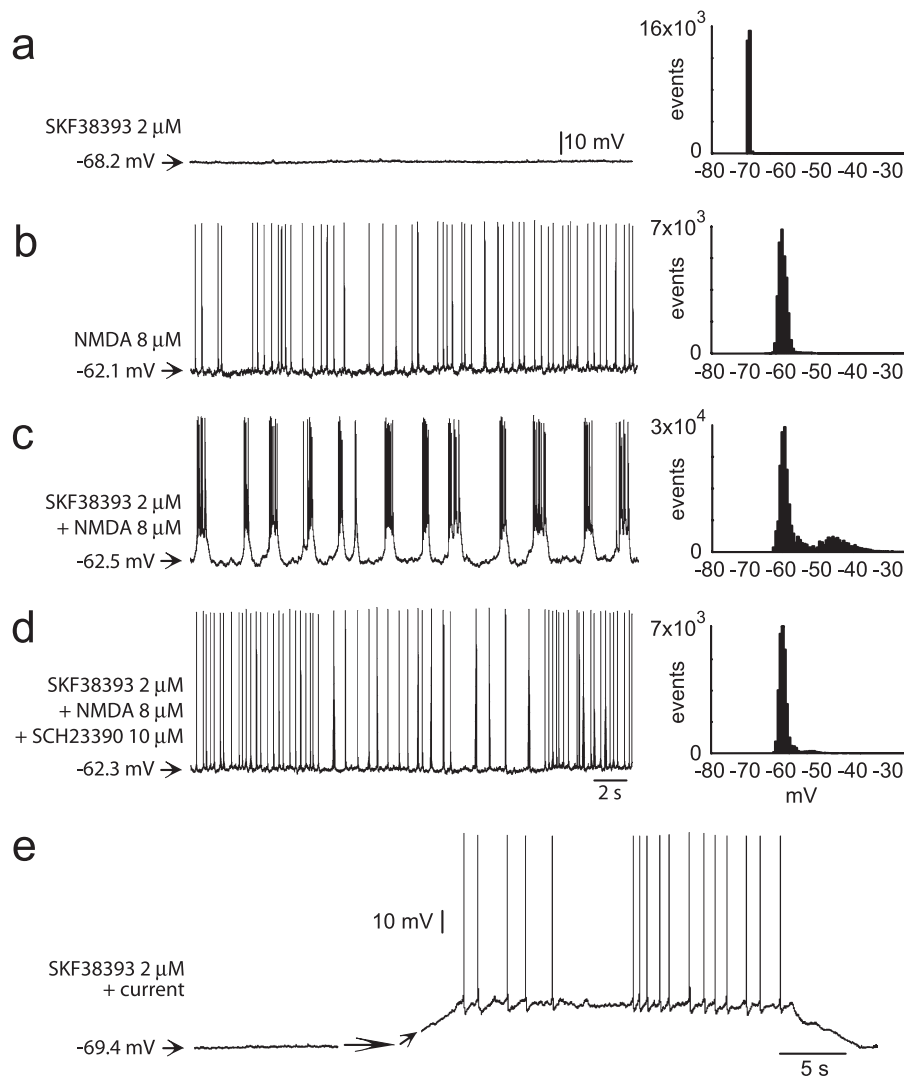


Figure 2. Enhanced frequency of plateau depolarizations with coadministration of NMDA and a D₁ agonist. (a) Left: tracing of spontaneous activity in the presence of 2 μ M SKF38393. Right: membrane potential histogram of the same trace revealing a very steady recording. (b) Trace and histogram showing a steady depolarization without plateaus in the presence of NMDA. (c) Combining the D₁ agonist with NMDA yielded frequent plateau depolarizations (left) that are reflected in a bimodal distribution in the histogram (right). (d) Trace and histogram showing absence of spontaneous plateaus when the D₁ antagonist SCH23390 (10 μ M) was present in the bath. (e) Tracings from a PFC neuron in the presence of the D₁ agonist (left) and after depolarizing the cell by intracellular current injection (right). No plateau depolarizations were observed.

(from -69.4 ± 3.1 mV to -62.3 ± 4.3 mV) yielding spontaneous cell firing (Fig. 2*b*). Combining SKF38393 (2 μ M) and NMDA (8 μ M) resulted in spontaneous depolarizing plateaus resembling the membrane potential fluctuations typically observed *in vivo* in 10 of 11 cells tested (90%; Fig. 2*c*). This proportion is significantly higher than what was observed in the presence of the D₁ agonist alone ($n = 0/8$, $P = 0.00012$, FET) or NMDA alone ($n = 2/12$, $P = 0.0002$, FET). The spontaneous plateaus recorded in the presence of SKF38393 + NMDA lasted 380.0 ± 169.3 ms ($P < 0.0003$ compared with NMDA: 102.2 ± 87.9 ms, Kruskal-Wallis ANOVA) and exhibited a frequency of 0.92 ± 0.58 Hz ($P < 0.00001$ compared with NMDA: 0.03 ± 0.08 Hz, Kruskal-Wallis ANOVA). The duration of these plateaus was not significantly different from those occasionally observed in control conditions. Pre-treatment with the D₁ antagonist SCH23390 (10 μ M) prevented the membrane potential oscillations when SKF38393 + NMDA were applied in six of eight cells tested ($P = 0.0044$ compared with NMDA + SKF38393, FET). What was left were the shorter (120.8 ± 118.5 ms; $P < 0.005$ compared with NMDA

+ SKF38393, Kruskal-Wallis ANOVA) sporadic (0.13 ± 0.21 Hz; $P < 0.002$ compared with NMDA + SKF38393, Kruskal-Wallis ANOVA) depolarizations resembling those recorded in control conditions. To address whether the increase in plateau depolarizations was dependent on NMDA receptor activation and not simply due to the depolarization elicited by NMDA, SKF38393 (2 μ M) was applied while the neuron was depolarized with somatic current injection instead of NMDA administration. Combining the D₁ agonist with positive current injection did not result in any depolarizing plateau, but elicited an increase in cell firing (Fig. 2*e*, $n = 6/6$). On the other hand, co-activation of D₁ and AMPA receptors (SKF38393 2 μ M + AMPA 0.4 μ M, $n = 7$) depolarized PFC pyramidal neurons causing cell firing, but not membrane potential oscillations (data not shown). These results indicate that D₁-NMDA receptor co-activation can induce spontaneous membrane potential fluctuations resembling up-down transitions in PFC pyramidal neurons.

Plateau depolarizations observed during D₁ + NMDA activation are typically accompanied by rapid action potential firing.

However, the threshold for action potential firing shifted to a more depolarized value (from -54.6 ± 2.5 mV to -51.5 ± 1.9 mV; $P < 0.005$, *t*-test; Fig. 3). This shift could be explained by an inhibitory action of D_1 receptors on Na^+ currents via PKA mediated phosphorylation of voltage-gated Na^+ channels (Cantrell and Catterall, 2001) or by an excitatory effect of D_1 receptors on interneurons yielding inhibitory postsynaptic currents that can in turn reduce pyramidal neuronal excitability (Zhou and Hablitz, 1999; Seamans *et al.*, 2001b; Gorelova *et al.*, 2002). Indeed, the membrane potential between plateaus in D_1 + NMDA activation is similar to that of NMDA alone (see Fig. 2*b,c*). Yet, at that membrane potential value NMDA causes rapid firing, but adding the D_1 agonist blocks firing. It is conceivable that the D_1 enhancement of postsynaptic NMDA actions is sufficient to periodically overcome this inhibition.

Plateau Depolarizations Induced by D_1 -NMDA Require Local Network Activity

D_1 + NMDA-induced membrane potential oscillations could reflect activity of a local neural network impinging on the recorded neuron. Holding the membrane potential to its baseline value failed to block plateau depolarizations induced by D_1 -NMDA in six of six cells tested (Fig. 4*a*), suggesting that voltage-dependent intrinsic currents may not be initiating the depolarizations (although they could still contribute to sustaining them). To investigate whether excitatory inputs arriving from other activated neurons contained in the slice were involved, subsets of experiments were conducted in the presence of the Na^+ channel blocker TTX or the AMPA receptor antagonist CNQX. Bath-application of TTX (0.05 μ M; Fig. 4*b*) or CNQX (10 μ M; Fig. 4*c*) prevented the D_1 + NMDA-induced membrane potential oscillations. Occasional plateau depolarizations were observed in only two of nine cells treated with TTX ($P = 0.0045$ compared with D_1 + NMDA, FET) and one of seven cells in presence of CNQX ($P = 0.0025$ compared with D_1 + NMDA, FET) every several minutes (~ 0.01 Hz). These results indicate that membrane potential oscillations induced by co-

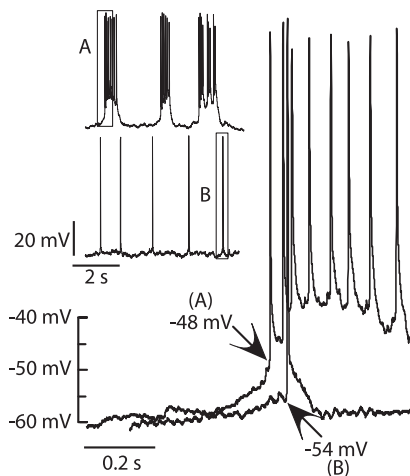


Figure 3. Action potential threshold shifts during spontaneous plateau depolarizations. Overlay of representative tracings obtained in the presence of the D_1 agonist and NMDA (A) and in the presence of NMDA alone (B). Arrows point to onset of first action potential in each trace. Insets show tracings at a slower time base, with the boxes indicating the portions of recording overlaid in the figure.

activation of D_1 and NMDA receptors in PFC pyramidal neurons may reflect the activity of a local neuronal network.

D_1 -NMDA-mediated Plateau Depolarizations Involve Intracellular Ca^{2+} , L-type Ca^{2+} Channels, Protein Kinase A and Voltage-gated Na^+ Channels

Several cellular mechanisms may also contribute to the depolarizing plateaus induced by D_1 -NMDA co-activation. For example, it has been shown that D_1 -NMDA synergism involves intracellular Ca^{2+} and activation of L-type Ca^{2+} channels (Cepeda and Levine, 1998; Cepeda *et al.*, 1998; Wang and O'Donnell, 2001). To test whether this applies to plateau depolarizations, a subset of recordings was conducted with electrodes containing the Ca^{2+} chelator BAPTA (2 mM) or in presence of nifedipine in the bath. Intracellular application of BAPTA ($n = 8$) reduced significantly the duration of plateau depolarizations (146.1 ± 109.6 ms; $P < 0.005$, compared with NMDA + SKF38393, Kruskal-Wallis ANOVA; Fig. 5*a*) to a value within the range of those observed in control conditions. The frequency of these depolarizations was also reduced to 0.07 ± 0.14 Hz ($P < 0.0006$ compared with NMDA + SKF38393, Kruskal-Wallis ANOVA). Similarly, bath application of nifedipine (10 μ M, $n = 7$) shortened the duration (184.5 ± 104.1 ms; $P < 0.02$ compared with NMDA + SKF38393, Kruskal-Wallis ANOVA) and decreased the frequency (0.19 ± 0.19 Hz, $P < 0.005$ compared with NMDA + SKF38393, Kruskal-Wallis ANOVA) of D_1 + NMDA-mediated plateau depolarizations (Fig. 5*b*). However, plateaus observed in the presence of nifedipine were more frequent than with normal aCSF. These results suggest that both intracellular Ca^{2+} and L-type Ca^{2+} channels may contribute to plateau depolarizations in PFC pyramidal neurons.

Second messenger cascades could also be involved in this interaction. D_1 receptors are coupled to the G protein G_s and

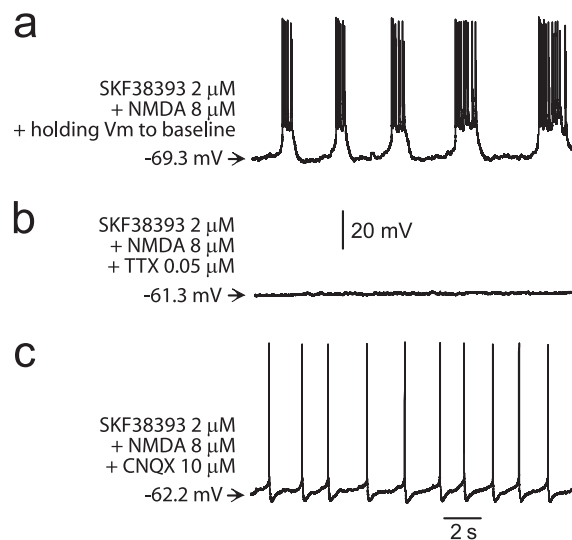


Figure 4. Plateau depolarizations induced by D_1 -NMDA activation require local network activity. (a) Representative tracing recorded in the presence of the D_1 agonist and NMDA with somatic current injection holding the membrane potential to baseline value. Hyperpolarizing the cell by intracellular current injection cannot abolish plateau depolarizations induced by D_1 + NMDA. (b) Tracing showing the absence of spontaneous plateaus when the D_1 agonist and NMDA were delivered with TTX. (c) Tracing obtained with bath application of the D_1 agonist SKF38393 and NMDA in presence of CNQX. The activity recorded is similar to that observed with NMDA alone.

adenylyl cyclase and their activation increases cyclic adenosine monophosphate (cAMP) formation and protein kinase A (PKA) activity (Surmeier *et al.*, 1995), stimulating the phosphoprotein DARPP-32, allowing phosphorylation of NMDA receptors (Snyder *et al.*, 1998). Indeed, synergistic interactions between NMDA and D₁ receptors in the PFC involve PKA (Wang and O'Donnell, 2001). Therefore, we tested whether D₁ + NMDA-dependent membrane potential oscillations in the PFC require a PKA-dependent cascade. Oscillations induced by co-activation of D₁ and NMDA receptors were prevented by application of a PKA inhibitor (KT-5720, 1 μM) in the bath in 9 of 10 cells tested (Fig. 5c). Plateau potentials had a duration of 107.6 ± 165.5 ms (*P* < 0.005, compared with NMDA + SKF38393, Kruskal-Wallis ANOVA) and their frequency was 0.03 ± 0.08 Hz (*P* < 0.0005 compared with NMDA + SKF38393, Kruskal-Wallis ANOVA). These results indicate that PKA mediates the D₁ + NMDA-induced membrane potential oscillations in PFC pyramidal neurons.

The blockade of plateau depolarizations with TTX does suggest that afferent activity may be needed, but it could also

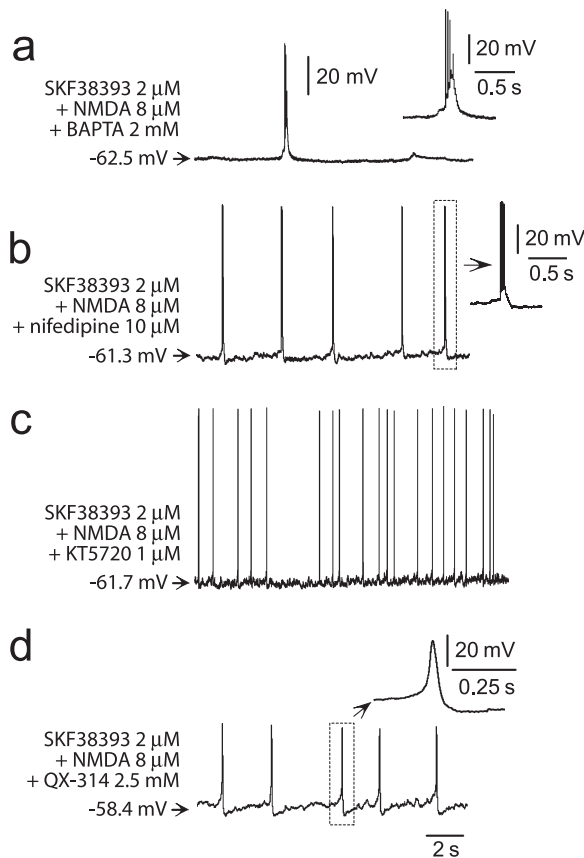


Figure 5. Plateau depolarizations induced by D₁-NMDA activation require calcium, PKA, and voltage-gated Na²⁺ channels. (a) Representative tracing from a neuron treated with the D₁ agonist and NMDA and being recorded with an electrode containing the Ca²⁺ chelator BAPTA. Spontaneous depolarizations were less frequent and shorter in duration, yielding short bursts of action potentials (inset). (b) Tracing showing short depolarizations (inset) with bath application of SKF38393, NMDA and the L-type Ca²⁺ channel antagonist nifedipine, (c) Tracing obtained in the presence of the D₁ agonist SKF38393, NMDA and a PKA inhibitor (KT 5720). The activity recorded is similar to that observed with NMDA alone. (d) Representative tracing recorded with electrodes containing the Na⁺ channel blocker QX-314 in the presence of the D₁ agonist and NMDA. Plateau depolarizations were replaced by 25 mV spikes resembling Ca²⁺ action potentials (Stafstrom *et al.*, 1985).

be explained by the participation of persistent Na⁺ currents. These currents are important for synaptic integration in pyramidal neurons (Crill, 1996; Yang *et al.*, 1996). To test whether slowly inactivating voltage-gated Na⁺ currents are required for plateau depolarizations, a subset of recordings was conducted with electrodes containing the Na⁺ channel blocker QX-314 (2.5 mM). Depolarizing plateaus were observed in only one of seven cells tested (*P* = 0.0012, compared with SKF38393 + NMDA, FET). Short spontaneous depolarizations resembling Ca²⁺ spikes (Stafstrom *et al.*, 1985) were observed instead of plateau depolarizations in the remaining six neurons. These depolarizations lasted 73.3 ± 82.1 ms (*P* < 0.002, compared with SKF38393 + NMDA, Kruskal-Wallis ANOVA; Fig. 5d) and exhibited a frequency of 0.61 ± 0.82 Hz. Thus, although slow Ca²⁺ events can be evoked by D₁ + NMDA when Na⁺ channels are blocked in the recorded neuron, plateau depolarizations were not observed. This suggests that slowly inactivating voltage-gated Na⁺ channels could contribute to sustaining synaptically induced depolarizations, although a role of back-propagating dendritic Na⁺ spikes cannot be ruled out. Figure 6 illustrates the frequency of plateau depolarizations in all treatment groups.

D₁-NMDA-mediated Plateau Depolarizations Require a Mature PFC

To determine the age at which plateau depolarizations elicited by D₁-NMDA interactions emerge, an additional set of experiments were conducted in slices from pre-pubertal rats. Eighteen PFC pyramidal neurons were recorded and displayed a negative resting membrane potential (PD 29–31, -65.5 ± 2.7 mV, *n* = 9; PD 36–38, -67.9 ± 2.4 mV, *n* = 9). None of them exhibited spontaneous action potential firing, but action potentials could be evoked in response to somatic depolarizing current injection (not shown). The sporadic plateau depolarizations

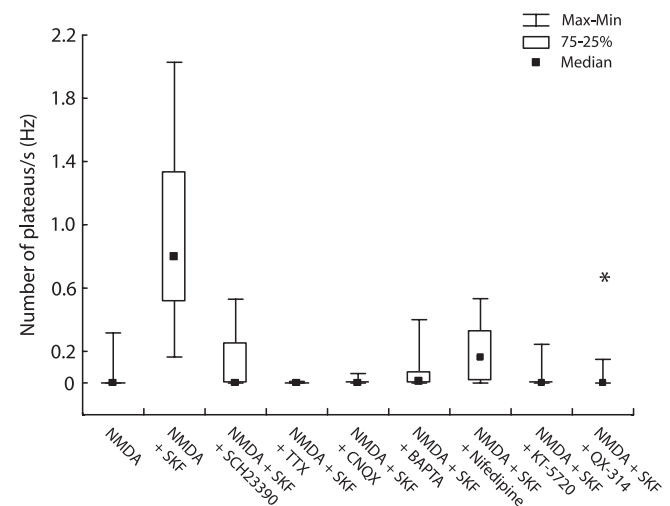


Figure 6. Box plots summarizing the frequency of plateau depolarizations observed with different pharmacological treatments. All data showed were obtained from the 2–3 min period following bath application of SKF38393 and NMDA. Only combining the D₁ agonist SKF38393 with NMDA resulted in depolarizing plateau fluctuations at ~0.8 Hz. When the experiments were conducted with electrodes containing the Na⁺ channel blocker QX-314 (2.5 mM), short depolarizations resembling Ca²⁺ spikes were observed instead of plateaus in six of seven cells tested. These Ca²⁺ spikes occur at ~0.6 Hz, similar to the frequency of plateau depolarizations induced by D₁ + NMDA and this is indicated in the figure with an asterisk.

observed during baseline recordings in brain slices obtained from adult animals could not be detected in these younger slices. Bath application of the D₁ agonist SKF38393 (2 μM) and NMDA (8 μM) resulted in membrane depolarization (PD 29–31, -57.9 ± 3.9 mV; PD 36–38, -59.3 ± 4.0 mV) with spontaneous action potential firing (Fig. 7), but failed to induce plateau depolarizations. Instead, short depolarizations were observed (PD 29–31, 41.6 ± 50.3 ms; PD 36–38, 51.4 ± 66.2 ms; *P* < 0.0001 compared with PD > 45, Kruskal-Wallis ANOVA; Fig. 7). A regression of all data points at all ages indicates that adult levels of plateau duration were achieved at around PD 45 (Fig. 7*a*). These findings indicate that the D₁ + NMDA-mediated plateau depolarizations occur in a mature, post-pubertal PFC.

Discussion

D₁-NMDA co-activation elicited plateau depolarizations resembling *in vivo* up states in slices obtained from adult animals. PFC pyramidal neurons exhibited a very stable and negative membrane potential with very sporadic plateau depolarizations that were increased in duration by a D₁ agonist. Combining the D₁ agonist with NMDA resulted in repeated plateau depolarizations in the PFC of post-pubertal, but not younger animals, that resembled *in vivo* up states, which were observed. These recurrent depolarizations required intracellular Ca²⁺, L-type Ca²⁺ channels and PKA signalling. Interrupting synaptic activity

with bath-application of TTX or CNQX blocked these plateaus. These events were also eliminated by intracellular administration of the Na⁺ channel blocker QX-314. Thus, co-activation of D₁ and NMDA receptors could enhance the activity of a mature PFC to the point of causing spontaneous depolarizing plateaus in individual neurons, an effect that may be mediated by both intrinsic and synaptic mechanisms.

Neocortical pyramidal neurons can exhibit persistent depolarizing activity *in vitro*. Recordings from visual and prefrontal cortices in mice and ferrets revealed plateau depolarizations when a bath solution that reflects more closely the K⁺ concentration of brain interstitial fluid *in situ* was used (Sanchez-Vives and McCormick, 2000; Cossart *et al.*, 2003). This persistent activity seems to be driven by local synaptic activity and intracellular Ca²⁺ increase, as indicated by Ca²⁺ transients observed during the depolarizations (Cossart *et al.*, 2003), probably reflecting activation of somatic voltage-sensitive Ca²⁺ channels (Smetters *et al.*, 1999; Mao *et al.*, 2001; Cossart *et al.*, 2003). The D₁ + NMDA-mediated plateau depolarizations reported here were prevented by interrupting local synaptic activity and shortened by adding the Ca²⁺ chelator BAPTA or the Na⁺ channel blocker QX-314 in the recording electrodes. These events were also shortened with bath application of the L-type Ca²⁺ channel blocker nifedipine. Thus, it seems likely that the cellular and synaptic mechanisms involved in these recurrent depolarizations induced by D₁ + NMDA interactions in the PFC are similar to those observed in the visual cortex (Mao *et al.*, 2001; Cossart *et al.*, 2003), involving a combination of increased network activity driving synaptic responses and slow intrinsic currents in pyramidal neurons sustaining the depolarizations.

The precise cellular or network mechanisms involved in generating and sustaining these *in vitro* depolarizations or the *in vivo* up states are not clear. Computational models have shown that increasing NMDA and GABA conductances by D₁ receptors could lead to persistent activity (Durstewitz *et al.*, 2000; Brunel and Wang, 2001) and that these interactions may play an important role in cognitive functions (for a review, see Cohen *et al.*, 2002). Even in control conditions, plateau depolarizations were sporadically observed in mature brain slices. In presence of the D₁ agonist, these events lasted longer. This suggests that there could be some active neurons in the slice in baseline conditions that may occasionally reach a critical level of firing, yielding spontaneous plateaus. If D₁ receptors are activated, these plateaus may increase their duration, probably via an enhancement of NMDA responses (Wang and O'Donnell, 2001). It has been suggested that plateau potentials may reflect both action potential backpropagation and NMDA receptor activity (Kerr and Plenz, 2002). Thus, it is possible that Ca²⁺ transients driven by action potential backpropagation mediate recurrent depolarizations induced by D₁ + NMDA interactions in the mature PFC. Although action potential backpropagation in an immature PFC (PD 21–35) is not affected by DA (Gulledge and Stuart, 2003), the possibility of a DA modulation of backpropagation in the PFC remains to be explored in mature animals. A D₁ + NMDA synergism would cause an enhanced level of activation in many pyramidal neurons in the recorded slice. Their activation would in turn increase synaptic drive of neighboring neurons (Beggs and Plenz, 2003), with the potential consequence of synaptic currents and slowly inactivating conductances sustaining the depolarization. The repeated depolarizations observed could therefore be a consequence of combination of network and cellular events.

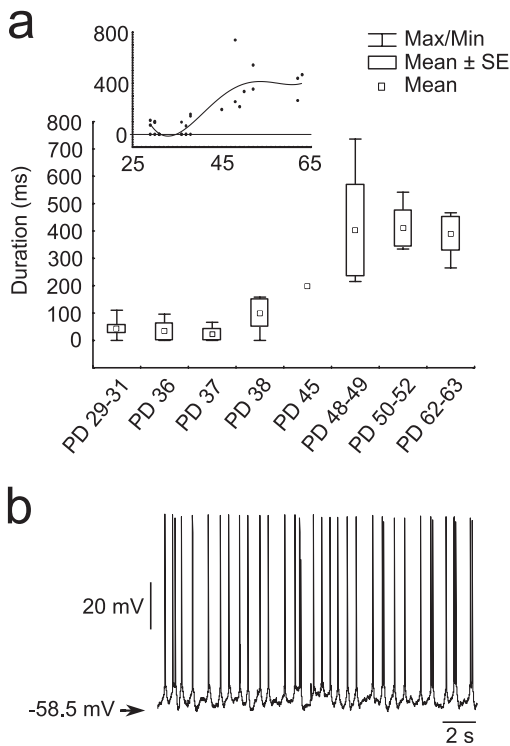


Figure 7. Spontaneous plateau depolarizations induced by co-activation of D₁-NMDA receptors in the medial PFC cannot be observed in pre-pubertal (PD 29–38) animals. (*a*) Box plots summarizing the duration of D₁-NMDA-induced depolarizing plateaus recorded in PFC pyramidal neurons from pre-pubertal (PD 29–38) and adult (PD > 45) rat brain slices. Inset shows a second-order polynomial regression for all data points summarized in *a*. (*b*) Representative tracing of a PFC pyramidal neuron recorded from a PD 36 rat brain slice in presence of the D₁ agonist SKF38393 (2 μM) and NMDA (8 μM).

The appearance of D₁ + NMDA-dependent membrane potential oscillations was prevented by impairing Ca²⁺ and PKA signalling as well as by blocking L-type Ca²⁺ channels. This is in line with recent data obtained from striatal and cortical slices indicating that D₁ receptors enhance NMDA responses (Cepeda and Levine, 1998; Cepeda *et al.*, 1998; Wang and O'Donnell, 2001) through a signalling cascade involving PKA activity (Surmeier *et al.*, 1995) and phosphorylation of DARPP-32 (Snyder *et al.*, 1998; Flores-Hernandez *et al.*, 2002). The observed increase in NMDA currents could be due to phosphorylation of NMDA receptors or events downstream to these receptors. Activation of the cAMP-PKA-DARPP32 pathway can also enhance Ca²⁺ currents (Surmeier *et al.*, 1995). Striatal neurons recorded from slices can exhibit sustained depolarization in response to intracellular current injection via a D₁ enhancement of L-type Ca²⁺ currents (Hernandez-Lopez *et al.*, 1997). It has been recently shown that D₁ receptors can enhance synaptically evoked NMDA currents in PFC pyramidal neurons from pre-pubertal rats (Seamans *et al.*, 2001a). In addition, D₁ receptors facilitate trafficking of striatal NMDA receptors to the postsynaptic membrane (Dunah and Standaert, 2001). Although there is not evidence linking D₁ receptors and trafficking of NMDA receptor subunits in neocortical pyramidal neurons, it is possible that a similar mechanism also plays a role in enhancing NMDA responses in the PFC. Thus, a number of intracellular events can contribute to a D₁-mediated enhancement of NMDA function.

The PFC plateau depolarizations reported here are not cortical paroxysmal discharges. Paroxysmal activity can be induced in neocortical brain slices from immature (PD 9–30) animals with bath application of high concentration of the GABA_A antagonists bicuculline or picrotoxin (Hablitz, 1987; Sutor *et al.*, 1994). D₁ + NMDA-mediated plateau depolarizations, on the other hand, require a mature cortical network. Moreover, paroxysmal cortical activity consists of depolarizing events of much longer duration with action potentials of progressively smaller amplitude (Rubinstein *et al.*, 2001). Thus, persistent activity elicited with D₁ and NMDA in mature PFC slices is likely to derive from activation of an assembly of cortical neurons (Beggs and Plenz, 2003), but not from exaggerated hyperexcitability.

It is well known that activation of the mesolimbic/mesocortical dopaminergic system is context-dependent and related to attention and salient stimuli (Horvitz, 2000; Cohen *et al.*, 2002; Schultz, 2002). A D₁-sustained plateau depolarization would therefore provide a temporal window during which context-relevant inputs can drive the system (O'Donnell, 2003) and NMDA-dependent synaptic plasticity would be enabled. Indeed, hippocampal-PFC long-term potentiation (LTP) is enhanced by activation of D₁, but not D₂, receptors (Gurden *et al.*, 2000). The dependence of LTP on an intact mesocortical projection is further evidenced by its absence in animals with DA depletion (Gurden *et al.*, 1999). Thus, cognitive functions such as working memory, learning and attention would be supported by this D₁-NMDA interaction. This concept is further supported by D₁ and NMDA receptors in the PFC being necessary for appetitive instrumental learning (Baldwin *et al.*, 2002) and by PFC D₁ receptors improving memory retrieval (Floresco and Phillips, 2001). It is possible that when mesocortical DA systems become active in response to salient stimuli, the relevant ongoing activity in the PFC (i.e. that mediated by NMDA receptors) becomes enhanced and reinforced by setting and maintaining

a population of neurons into the up state via activation of local D₁ receptors. Up states therefore are active periods of information processing that contribute to learning and cognitive functions (Cossart *et al.*, 2003; O'Donnell, 2003) and they may depend on potentiation of NMDA function by a number of mechanisms (including, but probably not limited to D₁ receptor activation) that ultimately enhance PKA activity and Ca²⁺ signalling.

The plateau depolarizations elicited by D₁-NMDA co-activation were observed only in slices from adult rats in our experimental conditions. It is possible that higher D₁ + NMDA concentrations could yield up states in PFC slices from younger animals, because it is well known that both D₁ and NMDA receptors change during postnatal development. But even if this were the case, it would still suggest that D₁-NMDA interactions impact differently on PFC activity depending on the level of maturation of the system, with prepubertal PFC neurons being less likely to exhibit strong plateaus. Indeed, *in vivo* intracellular recordings from pre-pubertal animals (PD 28–35) have shown PFC up states of smaller amplitude than similar recordings obtained from adult animals (O'Donnell *et al.*, 2002). Although neocortical neurons in pre-pubertal animals have many morphological and physiological properties of mature cells and most cortical synapses have been formed at pre-pubertal (PD 28–40) ages (Petit *et al.*, 1988; Zhu, 2000), there is also data suggesting that some elements in pyramidal cell physiology mature later. For example, dendritic Na⁺ and Ca²⁺ regenerative potentials (which may be important for persistent activity) become effective in coupling distal apical dendrites with somata at PD 42 (Zhu, 2000). Therefore, a post-pubertal appearance of persistent activity in the PFC may derive from a late maturation of intrinsic physiological properties in pyramidal neurons. Other factors that may play a critical role in converting pre-pubertal cortical synaptic organization into a mature network include the delayed acquisition of adult levels of D₁ dopamine receptors (Leslie *et al.*, 1991; Tarazi *et al.*, 1999) and NMDA receptor subunits (Williams *et al.*, 1993; Monyer *et al.*, 1994). The emergence of D₁ + NMDA-mediated persistent activity after puberty may contribute to developing and acquiring mature cognitive abilities in the PFC.

Notes

Supported by MH57683 and a NARSAD Independent Investigator Award (P.O'D.).

Address correspondence to Kuei-Yuan Tseng, Albany Medical College (MC-136), Center for Neuropharmacology and Neuroscience, Albany, NY 12208, USA. Email: tsengky@mail.amc.edu.

References

- Baldwin AE, Sadeghian K, Kelley AE (2002) Appetitive instrumental learning requires coincident activation of NMDA and dopamine D₁ receptors within the medial prefrontal cortex. *J Neurosci* 22:1063–1071.
- Beggs JM, Plenz D (2003) Neuronal avalanches in neocortical circuits. *J Neurosci* 23:11167–11177.
- Branchereau P, Van Bockstaele EJ, Chan J, Pickel VM (1996) Pyramidal neurons in rat prefrontal cortex show a complex synaptic response to single electrical stimulation of the locus coeruleus region: evidence for antidromic activation and GABAergic inhibition using *in vivo* intracellular recording and electron microscopy. *Synapse* 22:313–331.
- Brunel N, Wang XJ (2001) Effects of neuromodulation in a cortical network model of object working memory dominated by recurrent inhibition. *J Comput Neurosci* 11:63–85.

- Cantrell AR, Catterall WA (2001) Neuromodulation of Na⁺ channels: an unexpected form of cellular plasticity. *Nat Rev Neurosci* 2:397-407.
- Cepeda C, Levine MS (1998) Dopamine and *N*-methyl-D-aspartate receptor interactions in the neostriatum. *Dev Neurosci* 22:1-18.
- Cepeda C, Colwell CS, Itri JN, Chandler SH, Levine MS (1998) Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. *J Neurophysiol* 79:82-94.
- Cohen JD, Braver TS, Brown JW (2002) Computational perspectives on dopamine function in prefrontal cortex. *Curr Opin Neurobiol* 12:223-229.
- Cossart R, Aronov D, Yuste R (2003) Attractor dynamics of network UP states in the neocortex. *Nature* 423:283-288.
- Crill WE (1996) Persistent sodium current in mammalian central neurons. *Annu Rev Physiol* 58:349-362.
- Dunah AW, Standaert DG (2001) Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. *J Neurosci* 21:5546-5558.
- Durstewitz D, Seamans JK, Sejnowski TJ (2000) Neurocomputational models of working memory. *Nat Neurosci* 3(Suppl.):1184-1191.
- Floresco SB, Phillips AG (2001) Delay-dependent modulation of memory retrieval by infusion of a dopamine D₁ agonist into the rat medial prefrontal cortex. *Behav Neurosci* 115:934-939.
- Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, Calvert CR, Jokel ES, Fienberg AA, Greengard P, Levine MS (2002) Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. *J Neurophysiol* 88:3010-3020.
- Gonzalez-Islas C, Hablitz JJ (2003) Dopamine enhances EPSCs in layer II-III pyramidal neurons in rat prefrontal cortex. *J Neurosci* 23:867-875.
- Gorelova N, Seamans JK, Yang CR (2002) Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J Neurophysiol* 88:3150-3166.
- Goto Y, O'Donnell P (2001) Synchronous activity in the hippocampus and nucleus accumbens *in vivo*. *J Neurosci* 21(RC131):1-5.
- Gulledge AT, Stuart GJ (2003) Action potential initiation and propagation in layer 5 pyramidal neurons of the rat prefrontal cortex: absence of dopamine modulation. *J Neurosci* 23:11363-11372.
- Gurden H, Tassin JP, Jay TM (1999) Integrity of the mesocortical dopaminergic system is necessary for complete expression of *in vivo* hippocampal-prefrontal cortex long-term potentiation. *Neuroscience* 94:1019-1027.
- Gurden H, Takita M, Jay TM (2000) Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses *in vivo*. *J Neurosci* 20(RC106):1-5.
- Hablitz JJ (1987) Spontaneous ictal-like discharges and sustained potential shifts in the developing rat neocortex. *J Neurophysiol* 58:1052-1065.
- Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E (1997) D₁ receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca²⁺ conductance. *J Neurosci* 17:3334-3342.
- Horvitz JC (2000) Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience* 96:651-656.
- Kerr JN, Plenz D (2002) Dendritic calcium encodes striatal neuron output during up-states. *J Neurosci* 22:1499-1512.
- Leslie CA, Robertson MW, Cutler AJ, Bennett JP Jr (1991) Postnatal development of D1 dopamine receptors in the medial prefrontal cortex, striatum and nucleus accumbens of normal and neonatal 6-hydroxydopamine treated rats: a quantitative autoradiographic analysis. *Dev Brain Res* 62:109-114.
- Lewis BL, O'Donnell P (2000) Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D₁ dopamine receptors. *Cereb Cortex* 10:1168-1175.
- Linden DJ (1999) The return of the spike: postsynaptic action potentials and the induction of LTP and LTD. *Neuron* 22:661-666.
- Mahon S, Deniau JM, Charpier S (2001) Relationship between EEG potentials and intracellular activity of striatal and cortico-striatal neurons: an *In vivo* study under different anesthetics. *Cereb Cortex* 11:360-373.
- Mao BQ, Hamzei-Sichani F, Aronov D, Froemke RC, Yuste R (2001) Dynamics of spontaneous activity in neocortical slices. *Neuron* 32:883-898.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12:529-540.
- O'Donnell P (2003) Dopamine gating of forebrain neural ensembles. *Eur J Neurosci* 17:429-435.
- O'Donnell P, Grace AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. *J Neurosci* 15:3622-3639.
- O'Donnell P, Lewis BL, Weinberger DR, Lipska BK (2002) Neonatal hippocampal damage alters electrophysiological properties of prefrontal cortical neurons in adult rats. *Cereb Cortex* 12:975-982.
- Petersen CC, Hahn TT, Mehta M, Grinvald A, Sakmann B (2003) Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. *Proc Natl Acad Sci USA* 100:13638-13643.
- Petit TL, LeBoutillier JC, Gregorio A, Libstug H (1988) The pattern of dendritic development in the cerebral cortex of the rat. *Brain Res* 469:209-219.
- Rubinstein M, Cepeda C, Hurst RS, Flores-Hernandez J, Ariano MA, Falzone TL, Kozell LB, Meshul CK, Bunzow JR, Low MJ, Levine MS, Grandy DK (2001) Dopamine D₄ receptor-deficient mice display cortical hyperexcitability. *J Neurosci* 21:3756-3763.
- Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci* 3:1027-1034.
- Schultz W (2002) Getting formal with dopamine and reward. *Neuron* 36:241-263.
- Seamans JK, Durstewitz D, Christie BR, Stevens CF, Sejnowski TJ (2001a) Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. *Proc Natl Acad Sci USA* 98:301-306.
- Seamans JK, Gorelova N, Durstewitz D, Yang CR (2001b) Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J Neurosci* 21:3628-3638.
- Smetters D, Majewska A, Yuste R (1999) Detecting action potentials in neuronal populations with calcium imaging. *Methods* 18:215-221.
- Snyder GL, Fienberg AA, Huganir RL, Greengard P (1998) A dopamine/D₁ receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J Neurosci* 18:10297-10303.
- Spear LP (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav* 24:417-463.
- Stafstrom CE, Schwindt PC, Chubb MC, Crill WE (1985) Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex *in vitro*. *J Neurophysiol* 53:153-1570.
- Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol* 85:1969-1985.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* 367:69-72.
- Surmeier DJ, Bargas J, Hemmings HC, Nairn AC, Greengard P (1995) Modulation of calcium currents by a D₁ dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14:385-397.
- Sutor B, Hablitz JJ, Rucker F, ten Bruggencate G (1994) Spread of epileptiform activity in the immature rat neocortex studied with voltage-sensitive dyes and laser scanning microscopy. *J Neurophysiol* 72:1756-1768.
- Tarazi FI, Tomasini EC, Baldessarini RJ (1999) Postnatal development of dopamine D1-like receptors in rat cortical and striatolimbic brain regions: an autoradiographic study. *Dev Neurosci* 21:43-49.
- Wang J, O'Donnell P (2001) D₁ dopamine receptors potentiate NMDA-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. *Cereb Cortex* 11:452-462.

- Williams K, Russell SL, Shen YM, Molinoff PB (1993) Developmental switch in the expression of NMDA receptors occurs *in vivo* and *in vitro*. *Neuron* 10:267-278.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J Neurosci* 16:2397-2410.
- Yang CR, Seamans JK, Gorelova N (1996) Electrophysiological and morphological properties of layers V-VI principal pyramidal cells in prefrontal cortex *in vitro*. *J Neurosci* 16:1904-1921.
- Yang CR, Seamans JK, Gorelova N (1999) Developing a neuronal model for the pathophysiology of schizophrenia based on the nature of electrophysiological actions of dopamine in the prefrontal cortex. *Neuropsychopharmacology* 21:161-194.
- Zheng P, Zhang XX, Bunney BS, Shi WX (1999) Opposite modulation of cortical *N*-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. *Neuroscience* 91:527-535.
- Zhou FM, Hablitz JJ (1999) Dopamine modulation of membrane and synaptic properties of interneurons in rat cerebral cortex. *J Neurophysiol* 81:967-976.
- Zhu JJ (2000) Maturation of layer 5 neocortical pyramidal neurons: amplifying salient layer 1 and layer 4 inputs by Ca²⁺ action potentials in adult rat tuft dendrites. *J Physiol* 526:571-587.