Dopaminergic modulation of short-term synaptic plasticity in fast spiking interneurons of primate dorsolateral prefrontal cortex

Authors:

G. Gonzalez-Burgos¹, S. Kroener²,#, J.K. Seamans³, D.A. Lewis¹,², G. Barrionuevo²

Affiliations:

1: Department of Psychiatry, University of Pittsburgh.
2: Department of Neuroscience, University of Pittsburgh.
3: Department of Physiology and Neuroscience, Medical University of South Carolina
#: Present address: Department of Physiology and Neuroscience, Medical University of South Carolina

Running head: Dopamine and short-term plasticity in fast spiking cells

Contact information: G. Gonzalez-Burgos, Translational Neuroscience Program, Department of Psychiatry, University of Pittsburgh School of Medicine, Room W1651 Biomedical Science Tower, 3811 O'Hara St Pittsburgh, PA 15213. E-mail: gburgos@pitt.edu
Abstract

Dopaminergic regulation of primate dorsolateral prefrontal cortex (PFC) activity is essential for cognitive functions such as working memory. However, the cellular mechanisms of dopamine neuromodulation in PFC are not well understood. We have studied the effects of dopamine receptor activation during persistent stimulation of excitatory inputs onto fast-spiking GABAergic interneurons in monkey PFC. Stimulation at 20 Hz induced short-term EPSP depression. The D1 receptor agonist SKF81297 (5 µM) significantly reduced the amplitude of the first EPSP, but not of subsequent responses in EPSP trains, which still displayed significant depression. DA (10 µM) effects were similar to those of SKF81297, and were abolished by the D1 antagonist SCH23390 (5 µM), indicating a D1 receptor-mediated effect. DA did not alter miniature EPSCs, suggesting that its effects were activity- and presynaptic action potential-dependent. In contrast to previous findings in pyramidal neurons, in fast-spiking cells contribution of NMDA receptors to EPSPs at subthreshold potentials was not significant, and fast-spiking cell depolarization decreased EPSP duration. In addition, DA had no significant effects on temporal summation. The selective decrease in the amplitude of the first EPSP in trains delivered every 10 seconds suggests that in FS neurons DA reduces the amplitude of EPSPs evoked at low frequency, but not of EPSPs evoked by repetitive stimulation. DA may therefore improve detection of EPSP bursts above background synaptic activity. EPSP bursts displaying short-term depression may transmit spike-timing dependent temporal codes contained in presynaptic spike trains. Thus, DA neuromodulation may increase the signal-to-noise ratio at fast-spiking cell inputs.

Keywords: EPSP, short-term depression, D1 receptors, NMDA, temporal summation, GABAergic
Dopaminergic regulation of prefrontal cortex (PFC) activity is essential for cognitive functions such as working memory (Goldman-Rakic et al., 2004). Dopamine (DA) neurons innervate the primate PFC (Lewis et al., 1987; Williams and Goldman-Rakic, 1998) and release DA during working memory tasks (Watanabe et al., 1997). Activation of D1-like receptors is necessary for persistent firing in PFC (Sawaguchi, 2001; Williams and Goldman-Rakic, 1995), which is thought to be the cellular basis of information storage in working memory (Fuster, 1997). While DA appears to be essential for working memory, the cellular mechanisms of DA action in PFC are not well understood. In vitro, DA enhances the response of monkey PFC pyramidal neurons to injection of excitatory current. However, DA does not elicit pyramidal neuron firing in the absence of stimuli, and firing induced by depolarizing current pulses is not sustained after the stimulus ceases, in the presence or absence of DA (Gonzalez-Burgos et al., 2002; Henze et al., 2000). Thus, DA modulation of pyramidal cell excitability is not sufficient to produce persistent activity. Indeed computational models have suggested that the effects of DA on persistent activity may involve modulation of synaptic strength (Brunel and Wang, 2001; Durstewitz et al., 2000; Fellous and Sejnowski, 2003; Wang, 2001).

In PFC neurons, DA modulates the amplitude of EPSPs evoked by single stimuli (Gao et al., 2001; Gao and Goldman-Rakic, 2003; Gonzalez-Islas and Hablitz, 2003; Law-Tho et al., 1994; Seamans et al., 2001; Urban et al., 2002). However, persistent stimulation causes short-term synaptic plasticity, and thus EPSPs evoked by temporally isolated stimuli provide limited information on synaptic interactions during sustained activity. In PFC pyramidal neurons, stimulus trains elicit short-term EPSP depression (Gonzalez-Burgos et al., 2004; Hempel et al., 2000; Seamans et al., 2001). Synapses with short-term depression are not optimized to support persistent postsynaptic firing during persistent synaptic input, because their efficacy decreases markedly shortly after the onset of a presynaptic spike train (Pouille and Scanziani, 2004). However, recent data suggest that in PFC pyramidal neurons DA modulation counteracts EPSP depression by decreasing the initial EPSPs and potentiating the late postsynaptic responses in EPSP trains elicited by sustained presynaptic stimulation (Seamans et al., 2001). This effect of DA may contribute to the recurrent excitation thought to underlie sustained firing in local circuits of PFC (Durstewitz et al., 2000). Because recurrent excitation
requires inhibitory control, the effects of DA in PFC circuits may involve GABAergic neurons, in addition to pyramidal cells (Brunel and Wang, 2001; Durstewitz et al., 2000).

Inhibitory GABA neurons in monkey PFC include two electrophysiological subgroups: fast-spiking (FS) and non-FS cells (Gonzalez-Burgos et al., 2004; Gonzalez-Burgos et al., 2005). The electrophysiological properties of FS neurons in monkey PFC (Gonzalez-Burgos et al., 2004; Gonzalez-Burgos et al., 2005; Zaitsev et al., 2005) are similar to those described for FS cells in rat neocortex. In the frontal cortex of either rats or macaque monkeys, FS interneurons selectively express the calcium-binding protein parvalbumin (Kawaguchi and Kubota, 1997; Zaitsev et al., 2005). Compared to other interneuron classes, parvalbumin-containing FS cells express a higher density of DA receptors (Le Moine and Gaspar, 1998; Muly et al., 1998; Paspalas and Goldman-Rakic, 2005), are preferentially innervated by DA fibers (Sesack et al., 1995; Sesack et al., 1998) and selectively respond to DA receptor activation (Gorelova et al., 2002; Porter et al., 1999). Thus, FS cells may be the main interneuron target of DA in the PFC.

In monkey PFC, excitatory inputs onto FS cells and inhibitory connections made by FS neurons onto nearby pyramidal cells display short-term depression (Gonzalez-Burgos et al., 2004; Gonzalez-Burgos et al., 2005). Short-term depression decreases the efficiency of synaptic transmission during sustained activation and thus to encode the presynaptic firing rate \textit{per se}. However, synaptic depression enhances the ability to encode changes in presynaptic firing rate (Abbott et al., 1997; Tsodyks and Markram, 1997). Thus, the functional properties of their synaptic inputs and outputs suggest that FS cells in monkey PFC may constitute an inhibitory neuron system specialized to transiently signal changes in firing frequency (Beierlein et al., 2003; Pouille and Scanziani, 2004). However, DA may counteract short-term depression of excitatory inputs onto FS neurons, as it does in pyramidal cells (Seamans et al., 2001). If so, DA would enable FS neurons to produce sustained inhibition during persistent network activity. Here, we determined the effects of DA on short-term depression of excitatory synaptic inputs onto FS interneurons in monkey PFC.
Methods

Slice preparation and electrophysiological recording

All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Slices were prepared from tissue blocks containing portions of the dorsolateral PFC (areas 9 and 46) of young adult macaque monkeys, as described in detail elsewhere (Gonzalez-Burgos et al., 2004). Experiments were conducted in 43 slices obtained from 11 animals. Slices obtained from the dorsolateral PFC of these same animals were used to collect data for two other studies (Gonzalez-Burgos et al., 2004; Gonzalez-Burgos et al., 2005). For recording, slices were placed in a submersion chamber and superfused at 32-33°C with an oxygenated solution of the following composition (in mM): 126 NaCl, 2.5 KCl, 1.2 Na₂HPO₄, 25 NaHCO₃, 2.0 CaCl₂, 1.0 MgCl₂, 10 glucose. Voltage recordings were obtained with patch pipettes, from neurons identified visually in layers 2/3 of either the medial or lateral bank of the principal sulcus (area 46), using infrared differential interference contrast video microscopy. Recordings were obtained using Axoclamp 2B (Axon Instruments Inc, Union City, CA) or BVC700A (Dagan Corporation, Minneapolis, MN) amplifiers operating in current clamp mode, using bridge balance and pipette capacitance compensation. Pipettes had a resistance of 4-6 MOhm when filled with a solution of the following composition (in mM): 120 KMethylsulphate, 10 HEPES, 0.2 EGTA, 4.5 ATP, 0.3 GTP, 14 phosphocreatine and 0.5 % biocytin, pH adjusted to 7.2-7.3. Focal stimulation was applied using theta glass pipettes pulled to a tip diameter or 3-5 µm and filled with extracellular solution. A chlorided silver wire was inserted into each compartment of the theta glass stimulation pipette and connected to a stimulus isolation unit. External trigger pulses were generated by a computer-controlled data acquisition board and were used to command the stimulus isolation unit. Focal stimulation was used to elicit small EPSPs (mean amplitude smaller than 5 mV) in the presence of 10 µM bicuculline (Gonzalez-Burgos et al., 2004). The duration of the stimulus pulses was 100 µsec and the stimulation current intensity varied from ~20 to ~ 150 µA. Stimulation parameters were adjusted in each experiment to elicit single EPSPs with characteristics of monosynaptic responses, i.e. a short latency (less than 3 msec)
with small trial-to-trial variability and absence of polysynaptic components overlapping with the EPSP decay phase (Gonzalez-Burgos et al., 2000). Once a satisfactory response was obtained, the stimulation parameters were kept constant throughout the experiment. EPSPs were elicited first at a frequency of 0.1 Hz; next, stimulus trains of 20 Hz frequency were delivered every 10 sec, using the same stimulus parameters. To determine if in addition to short-term depression, stimulus trains had long-term effects on EPSP amplitude, in 12 experiments EPSPs were initially elicited by single stimulus shocks delivered every 10 sec (0.1 Hz) during a ~ 5 minute baseline recording period. After this period, 20 Hz stimulus trains were delivered every 10 sec for at least 10 minutes.

In current clamp recordings, injection of steady-state current was employed to compensate for any significant changes in the FS cell membrane potential induced by application of DA or D1 agonists, relative to control resting membrane potential. In voltage-clamp experiments, the membrane potential was held at -70 mV, and EPSCs were pharmacologically isolated by adding 20 µM bicuculline to the bath. Spontaneous excitatory post-synaptic currents (sEPSCs) and miniature EPSCs (mEPSCs) were recorded under the same conditions except that mEPSCs were recorded in the presence of 1 µM of the sodium channel blocker tetrodotoxin (Sigma, St. Louis, MO).

Fresh stock solutions of all dopaminergic pharmacological compounds were prepared on the day of each experiment, and were protected from light exposure until bath application. DA stock solutions were prepared by dissolving the compound shortly before application to each slice. The stocks and bath solutions contained the anti-oxidant sodium metabisulfite, at a final concentration of 75 µM. All pharmacological compounds used in this study were obtained from Research Biochemicals Incorporated, unless otherwise indicated.

Data analysis

To determine if stimulus trains had long-term effects on EPSP amplitude, we compared the amplitude of single EPSPs with the amplitude of the first EPSP (EPSP1) in 20 Hz trains elicited in the same cells. Single EPSPs were elicited by stimulus shocks delivered every 10 sec (0.1 Hz). EPSP trains were elicited by trains of 10 stimulus pulses at 20 Hz.
(unless otherwise indicated) and delivered at an inter-train frequency of 0.1 Hz (Figure 1F). The amplitude of single EPSPs elicited at 0.1 Hz was estimated by averaging the EPSPs recorded during a 5 minute baseline period of stimulation with single shocks. The amplitude of EPSP1 in trains was estimated by averaging the first EPSPs of trains recorded in the same neurons, during a 10 minute period of stimulation with 20 Hz trains.

Analysis of the effect of DA agonists on EPSP trains was based on monitoring the amplitude of the first EPSP in the trains before, during a 5-min drug application and after washout. In most recorded neurons (SKF81297, 9 of 12 cells; DA 5 of 7 cells), shortly after application of DA agonists, a significant reduction of the first EPSP amplitude was observed in the FS neuron (Fig. 2). In the remaining cases (n= 5 FS neurons), in contrast, DA agonists produced no detectable decrease or apparently an increase in EPSP amplitude compared to the baseline EPSP amplitude before drug application. The effect of DA receptor activation was somewhat weaker in the entire population (EPSP1 reduced to 87 ± 7 % of control) than in the 14 cells showing a decrease of EPSP1 amplitude (EPSP1 reduced to 70 ± 5 % of control). Nevertheless, DA receptor activation had a statistically significant effect on the first EPSP in trains (P < 0.05) in the entire population (n=19) of FS cells in which EPSP trains were tested with DA agonists. Previous studies suggest the presence of heterogeneous subpopulations of FS neurons in PFC, with regard to the effects of DA on excitatory synaptic inputs (Gao and Goldman-Rakic, 2003); C.D. Paspalas, personal communication; see Discussion). In light of these previous studies suggesting heterogeneity, the results presented here are based on the analysis of short-term plasticity in the group of FS neurons in which D1 receptor activation produced a decrease in the amplitude of the first EPSP in the trains, the decrease being greater than 10 % of the baseline EPSP amplitude during control period.

The effect of DA receptor activation on the EPSP trains was determined on traces obtained by averaging the last 10-20 consecutive EPSP trains recorded in the presence of the drugs and before the onset of washout. These average traces were compared with control average traces obtained just prior to drug application. In either experimental and control average traces, EPSP amplitude was normalized relative to that of the first EPSP in the trains recorded in control conditions. The effects of DA receptor activation were typically reversed by
drug washout (Fig. 2B). However, in some slices the effects were long-lasting, as described previously for certain D1 receptor-mediated effects (Seamans and Yang, 2004). Potential differences between FS neuron EPSP trains exhibiting short- versus long-lasting D1 receptor effects were not investigated further and results were pooled. To determine if DA receptor activation changed the rate of synaptic depression after the first EPSP in the trains, EPSPs were also normalized to the second control EPSP (Fig. 2D).

Analysis of mEPSCs and sEPSCs data (event frequency, amplitude and kinetics) was performed using MiniAnalysis (Synaptosoft, Decatur, GA). Events were detected when they crossed a threshold set at three times the standard deviation of the baseline noise. The detected events were confirmed as synaptic events by visual inspection. For sEPSCs and mEPSCs, the total number of events from 8 minutes of continuous recordings each before and~2 min after DA application was compared.

Results are shown as mean and standard error of the mean, unless otherwise indicated. The statistical significance of differences between group means was tested using Student’s t-test or ANOVA followed by Dunnett’s test. Differences between group means were considered significant if P<0.05.

The morphological properties of neurons filled with biocytin were assessed after staining and reconstruction. Following recording, slices were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline and resectioned at 50 µm on a microtome. The biotin label was visualized by standard 3,3'-diaminobenzidine histochemistry, using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). Individual cells were reconstructed using Neurolucida software (Microbrightfield, Williston, VT). As reported previously (Gonzalez-Burgos et al., 2005), FS cells were classified into the chandelier cell group based on the presence of vertical cartridges of axonal boutons (Fig 1D; left), which are distinctive of this cell type. FS neurons lacking cartridges had axon varicosities more evenly distributed throughout the axonal tree and were classified as basket cells (Fig. 1D; right).
Results

D1 receptor activation reduces the initial EPSP but not later responses in EPSP trains

To focus the present study on FS interneurons, we included in the data analysis only layers 2/3 interneurons having spike duration shorter than 0.5 ms at half-amplitude, no significant spike frequency adaptation, and spike afterhyperpolarizations with large amplitude (Fig. 1A). The results of this study were based on recordings from a total of 44 FS neurons from the superficial layers of the principal sulcus region (area 46) of macaque monkey PFC. The morphology of the recorded FS cells (Fig. 1B) was consistent with either chandelier or basket types, as described previously (Gonzalez-Burgos et al., 2004; Gonzalez-Burgos et al., 2005; Zaitsev et al., 2005). Stimulus trains of 20 Hz frequency, which corresponds to typical mean frequencies of persistent firing in monkey PFC during working memory tasks (Sawaguchi, 2001; Wang, 2001; Williams and Goldman-Rakic, 1995), were delivered every 10 seconds. These 20 Hz input trains produced significant EPSP depression (P<0.001) with weak temporal summation, due to a fast EPSP decay (Fig. 1C). During synaptic depression induced by 20 Hz stimulation, the amplitude of the tenth response (EPSP10) was reduced to 20-50% of the first response (EPSP1) amplitude (Fig. 1D). Between subsequent stimulus trains, the EPSP1 amplitude completely recovered from depression (Fig. 1E). Furthermore, the amplitude of EPSP1 in trains delivered every 10 sec did not differ significantly from the amplitude of single EPSPs evoked every 10 sec in the same neurons, prior to the onset of stimulation with 20 Hz trains (Figure 1F). The results displayed in Figures 1E and 1F suggest that stimulus trains did not have long-term effects on the EPSP amplitude and that EPSP1 in trains delivered at a low frequency (0.1 Hz) equals single EPSPs elicited at the same low frequency.

In monkey dorsolateral PFC, D1-like receptors are significantly more abundant than receptors of the D2 family (Goldman-Rakic et al., 1990). Thus, to determine the effects of DA receptor activation on the 20 Hz EPSP trains recorded from FS cells (n=9), we first applied the selective D1 receptor agonist SKF81297 (5 µM). SKF81297 application significantly reduced the EPSP1 amplitude (to ~80-50% of EPSP1 control), whereas the depolarization produced by later EPSPs appeared to be unaffected (Fig. 2A). Figure 2B shows the time course of the D1
agonist effect on EPSP1 and EPSP10 amplitudes. As displayed in the top graph of Figure 2B, in some cells the effect elicited by DA agonists was short-lasting, whereas in other neurons the DA agonist effect was persistent and did not reverse upon washout (Figure 2B, bottom graph). As summarized in Figure 2C, D1 receptor stimulation significantly reduced the EPSP1 amplitude, to about 70% of the EPSP1 recorded in control conditions (Fig. 2C; P<0.05). In contrast, compared to control conditions, D1 receptor activation did not change significantly the depolarization elicited by EPSP2 or the late, stationary EPSPs (Fig. 2C; P>0.1). Despite reduction of the first EPSP, short-term EPSP depression induced by input trains in the presence of SKF81297 was statistically significant (Fig. 2C, P<0.001). However, the reduction in EPSP1 amplitude without significant change in stationary EPSPs suggests that repetitive stimulation elicits less depression during D1 receptor activation than in control conditions. Indeed, SKF81297 significantly increased the EPSP10 / EPSP1 ratio within a train (Fig. 2D). To further examine the effect of D1 receptor stimulation on the time course of depression, we fit a single exponential decay function to the changes in EPSP amplitude during trains. The time constant of decay increased following D1 receptor stimulation, although the difference was not significant (control: 79 ± 19 msec; SKF81297: 142 ± 58 msec, n=8, P>0.3, Paired Student’s t-test). Because D1 receptor activation significantly decreased EPSP1 but not EPSP2 or later responses (Fig. 2C), we next examined whether the rate of EPSP depression after EPSP2 was changed by 5 µM SKF81297. Normalization of EPSP amplitude relative to EPSP2, showed that synaptic depression after the second EPSP in the trains was not significantly altered by D1 receptor activation, compared to control conditions (Fig. 2E). Moreover, the time constant of exponential decay after EPSP2 was not altered by D1 receptor activation (control: 85 ± 28 msec; SKF81297: 108 ± 37 msec, n=8, P>0.3, Paired Student’s t-test). Thus, the increase in EPSP10 / EPSP1 ratio depicted in Figure 2D, results only from the reduction of EPSP1 by D1 receptor activation, without additional changes in the temporal features of synaptic depression. Together, these data show that the main effect of D1 receptor stimulation on EPSP trains recorded from FS neurons is a reduction of the first EPSP, without changes in the absolute depolarization elicited by the following EPSPs in the trains, once stimulation switches to a repetitive regime. In agreement with these results, D1 receptor activation selectively reduced, in a frequency-dependent manner, the first response during
repetitive stimulation of depressing excitatory inputs in nucleus accumbens neurons (Hjelmstad, 2004).

Application of the endogenous agonist DA (10 µM), also reduced EPSP1 without affecting the repetitive EPSPs in trains recorded from FS neurons (n=5), thus having effects similar to those of the D1 agonist SKF81297 (Fig. 2F-H). In the presence of the D1 receptor antagonist SCH23390 (5 µM), DA (10 µM) failed to decrease the amplitude of the first EPSP (EPSP1 was 90 ± 13 % of control; n=3, p=0.48) or subsequent EPSPs in the trains (data not shown). Moreover, in the presence of SCH23390, DA failed to increase the EPSP10 / EPSP1 ratio within a train (control ratio, 0.31 ± 0.09; DA+SCH23390 ratio, 0.32 ± 0.04; n=3, p=0.91). Taken together, these results suggest that the effects of DA on EPSP trains in FS interneurons are mediated by activation of D1 receptors.

D1 receptor activation had differential effects on the first response in EPSP trains delivered every 10 sec. These results suggest that DA modulation of excitatory synaptic inputs onto FS neurons is activity-dependent, selectively decreasing the amplitude of EPSPs evoked at low frequency (0.1 Hz). We therefore tested the effects of DA on miniature EPSCs (mEPSCs), which result from low-frequency spontaneous release of glutamate at single synapses. As shown in Figure 3, DA did not have significant effects on the frequency, amplitude or kinetics of mEPSCs in FS neurons. Miniature EPSCs are recorded in the presence of the sodium channel blocker tetrodotoxin and result from action potential-independent (and possibly extracellular calcium-independent) spontaneous release mechanisms (Simkus and Stricker, 2002). In a previous study of excitatory synaptic inputs onto pyramidal cells during normal aging of the monkey PFC, the frequency of mEPSCs was apparently lower than that of spontaneous EPSCs (sEPSCs) recorded in the absence of tetrodotoxin (Luebke et al., 2004). These results suggest that low frequency spontaneous action potential firing occurred in cells providing excitatory inputs onto pyramidal neurons. Thus, we also tested the effects of DA on spontaneous EPSCs (sEPSCs) recorded from FS neurons in the absence of tetrodotoxin. As shown in Table 1, DA application did not alter the frequency, amplitude or kinetics of sEPSCs recorded from FS cells. In addition, we found no significant differences in frequency, amplitude or kinetics of sEPSCs recorded from individual
FS neurons before and after tetrodotoxin application, respectively (Table 1). These results suggest that in our experimental conditions there is no significant spontaneous presynaptic action potential firing in excitatory inputs onto FS neurons and that therefore sEPSCs essentially represent mEPSCs, as reported previously for layers 2/3 neurons in rat neocortex (Simkus and Stricker, 2002). In addition, the mEPSC and sEPSC data indicate that DA does not change the postsynaptic response to synaptic release of glutamate and that the effects of DA are exclusively associated with action potential-evoked glutamate release.

The effects of DA receptor activation do not involve temporal EPSP summation or activation of NMDA receptor channels

In rat PFC pyramidal neurons, the depolarization elicited by late EPSPs in trains is enhanced by DA via a D1 receptor-mediated increase in temporal summation (Seamans et al., 2001). Here we found that in PFC FS neurons, D1 receptor activation did not enhance the depolarization elicited by late EPSPs. Thus, we tested whether this difference is due to a lack of DA effect on temporal summation in FS cell EPSP trains. To estimate the effect of temporal summation, we calculated the fraction of the EPSP-induced depolarization, relative to the resting potential, that was due to summation of each EPSP with the decay phase of the prior response in the train (Fig. 4A). On average, EPSP summation accounted for only 15 to 30 % of the depolarization elicited by the EPSPs, and did not change significantly throughout the trains (Fig. 4B). In addition, a comparison between EPSP trains recorded before and after DA receptor activation, showed that while reducing EPSP1 (Fig. 2), activation of D1 receptors produced no significant differences in temporal summation in the same cells (Fig. 4B). Thus, in contrast to the effect observed in PFC pyramidal cells (Seamans et al., 2001), D1 receptor activation did not increase summation in EPSP trains recorded from FS neurons.

The D1 receptor-mediated increase in EPSP summation that enhances late responses in pyramidal cell trains, is NMDA receptor-dependent (Seamans et al., 2001). Previous studies of rat neocortex and hippocampus suggest that excitatory synapses in FS interneurons have a low NMDA receptor content (Angulo et al., 1999; Geiger et al., 1997; Goldberg et al., 2003b; Goldberg et al., 2003a; Nyiri et al., 2003). Therefore, the lack of effect of D1 receptor activation on temporal summation in PFC FS neurons may be explained by a small NMDA EPSP.
component. To determine the contribution of NMDA receptors to EPSP trains, we tested the effects of the NMDA receptor antagonist DL-APV. Figure 5A shows that application of APV (100 µM) did not alter significantly the EPSP trains, suggesting a small or absent NMDA EPSP component in FS interneurons from monkey PFC.

A smaller NMDA component in FS neurons than in pyramidal cells may result from a more significant voltage-dependent \( \text{Mg}^{2+} \) block of NMDA channels. In monkey dorsolateral PFC in vitro, the resting potential is significantly depolarized in FS cells (-67.7 ± 1.2 mV) compared to pyramidal neurons (-74.4 ± 1.1 mV) (Gonzalez-Burgos et al., 2004). In the present study, FS cells showing D1 effect and FS cells tested with APV had resting potentials of -66.7 ± 5.2 mV and -67.2 ± 2.9 mV, respectively. However, the resting potential of FS cells in monkey PFC is actually hyperpolarized compared to the range of potentials (-65 to -58 mV) at which D1 receptor modulation of EPSP trains in pyramidal cells is NMDA-dependent (Seamans et al., 2001). Therefore, subthreshold membrane depolarization may reveal an NMDA-mediated increase in EPSP duration that could enhance EPSP summation. As shown in Figure 5B, depolarization of FS neurons, however, produced effects opposite to those of NMDA channel activation, because EPSP duration was significantly shortened at potentials near spike threshold (Fig. 5B). These results confirm previous findings indicating that in FS neurons voltage-dependent conductances curtail the EPSP duration and decrease temporal summation at depolarized subthreshold potentials (Galarreta and Hestrin, 2001; Gonzalez-Burgos et al., 2004). In contrast, in cortical pyramidal cells from both rat and monkey, voltage-gated channels increase EPSP duration, via an effect that is independent of, but potentially synergistic with, the effect of NMDA receptors (Gonzalez-Burgos et al., 2004; Gonzalez-Burgos and Barrionuevo, 2001; Stuart and Sakmann, 1995). Therefore, the actions of voltage-gated and synaptic conductances give rise to significant differences in temporal EPSP summation in FS neurons and pyramidal cells. Together, this and previous studies suggest that, in FS neurons at the subthreshold membrane potential range, NMDA receptors have a small impact on somatic EPSP duration and thus on temporal summation.
Discussion

In this study, we found that at excitatory inputs onto FS neurons of monkey PFC, DA modulated EPSP amplitude in an activity-dependent manner. Specifically, when excitatory inputs were stimulated with trains at inter-train intervals of 10 sec, D1 receptor activation decreased the amplitude of the first EPSP, but not the amplitude of later EPSPs in the train. The repetitive EPSPs (EPSPs2-10 in the trains), showed similar degrees of short-term depression in control or DA conditions. This suggests that the basic mechanisms of temporal coding and gain control are retained during DA neuromodulation of FS cell inputs. In contrast with previous findings in PFC pyramidal cells, we found that in FS neurons D1 receptor activation did not change temporal summation of EPSPs. In addition, NMDA receptors did not contribute significantly to EPSPs in the subthreshold potential range. This suggests that NMDA receptors are an unlikely substrate for DA modulation of EPSP integration in FS neurons. Thus, D1-NMDA receptor interactions in PFC microcircuits appear to be specific to pyramidal cell inputs.

DA modulation of short-term plasticity in FS neurons.

At excitatory connections onto neocortical neurons, depression predominates if the synapses display high probability of glutamate release (Pr) in response to single stimuli (Atzori et al., 2001; Rozov et al., 2001). Manipulations that reduce Pr, decreased the amplitude of EPSP1 but not of subsequent EPSPs in trains elicited at depressing synapses (Markram and Tsodyks, 1996; Tsodyks and Markram, 1997). Similarly, in this study D1 receptor activation selectively reduced EPSP1, suggesting that DA may produce a reduction in Pr via presynaptic effects. Because the amplitude of EPSP2 and subsequent responses in the trains were not altered by DA relative to control conditions, short-term synaptic depression of repetitive EPSPs seemed to be independent of the DA-induced decrease in EPSP1. To examine the complex mechanisms underlying this effect was beyond the scope of the present study. A mechanism that could yield depressed EPSPs independent of prior responses in the trains is release-independent depression (RID). During RID, EPSPs depress independent of whether preceding stimuli fail to evoke release (Thomson and Bannister, 1999). The mechanisms of RID imply significant differences between release evoked by single stimuli or repetitive stimulation.
(Fuhrmann et al., 2004). Thus, during RID glutamate release may be refractory to DA effects that, however, decrease release evoked by temporally isolated spikes, i.e. the first spike in a train. The contribution of RID to repetitive EPSPs in FS neurons remains to be determined.

If D1 receptor activation modulates glutamate release at FS cell inputs, then DA effects should be at least in part presynaptic. In the deep layers of monkey PFC, D1 receptors are localized in excitatory boutons synapsing with pyramidal cells, but are distinctively absent from excitatory boutons targeting parvalbumin-positive interneurons (Paspalas and Goldman-Rakic, 2005). These data argue against presynaptic effects of DA at glutamate synapses onto FS neurons. However, in the superficial layers of monkey PFC area 46, certain excitatory boutons synapsing with parvalbumin-containing dendrites overtly express D1 receptors and contain numerous D1 receptor immunoparticles (C. D. Paspalas, personal communication). Ultrastructural studies thus imply both target- and layer-specific heterogeneity in the expression of D1 receptors in excitatory axons synapsing onto PFC neurons. Likewise, our physiological experiments also suggest heterogeneity because, in a minority of the recorded FS cells, DA caused no reduction of EPSP1 amplitude (see Methods). This subgroup included both basket and chandelier cells, suggesting no correlation between heterogeneity in DA effects and morphological subpopulations of FS neurons. In contrast with our findings, in layer 5 of ferret PFC, DA did not affect EPSPs in most FS neurons, but strongly reduced EPSP amplitude in a minority of these cells (Gao and Goldman-Rakic, 2003). Besides differences between species, the discrepancy between our results and those of Gao and Goldman-Rakic (2003) may be explained by a greater incidence of D1 receptor expression in terminals contacting FS neurons in the superficial compared to the deep cortical layers of PFC (C. D. Paspalas, personal communication).

Our results suggesting a small NMDA receptor contribution to EPSP trains in PFC FS neurons are consistent with previous studies indicating that a small NMDA EPSC component and a low NMDA content are found in excitatory synapses onto FS neurons from rat hippocampus and neocortex (Angulo et al., 1999; Geiger et al., 1997; Nyiri et al., 2003). In FS neurons, synaptically-evoked dendritic Ca\(^{2+}\) transients are not sensitive to NMDA antagonists, or are significantly less sensitive than in other interneuron subtypes (Goldberg et al., 2003b;
Goldberg et al., 2003a). In some FS cell synapses, NMDA antagonists partially reduced the dendritic Ca$^{2+}$ transients, without simultaneously altering the amplitude or time course of somatic EPSPs (Goldberg et al., 2003b; Goldberg et al., 2003a). Thus, NMDA receptors may mediate Ca$^{2+}$ influx at certain FS cell synapses, without having a significant impact on somatic EPSP time course. Voltage-clamp experiments suggest that some NMDA receptor activation occurs at very depolarized potentials in FS neurons from rat neocortex (Angulo et al., 1999). However, our data suggest that DA regulation of short-term depression of subthreshold EPSPs in FS cells is unlikely to involve D1 modulation of synaptically-activated NMDA receptors, in contrast to PFC pyramidal cells (Seamans et al., 2001).

**Functional Implications**

In computational and in vitro experimental models, persistent firing in PFC is strongly influenced or dominated by recurrent inhibition (Fellous and Sejnowski, 2003; McCormick et al., 2003; Seamans et al., 2003; Wang et al., 2004). Modeling studies indeed show that DA-mediated modulation of GABA-mediated inhibition is critical for the stability of persistent activity (Brunel and Wang, 2001; Durstewitz et al., 2000; Durstewitz and Seamans, 2002). The prevalent synaptic depression in their inputs and outputs suggests that FS neurons generate transient inhibition but are not a significant source of sustained recurrent inhibition (Beierlein et al., 2003; Pouille and Scanziani, 2004). In pyramidal cells, DA counteracts EPSP depression by increasing the amplitude of late, stationary EPSPs, potentially favoring sustained postsynaptic firing by sustained presynaptic stimulation. In FS cells, in contrast, DA did not potentiate the late EPSPs in trains, suggesting that DA modulation does not favor sustained recruitment of FS neurons by input trains. Recurrent inhibition during persistent firing may thus be mediated by non-FS interneurons, which typically exhibit short-term facilitation instead of depression, in either inputs or outputs (Beierlein et al., 2003; Gonzalez-Burgos et al., 2004).

The DA effects found here are consistent with a reduction of EPSPs elicited by temporally isolated presynaptic spikes (EPSP1), and no reduction of EPSPs elicited by repetitive spikes (EPSPs2-10), i.e. spikes elicited shortly after the preceding one. Repetitive spikes elicited short-term depression of EPSPs2-10 and, because the absolute amplitude of EPSPs2-10 was not affected by D1 receptor activation, this depression was not altered by DA.
Temporal coding by depressing synapses depends strongly on the effect of the initial EPSPs after presynaptic firing frequency switches from low to high, because the initial EPSPs are not significantly depressed, compared to the stationary responses (Abbott et al., 1997; Tsodyks and Markram, 1997). In either control conditions or following D1 receptor stimulation, EPSPs1-3 were significantly different from stationary EPSPs (P<0.05, ANOVA followed by contrasts), despite of the reduction of EPSP1 amplitude by DA receptor activation. In addition, the absence of DA modulation of stationary EPSPs during 20 Hz stimulation, suggests that DA does not enhance the ability of synapses onto FS neurons to code presynaptic firing rate per se (Abbott et al., 1997; Tsodyks and Markram, 1997). As suggested previously (Abbott et al., 1997), these results indicate that the basic temporal coding and gain control mechanisms at depressing synapses onto FS neurons are retained during DA neuromodulation. Spike trains recorded from the monkey PFC during working memory tasks display increased inter-spike interval variability, approximating a Poisson process, and suppression of specific frequencies in the power spectra (Compte et al., 2003). Thus, spike trains in PFC may contain spike timing-dependent information relevant for task-related events, although the precise nature of this information remains to be determined.

If spike timing-dependent information is transmitted via presynaptic action potential bursts at depressing synapses, then EPSP bursts in FS neurons may contain an activity-dependent signal related to transitions in the level of network activity. Because the effects of DA described here may favor detection of bursts relative to low-frequency background synaptic activity, then DA may actually enhance the signal-to-noise ratio at FS cell inputs. The results reported here as well as those of a previous electrophysiological and ultrastructural studies (Gao and Goldman-Rakic 2003; Paspalas and Goldman-Rakic 2005; C. Paspalas personal communication) suggest heterogeneity in the effects of DA on FS cells. Therefore, it is possible that the potential enhancement of the signal-to-noise ratio is observed only at particular subtypes of depressing synaptic inputs onto FS cells. Similar mechanisms may operate at depressing synapses onto pyramidal neurons, so long as transmission is mediated mostly by non-NMDA receptors, as in hyperpolarized states. When PFC pyramidal cells are depolarized, activation of NMDA receptors, together with the subthreshold effects of Na\(^+\) channels counteract synaptic depression, actually converting it into facilitation (Gonzalez-
Burgos and Barrionuevo, 2001; Williams and Stuart, 1999). This NMDA-dependent facilitation may effectively increase the strength of connections above a critical threshold necessary for the stability of persistent firing (Brunel and Wang, 2001; Wang, 2001). In contrast, in the absence of significant NMDA receptor contribution, in FS cells depolarization decreases EPSP duration and thus enhances the effects of synaptic depression. Our results therefore suggest that the contribution of D1-NMDA receptor synergy to temporal EPSP integration is pyramidal cell-specific, and that DA has different physiological effects at different components of the local PFC circuits.

Acknowledgements: We thank Drs. Daniel Durstewitz, Edda Thiels and Etienne Sibille for their useful comments on a previous version of this manuscript. This work was supported by NIMH grants MH45156 and MH51234 and by a NARSAD Young Investigator Award to G. Gonzalez-Burgos.
Figure legends

Figure 1. Basic properties of fast-spiking neurons and excitatory synaptic responses evoked by repetitive stimulation. A, The response of the FS cell membrane potential to injection of hyperpolarizing and depolarizing current (top graph), showed fast spikes with large afterhyperpolarizations and typical absence of spike frequency adaptation. The absence of significant adaptation is also revealed by an analysis of the instantaneous firing frequency as a function of interspike interval number, for different levels of suprathreshold current injection. (bottom graph). B, The morphological properties of FS neurons filled with biocytin during recording, showed cells with smooth aspiny dendrites. The axonal features divided the cells into basket (left) and chandelier neurons (right). Shown are partial reconstructions of the morphology of two FS cells, obtained from three adjacent 50 µm sections. Additional axonal label observed in other sections, distal to the section containing the soma was not included in the reconstruction. Dendritic trees are shown with thick red lines and axonal arborization with thin black lines. C, Focal extracellular stimulation within layers 2/3 elicited EPSPs with features consistent with those of monosynaptic responses. In response to 20 Hz stimulation, EPSPs showed significant depression. Temporal summation was weak, due to a rapid EPSP decay. D, Repetitive stimulation at 20 Hz caused significant EPSP depression (P<0.001), reducing the EPSP10 amplitude to about 20-50 % of EPSP1. E, The amplitude of the first and tenth EPSPs in trains were plotted over time in a representative experiment, showing that EPSPs recovered completely from depression between subsequent applications of the 20 Hz stimulus trains at 0.1 Hz. F, The amplitude of EPSPs elicited by single stimulus shocks delivered every 10 sec was compared with the amplitude of EPSP1 in ten-stimulus 20 Hz trains delivered in the same neurons at inter-train intervals of 10 sec (n=12 FS neurons). The absence of significant differences in the amplitude of single EPSPs and EPSP1 suggests that stimulus trains produced no long-term effects on EPSP amplitude.

Figure 2. Effects of DA receptor activation on EPSP trains recorded from FS neurons. A, Representative example showing the effects of the D1 agonist SKF81297 (5 µM) on EPSP trains recorded from FS neurons. Note the significant reduction in the amplitude of the first EPSP without significant change in the EPSPs evoked by repetitive stimulation. Traces shown
are the average of 10 consecutive sweeps right before SKF81297 application (control) and right before beginning of washout (SKF81297). B, Plot of the amplitude of the first and tenth EPSPs in trains versus time, in two representative experiments. Note the significant reduction in EPSP1 amplitude shortly after beginning of SKF81297 application (indicated by the bar), and the absence of significant change in the EPSP10 amplitude. In most neurons, the effect of SKF81297 was reversed after a short period of washout (top graph); however, in some cells the effect of a brief application of SKF81297 was long lasting (bottom graph). C, Summary graph showing the effects of SKF81297 on EPSP depression in FS neurons (n=9). The depolarization elicited by each EPSP in the trains was divided by the amplitude of the first EPSP recorded in control conditions and plotted versus stimulus number. ANOVA followed by contrasts indicated a significant reduction of the EPSP1 amplitude by SKF81297 (P<0.05), but no significant change of subsequent EPSPs in the trains (P>0.1). In either control recording conditions or after SKF81297 application, repetitive stimulation caused significant depression of the EPSP amplitude (P<0.001) and, EPSPs1-3 were significantly different from EPSP10 (P<0.01). These data show that D1 receptor stimulation reduced EPSP1, but did not significantly alter the rate of depression of EPSPs elicited by repetitive presynaptic spikes (i.e. EPSPs2-10). D, The ratio between EPSP10 and EPSP1 within a train, was increased significantly during D1 receptor activation (P<0.05). E, Relative to EPSP2 recorded in control conditions, the amplitude of EPSP2 and subsequent EPSPs in trains was not altered by D1 receptor stimulation. F-H, The effects of Dopamine (10 µM) on the first and subsequent EPSPs in FS cell EPSP trains (n=5) were similar to those of SKF81297. DA produced a significant reduction of EPSP1 amplitude (P<0.05), but no significant change of subsequent EPSPs in the trains (P>0.1). Depression of the EPSP amplitude repetitive stimulation caused significant (P<0.001) and, EPSPs1-3 were significantly different from EPSP10 (P<0.01). These data show that D1 receptor stimulation reduced EPSP1, but did not significantly alter the rate of depression of EPSPs elicited by repetitive presynaptic spikes (i.e. EPSPs2-10).

**Figure 3.** Dopamine receptor activation does not alter spontaneous glutamate release at synapses onto FS neurons. A, Representative recordings of mEPSCs from a PFC FS neuron. Application of DA (10 µM) did not significantly change mEPSC frequency or amplitude compared to baseline conditions. B, An analysis of the distribution and cumulative distribution
of mEPSC amplitude indicated that DA had no significant effects. C, Left, The averages of all mEPSCs recorded from a representative FS neuron in control versus DA condition, show no significant effects of DA application on mEPSCs amplitude and time course. Right, Bar graphs showing the absence of effects of DA receptor stimulation on the mean mEPSC frequency, amplitude, 10-90% rise time and monoexponential decay time constant. Results were obtained after recording from n=6 FS neurons.

**Figure 4.** Temporal summation during FS cell EPSP trains was not influenced by dopamine receptor activation. A, The contribution of temporal summation to the depolarization produced by EPSPs in trains was estimated by calculating the fraction of the total depolarization (2) that was accounted by summation with the decay phase of the previous EPSP in the train (1). B, Temporal summation during FS cell EPSP trains was calculated as described in A for trains recorded before and after DA receptor stimulation. Data were pooled for experiments in which either SKF81297 or DA was applied to the slices an produced significant changes in EPSP1 amplitude (see Fig. 2). Note the absence of significant effects of DA receptor activation on the degree of temporal summation.

**Figure 5.** Contribution of NMDA receptors to excitatory transmission in FS neurons. A, Application of the NMDA receptor blocker APV (100 µM) did not affect the EPSPs trains recorded at the FS cell resting membrane potential. The traces show EPSP trains recorded from a FS neuron that exhibited one of the greatest degrees of temporal summation during the trains. B, Depolarization of FS neurons in the subthreshold membrane potential range had effects opposite to those expected for NMDA channel activation, since it reduced the EPSP duration in FS neurons (n=3); * indicates a truncated action potential.
Table 1. Effects of dopamine receptor activation and tetrodotoxin on spontaneous EPSCs recorded from FS neurons in monkey PFC.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>3.18 ± 0.93</td>
<td>3.10 ± 0.78</td>
</tr>
<tr>
<td>Amplitude (pA)</td>
<td>13.4 ± 2.1</td>
<td>13.3 ± 1.9</td>
</tr>
<tr>
<td>Rise time (msec)</td>
<td>0.85 ± 0.04</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td>Decay time (msec)</td>
<td>1.84 ± 0.16</td>
<td>1.85 ± 0.18</td>
</tr>
</tbody>
</table>

Two groups of experiments were performed. In Experiment 1 (second and third columns from left), sEPSCs were recorded from FS neurons (n=5) before and after a 5 min application of DA (10 µM). In Experiment 2, (fourth and fifth columns from left), sEPSCs were recorded from a different group of FS neurons (n=4) before and after addition of tetrodotoxin (1 µM). Paired t-test was used to compare sEPSC parameters in control versus DA or tetrodotoxin conditions. No significant differences were observed between group means (P>0.05). Data are expressed as means ± S.E.M.
Reference List


Goldman-Rakic PS, Lidow MS and Gallager DW. Overlap of dopaminergic, adrenergic, and serotonergic receptors and complementarity of their subtypes in primate prefrontal cortex. Journal of Neuroscience 10: 2125-2138, 1990.


Zaitsev AV, Gonzalez-Burgos G, Povysheva NV, Kroner S, Lewis DA and Krimer LS.

Figure 1

A) A plot showing interspike interval number against frequency (Hz).

B) Images of neural structures with a scale of 100 µm.

C) A graph displaying EPSP amplitudes over time (min).

D) A graph showing the ratio of EPSP (n) to EPSP (1) against EPSP number.

E) A scatter plot comparing EPSP (1) and EPSP (10) amplitudes over time.

F) A graph comparing amplitudes of single EPSPs and EPSPs in trains.
Figure 2

A  Control

SKF81297

200 ms

2 mV

B  ● EPSP1  ○ EPSP10

EPSP amplitude (mV)

Time (min)

SKF81297

C  ● Control  ○ SKF81297

EPSPn / EPSP1-control

Stimulus number

D  ● Control  ○ SKF81297

EPSP10 / EPSP1

Stimulus number

E  ● Control  ○ SKF81297

EPSPn / EPSP2-control

Stimulus number

F  ● Control  ○ Dopamine

EPSPn / EPSP1-control

Stimulus number

G  ● Control  ○ Dopamine

EPSP10 / EPSP1

Stimulus number

H  ● Control  ○ Dopamine

EPSPn / EPSP2-control

Stimulus number
Figure 3

A

Control

Dopamine

B

Number of events

Amplitude (pA)

Cumulative fraction

Amplitude (pA)

C

Control

Dopamine

2 pA

5 ms

Frequency (Hz)

Amplitude (pA)

Rise time (ms)

Decay time (ms)
Figure 4

A

B

- Control
- Dopamine / SKF81297

EPSP Summation (fraction)

Stimulus number
Figure 5