Dopaminergic Modulation of Short-Term Synaptic Plasticity in Fast-Spiking Interneurons of Primate Dorsolateral Prefrontal Cortex

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INTRODUCTION

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). Although 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 excitatory 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 excitatory postsynaptic potentials (EPSPs) evoked by single stimuli (Gao and Goldman-Rakic 2003; Gao et al. 2001; 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, 2005). The electrophysiological properties of FS neurons in monkey PFC (Gonzalez-Burgos et al. 2004, 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 with 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, 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, 2005). Short-term depression decreases the efficiency of synaptic transmission during sustained activation and thus to encode the presynaptic firing rate 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 National Institutes of Health 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, 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, and 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, Union City, CA) or BVC700A (Dagan, Minneapolis, MN) amplifiers operating in current-clamp mode, using bridge balance and pipette capacitance compensation. Pipettes had a resistance of 4–6 MΩ when filled with a solution of the following composition (in mM): 120 NaCl, 2.5 KCl, 1.2 Na2HPO4, 25 NaHCO3, 2.0 CaCl2, 1.0 MgCl2, and 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, Union City, CA) or BVC700A (Dagan, Minneapolis, MN) amplifiers operating in current-clamp mode, using bridge balance and pipette capacitance compensation. Pipettes had a resistance of 4–6 MΩ when filled with a solution of the following composition (in mM): 120 NaCl, 2.5 KCl, 1.2 Na2HPO4, 25 NaHCO3, 2.0 CaCl2, 1.0 MgCl2, and 10 glucose.

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 neurons. Single EPSPs were elicited by stimulus shocks delivered every 10 s (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 (Fig. 1F). The amplitude of single EPSPs elicited at 0.1 Hz was estimated by averaging the EPSP recorded during a 5-min 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-min 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 with 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 >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 m- and pEPSC 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 SD of the baseline noise. The detected events were confirmed as synaptic events by visual inspection. For s- and mEPSCs, the total number of events from 8 min of continuous recordings each before and ~2 min after DA application was compared.

Results are shown as means ± SE 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. After 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. 1B, right), 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. 1B, left).

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 <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, 2005; Zait-
sev 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 s. 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 10th 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 s did not differ significantly from the amplitude of single EPSPs evoked every 10 s in the same neurons, prior to the onset of stimulation with 20-Hz trains (Fig. 1F). The results displayed in Fig. 1, E and F, 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 $\mu$M). SKF81297 application significantly reduced the EPSP1 amplitude (to $\sim 80-50\%$ 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 Fig. 2B, top, 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 on washout (Fig. 2B, bottom). As summarized in Fig. 2C, D1 receptor stimulation significantly reduced the EPSP1 amplitude to $\sim 70\%$ of the EPSP1 recorded in control conditions (Fig. 2C; $P < 0.05$). In contrast, compared with 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: $79 \pm 19$ ms; SKF81297: $142 \pm 58$ ms, $n = 8$, $P > 0.3$, 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 $\mu$M 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 with control conditions (Fig. 2E). Moreover, the time constant of exponential decay after EPSP2 was not altered by D1 receptor activation (control: $85 \pm 28$ ms; SKF81297: $108 \pm 37$ ms, $n = 8$, $P > 0.3$, paired Student’s t-test). Thus the increase in EPSP10/EPSP1 ratio depicted in Fig. 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 $\mu$M), 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. 2, F-H). In the presence of the D1 receptor antagonist SCH23390 (5 $\mu$M), DA (10 $\mu$M) failed to decrease the amplitude of the first EPSP (EPSP1 was $90 \pm 13\%$ 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, $0.31 \pm 0.09$; DA + SCH23390 ratio, $0.32 \pm 0.04$; $n = 3$, $P = 0.91$). 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 s. 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 mEPSCs, which result from low-frequency spontaneous release of glutamate at single synapses. As shown in Fig. 3, DA did not have significant effects on the frequency, amplitude, or kinetics of mEPSCs in FS neurons. mEPSCs 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 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 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 neu-
DOPAMINE AND SHORT-TERM PLASTICITY IN FAST-SPIKING CELLS

Tetrodotoxin (1 μM) significantly decreases mEPSC frequency, amplitude, rise time, and decay time constant. Results were obtained after recording from a representative FS neuron in control vs. DA condition show no significant effects of DA. The absence of effects of DA receptor stimulation on mEPSCs amplitude and time course is indicated by the mean mEPSC frequency, amplitude, 10–90% rise time, and exponential decay time constant. Results were obtained after recording from a monkey PFC from a PFC FS neuron. Application of DA (10 μM) 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 15–30% 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 pyramid cell trains, is N-methyl-D-aspartate (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. 2003a,b; 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 EPSPs, we tested the effects of the NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid (D,L-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.

Effects of DA receptor activation do not involve temporal EPSP summation or activation of N-methyl-D-aspartate 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 15–30% 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.

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Two groups of experiments were performed. In experiment 1, sEPSCs were recorded from fast-spiking (FS) neurons (n = 5) before and after a 5-min application of dopamine (DA) (10 μM). In experiment 2, 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 vs. DA or tetrodotoxin conditions. No significant differences were observed between group means (P > 0.05). Data are expressed as means ± SE.

![Graphs showing the absence of effects of DA receptor stimulation on mEPSCs amplitude and time course.](Image)

FIG. 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 with baseline conditions. B: analysis of the distribution and cumulative distribution of mEPSC amplitude indicated that DA had no significant effects. C, left: averages of all mEPSCs recorded from a representative FS neuron in control vs. 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.

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tr>
<td>Control</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>3.18 ± 0.93</td>
</tr>
<tr>
<td>Amplitude, pA</td>
<td>13.4 ± 2.1</td>
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<tr>
<td>Rise time, msec</td>
<td>0.85 ± 0.04</td>
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<tr>
<td>Decay time, msec</td>
<td>1.84 ± 0.16</td>
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The m- and sEPSCs 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.

Effects of DA receptor activation do not involve temporal EPSP summation or activation of N-methyl-D-aspartate 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 15–30% 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.

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A smaller NMDA component in FS neurons than in pyramidal cells may result from a more significant voltage-dependent Mg2+/H11001 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 with 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 and 67.2 ± 2.9 mV, respectively. However, the resting potential of FS cells in monkey PFC is actually hyperpolarized compared with 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 Fig. 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 and Barrionuevo 2001; Gonzalez-Burgos et al. 2004; 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 s, D1 receptor activation decreased the amplitude of the first EPSP, but not the amplitude of later EPSPs in the train. The repetitive EPSPs (EPSPs 2-10 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 sub-populations 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 with 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 Ca2+ transients are not sensitive to NMDA antagonists or are significantly less sensitive than in other interneuron subtypes (Goldberg et al. 2003a,b). In some FS cell synapses, NMDA antagonists partially reduced the dendritic Ca2+ transients, without simultaneously altering the amplitude or time course of somatic EPSPs (Goldberg et al. 2003a,b). Thus NMDA receptors may mediate Ca2+ 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 and Seamans 2002; Durstewitz et al. 2000). 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 with the stationary responses (Abbott et al. 1997; Tsodyks and Markram 1997). In either control conditions or after D1 receptor stimulation, EPSPs1-3 were significantly different from stationary EPSPs (P < 0.05, ANOVA followed by contrasts) despite 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; Pasпалas and Goldman-Rakic 2005; C. Pasпалas, 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.

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