Dopamine Modulates Temporal Dynamics of Feedforward Inhibition in Rat Prefrontal Cortex In Vivo

Midbrain dopamine (DA) neurons project to pyramidal cells and interneurons of the prefrontal cortex (PFC). At the microcircuit level, interneurons gate inputs to a network and regulate/pattern its outputs. Whereas several in vitro studies have examined the role of DA on PFC interneurons, few in vivo data are available. In this study, we show that DA influences the timing of interneuron firing. In particular, DA had a reductive influence on interneuron spontaneous firing, which in the context of the excitatory response of interneurons to hippocampal electrical stimulation, lead to a temporal focalization of the interneuron response. This suggests that the reductive influence of DA on interneuron excitability is responsible for filtering out weak excitatory inputs. The increase in the temporal precision of interneuron firing is a mechanism by which DA can modulate the temporal dynamics of feedforward inhibition in PFC circuits and can thereby influence cognitive information processing.

Keywords: hippocampus, interneuron, schizophrenia, synchronization, timing, ventral tegmental area

Introduction

The prefrontal cortex (PFC) is the brain region most importantly implicated in executive processes (Grafman 2002). It receives inputs from virtually all neocortical areas and many subcortical nuclei including limbic structures and is thus well placed to coordinate the actions of an organism in relation to both its internal and external environment. An example of such an integrative function is the reward/novelty-related modulatory inputs from midbrain dopamine (DA) neurons and the spatial/mnemonic-related inputs from the hippocampus that project onto the very same PFC circuits. The PFC of rodents and primates receives a direct projection from the ventral CA1 subiculum region of the hippocampus (Rosenzweig and Van Hoesen 1977; Swanson 1981) that directly activates pyramidal cells (Degenaerts et al. 2003) and interneurons (Tierney et al. 2004), thereby mediating a feedforward inhibition of PFC networks. This pathway is important for the temporal organization of behavior and for some short-term memory processes (Seamans et al. 1995; Floresco et al. 1997). Interestingly, correlated firing and coherence in the theta band is selectively increased during behaviors that recruit spatial working memory (Jones and Wilson 2005; Siapas et al. 2005), and it is suggested that the feedforward activation of PFC interneurons by the hippocampus could be a mechanism by which hippocampal theta rhythms could entrain the activity of PFC pyramidal cells.

The mesocortical DA projection, originating in the ventral tegmental area (VTA), innervates the PFC of primates as well as of rodents (Thierry et al. 1973; Lewis et al. 1987), and both pyramidal cells and interneurons express DA receptors (Vincent et al. 1993; Le Moine and Gaspar 1998). In terms of the effect of DA on PFC cellular activity, in vivo experiments in rodents predominantly find that DA decreases the spontaneous firing of pyramidal cells (Sesack and Bunney 1989; Parfitt et al. 1990; Pirot et al. 1992). However, little is known on the in vivo effect of DA on cortical GABAergic interneurons, a neuronal population that plays an important role in the modulation of pyramidal cell excitability, oscillatory firing, the synchronization of cell firing, and the gating of inputs to local microcircuits by feedforward processes (Traub et al. 1996; Galaretta and Hestrin 2001; Swadlow 2003). In vitro studies find that DA increases the excitability of PFC interneurons (Zhou and Hablitz 1999; Gorelova et al. 2002; Gao and Goldman-Rakic 2003), and in the only in vivo study (Tseng et al. 2006), VTA chemical stimulation increases the firing of PFC parvalbumin (PV) containing interneurons. In terms of function, when prefrontal DA signaling is exaggerated or blocked, cognition as a whole is impaired (Brozoski et al. 1979; Weinberger et al. 1988).

Given the important role of interneurons in the temporal structuring of neural activity and the role of DA in modulating the cognitive functions mediated by prefrontal cortices, we sought to study, in anesthetized rats, the effect of DA on interneuron activity. To this end, we studied the effect of DA microiontophoresis and VTA electrical stimulation on 1) PFC interneuron spontaneous activity and 2) their response to a well-defined synaptic input by electrically stimulating the ventral hippocampal CA1 field. Interneurons were identified morphologically by the juxtacellular injection of neurobiotin or the basis of electrophysiological criteria (Tierney et al. 2004) and in a subset of experiments were tested for PV immunoreactivity.

Materials and Methods

Animal preparation

Experiments were conducted in 73 male Sprague-Dawley rats (Charles River, L’Arbresle, France) weighing 280–350 g. All experiments were performed in accordance with European Ethical Standards for the care and use of laboratory animals (86/609-EEC). Animals were placed in a stereotaxic apparatus (Unimécanique, Asnières, France) after anesthesia induction with a 400-ng/kg intraperitoneal injection of chloral hydrate. Anesthesia maintenance was ensured by intraperitoneal infusion of chloral hydrate with a peristaltic pump set at 60 mg/kg/h turned on 1 h after induction. Proper depth of anesthesia was assessed regularly by testing the limb withdrawal reflex and monitoring of electroencephalography for signs of arousal. To increase recording stability, the cistern was drained. Body temperature was maintained at 36.5 °C by a homeothermic blanket.
**Electrophysiological Procedures**

The VTA was stimulated with a concentric bipolar electrode (300 μm diameter, 300 μm tip-barrel distance) stereotaxically positioned at the following coordinates: −5.8 mm from bregma, 0.6 mm from the midline, and 8.1 mm from the cortical surface (Paxinos and Watson 1986, Fig. 3.4). An identical electrode was stereotaxically positioned at anterior, −6.3 mm from bregma; lateral, 5.3 mm from the midline; and depth, 5.5 mm from the cortical surface to stimulate the hippocampal CA1/ventral subicular region (Fig. 7C). In the VTA modulation of hippocampal inputs experiments, the latency of hippocampal stimulation was timed such that the hippocampal-induced excitatory response occurred halfway through the VTA-induced inhibitory period. Due to the variability in the duration of the VTA-induced inhibition, the hippocampus was stimulated 5–35 ms after VTA stimulation. For most cells (8/12), the hippocampus was stimulated between 20 and 35 ms after VTA stimulation. For 2 cells, the hippocampus was stimulated 10 ms after the VTA, and for the remaining 2 cells, the hippocampus was stimulated 5 ms after the VTA.

Single-pulse stimulation (300 μs duration) of the VTA was applied at a frequency of 0.5 Hz for a minimum of 80 s (i.e., 40 sweeps). For each cell, poststimulus time histograms were generated. Stimulation intensity was initially set at 0.2 mA and gradually increased up to a maximum of 0.8 mA until the threshold for excitatory or inhibitory responses, if present, was obtained. At the end of the experiment, the position of the stimulating electrodes was marked by an electrical deposit of iron (4 μA positive current, 20 s) and observed on histological sections following a ferri-ferricyanide reaction.

Extracellular unit recordings were performed in the PL/MO areas of the PFC (anterior: + 2.8–4 mm from bregma; lateral: 0.4–1 mm from the midline; depth: 2.5–4 mm from the cortical surface (Paxinos and Watson 1986) using glass pipettes (20–30 MΩ) containing 2% neurobiotin (Vector Labs, Burlingame, CA).

For the iontophoresis experiments, 5-barrel glass pipettes (Harvard Apparatus, Kent, UK) were used (10–40 MΩ). The glass recording pipette was glued under microscopic control 15–30 microns below the tip of the 5-barrel iontophoresis pipette. This particular preparation reduces current artifacts (Crossman et al. 1974). Furthermore, in preliminary control experiments, we tested for current and hydronium ion effects with a solvent-only filled barrel and observed no effect on interneuron firing rates with this particular preparation.

All recordings were obtained using an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA) operated in bridge mode, amplified (×1000) and filtered (0.3–3 kHz) with an AC/DC amplifier (DAM 50, World Precision Instruments, Hertfordshire, UK). The electrical activity was directly sampled with a CED Micro1401 (Cambridge Electronic Design, Cambridge, UK) and saved for offline analysis with Spike2 software (Cambridge Electronic Design). Five high-impedance current control units (Bionic Instruments, Bris sur Forges, France) were used to deliver currents for the iontophoresis experiments. Retention currents were set at 8–10 nA. Ejection currents were adjusted for each cell. In brief, DA was initially iontophoresed with a current of 10 nA and increased in increments of 10–20 nA until an effect was observed. The current for antagonists was initially set at 20 nA greater than that of the DA ejection current and eventually increased by steps of 10 nA up to 80 nA.

**Drug Preparation and Administration**

For the intravenous (I.V.) experiments, the DA-D1 antagonist SCH 23390 (0.2 mg/kg at 0.35 mg/ml) and the DA-D2 antagonists (-)-sulpiride (30 mg/kg at 52.5 mg/ml) or (S)-raclopride tartrate (0.2 mg/kg at 0.35 mg/ml) (Sigma-Aldrich, Lyon, France) were combined into a single syringe and injected via a lateral tail vein. For iontophoresis experiments, individual barrels were filled with 1 of the following solutions: DA-HCl (500 mM, pH 4.0), SCH 23390 (20 mM, pH 3), (-)-sulpiride (50 mM, pH 4.0), raclopride (100 mM, pH 4.0), (-)-bicuculline methiodide (100 mM, pH 3), gamma-amino butyric acid (GABA, 1000 mM, pH 4.0), kynurenic acid (200 mM, pH 8.0). All drugs were purchased from Sigma-Aldrich and were expelled with a positive current, except for kynurenic acid. Hydronium ion effects were tested using a saline filled barrel at pH 3 and no such effect was observed.

**Juxtacellular Labeling Procedure**

Single units were labeled using the technique of juxtacellular injection of neurobiotin (Pinault et al. 1996). Positive pulses of current (1–6 nA,
200 ms duration) were delivered at a frequency of 2.5 Hz through the bridge circuit of the amplifier. The current was slowly increased and the electrode was advanced by steps of 1 μm (LS-1000 Inchworm Motor Positioning System, Burleigh Instruments, Fishers, NY) until cell discharge was driven by the injected current. Current pulses were applied for a 10- to 30-min period to obtain a reliable labeling of neuronal processes. When 2 cells (e.g. one with a short action potential and the other with a larger one) were simultaneously recorded, an attempt was made to juxtacellularly label only 1 of the cells. Cell discharge was monitored during the injection procedure to determine which cell from the pair was injected.

One to 2 hours after neurobiotin injection, the animal was deeply anesthetized with sodium pentobarbital (200 mg/kg, intraperitoneally) and was perfused with 250 ml of saline followed by 500 ml of 4% paraformaldehyde in phosphate buffer (PB), 0.1 M, pH 7.4. In all experiments, brains were postfixed for 1 h in the same fixative solution and was then converted to a permanent nickel-enhanced diaminobenzidine product. Briefly, after several rinses in PB, neurobiotin was revealed by incubation of the sections in the avidin-biotin peroxidase complex (Vector Labs; 1:100) in PB containing 0.3% Triton X-100 for at least 12 h at 4 °C. Incubated sections were washed in PB (2 × 30 min) before immersion in a solution containing 0.05% 3,3' -diaminobenzidine tetrahydrochloride (Sigma), 0.4% nickel ammonium sulfate, and 0.0006% H2O2. After several washes in PB, sections were mounted on gelatin-coated slides, counterstained with safranine, and dehydrated through alcohol to xylene for light microscopic examination.

**Immunohistochemistry and Neurobiotin Revelation**

Cells were tested for PV immunoreactivity. To this end, streptavidin conjugated to Alexafluor 488 (Invitrogen, Carlsbad, CA) was used as a fluorescent marker of neurobiotin. Brains were cut at 70 μm using a freezing microtome, sections were initially immersed in a blocking solution composed of 2% bovine serum, 10% goat serum, and 0.2% Triton-X solution for 2 × 30 min. Sections were then placed for 1 h at room temperature in a bath composed of 1% bovine serum, 5% goat serum, 0.2% Triton-X, and the primary antibody: mouse monoclonal anti-parvalbumin (Sigma, St Louis, MO; 1:4000), and then incubated overnight at 4 °C. On the next day, sections were rinsed 3 × 5 min in phosphate-buffered saline (PBS) and then incubated 2-3 h in a solution of 1% bovine serum, 5% goat serum, 0.2% Triton-X, and the secondary antibodies: TRITC (Southern Biotech, Birmingham, AL; 1:200) and Alexafluor 488 conjugated to streptavidin (1:200).

Sections were individually mounted in PB. Once the section with the recorded neuron was located, photographs were taken with a digital camera and acquired onto a computer. For morphological analysis, coverslips were removed and the sections were rinsed 3 × 5 min in PBS and returned to a PBS solution overnight. The next day, fluorescence was then converted to a permanent nickel-enhanced diaminobenzidine product. Briefly, after several rinses in PB, neurobiotin was revealed by incubation of the sections in the avidin-biotin peroxidase complex (Vector Labs; 1:100) in PB containing 0.3% Triton-X 100 for at least 12 h at 4 °C. Incubated sections were washed in PB (2 × 10 min) before immersion in a solution containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Sigma), 0.4% nickel ammonium sulfate, and 0.0006% H2O2. After several washes in PB, sections were mounted on gelatin-coated slides, counterstained with safranine, and dehydrated through alcohol to xylene for light microscopic examination.

**Data Analysis**

Action potential duration was measured on the 2nd phase of the spike and the criteria for identification as an interneuron was that the action potential be inferior to 0.6 ms. Unless specified otherwise, in the I.V. injection experiments, data from the 5- to 20-minutes post-injection period was used for comparison to the control condition. For the determination of excitatory responses and periods of inhibition in the post-stimulus time histogram, 2 ms bins were used. Baseline activity was defined as the mean bin value of the 300 ms preceding stimulation. For early excitatory responses, an average was calculated for each bin that corresponded to the mean of that bin and the subsequent 2 bins. The threshold for determining an excitatory response was done on the average bin values and was defined as the 1st bin of: (4 consecutive

---

**Figure 2.** (A) Histogram of binned activity of a single interneuron illustrating the immediate GABA-mediated inhibition in contrast to the more progressive and prolonged reduction in activity induced by DA application. (B) For the same interneuron, application of a GABA_A receptor antagonist does not affect the DA-induced reduction in activity, suggesting a direct effect of DA on interneuron activity. (C) For this cell, only the application of D1 and D2 receptor antagonists blocked the DA-mediated reduction in activity. (D) Individual histogram bars represent the mean activity of all recorded cells for each condition as a percentage of the baseline activity that preceded drug application. For the majority of cells (n = 8; left bars) GABAA receptor antagonist had no significant effect on the DA-induced inhibition. For the remaining cells (n = 5; right bars) a GABA_A receptor antagonist blocked the DA-induced inhibition (significant difference denoted by asterisk).
bins with values) > (mean baseline bin value + 2 standard deviations). Excitatory response offset was defined as the 1st of 4 consecutive bins with values below this threshold. For the late excitatory responses, the same method was used with the exception that 10 ms bins were used. For inhibitory periods, 5 bin averages were computed. Due to the relatively low levels of spontaneous activity for some of the cells and...
their irregular firing rate, the threshold for an inhibition was defined as the 1st bin of 6 consecutive bins with values < (66% of mean baseline bin value). Inhibition offset was defined as the 1st bin of 6 consecutive bins with values above threshold. To quantify the efficacy of the inhibition, a failure rate was calculated for each cell and was defined as the number of sweeps for which an inhibition was absent as a percentage of the total number of sweeps. Drug effects were quantified by calculating the failure rate observed during the period of time corresponding to the interval between the onset and offset of the inhibition in the control condition. Because GABA antagonists have a tendency to increase spontaneous activity, we calculated a normalized inhibition efficacy score in addition to the failure rate for the GABA antagonist experiments by dividing the failure rate by the average number of spikes during 300 ms of baseline activity multiplied by the duration of the inhibition. For the temporal variability study, the latency of the 1st action potential of the excitatory response to each pulse of hippocampal electrical stimulation was used to determine the mean latency and standard deviation for the response of each cell before and after VTA stimulation or DA iontophoresis. To further quantify the temporal modulation of the response, the coefficient of variability (standard deviation divided by the mean) of the response for each cell was also calculated based on the latency of the 1st action potential.

For the iontophoresis experiments, DA was iontophoresed for 60 s. Antagonists were iontophoresed 60 s prior to DA, maintained during DA administration, and terminated 60 s after DA. This was done to account for lingering extracellular DA still present once DA administration was terminated. Subsequent trials on the same cell were only performed once activity levels returned to baseline levels (defined as average activity in the 2 min preceding the current trial), with a minimum of 5 min between trials. The duration of the inhibition induced by DA iontophoresis was measured and the firing rate during that period was calculated. To quantify the effect of DA antagonists, using the onset and offset times from the DA condition, the firing rate of the interneuron was calculated during that period.

For all comparisons, paired Student’s t-tests were used to determine if treatment condition had a statistically significant effect on the response in comparison to control. For some experiments, multiple paired t-tests were performed because some cells did not receive the full battery of tests. To adjust for the increased probability of a Type I error due to these multiple comparisons on our partially overlapping data set, stated P-values were adjusted using the Bonferroni method. For the comparison of inhibition duration changes induced by GABAA-R antagonists, the 2 groups did not have equal variance so a Wilcoxon-signed rank test was used. All means are expressed with standard deviation.

Results

Expression of PV
Of the 52 recorded interneurons, 23 were labeled by juxta-cellular injection, and a subset of these labeled cells were tested for PV immunoreactivity (n = 11). All the interneurons...
were located in laminar layer V. Seven interneurons were positive for PV, whereas 4 were negative. We did not observe any difference between the PV positive or negative neurons with respect to their response to VTA stimulation, iontophoresis of DA, the sensitivity of the DA-mediated inhibition to D₁ or D₂-R blockade, or response to hippocampal stimulation. However, this could be due to the relatively small sample size and/or the fact that only layer V cells were studied. Since all fast-spiking interneurons express PV and we also observed DAergic modulation of interneurons that did not express PV, these results suggest that our recordings were not biased for a certain type of interneuron and that presumably other electrophysiological types of interneurons were modulated by DA.

**Effect of DA Iontophoresis on Interneuron Activity**

The iontophoretic application of DA consistently reduced, in a reversible manner, the firing rate of all recorded interneurons (reduction of $60 \pm 16\%$, $n = 41$). The inhibitory effect was proportional to the ejection current, where at low intensities no effect was observed and with progressively greater intensities the inhibition intensity and duration increased (Fig. 1A). In most cases, the interneuron’s firing rate decreased 10-20 s after the onset of DA application and returned gradually over 1-2 min to its basal firing rate after DA application was stopped. The iontophoresis of either a D₁-R and/or D₂-R antagonists on the same cell consistently reduced the DA-induced inhibition ($n = 14$; Fig. 1B). The application of the D₁-R antagonist (SCH 23390) reduced the inhibition by an average of $60 \pm 31\%$. The application of a D₂-R antagonist (sulpiride or raclopride) on the same cell showed a tendency to more potentmly reduce the inhibition by an average of $80 \pm 26\%$ (Fig. 1C). The reduction of the inhibition induced by application of a D₁-R or a D₂-R antagonist was significant (paired t-test, adjusted $P = 0.004$). However, the difference between the effectiveness of D₁-R and D₂-R antagonists was not statistically significant (paired t-test, adjusted $P = 0.55$). In sum, both classes of DA-Rs mediated the inhibition of interneuron activity.

DA could operate directly on interneurons or presynaptically on GABAergic axon terminals to favor the release of GABA. To test this possibility, GABA₂-R were blocked during DA application ($n = 13$). As illustrated in Figure 2A-C, despite GABA₂-R blockade (bicuculline) and the concomitant increase in interneuron activity, DA application still decreased the spontaneous activity of a majority of cells ($n = 8$). The decrease was of $57 \pm 11\%$ under DA alone and of $45 \pm 16\%$ under GABA₂-R antagonists + DA; this difference was not statistically significant (paired t-test, adjusted $P = 0.50$). In 6 of these cells, DA application induced an equivalent inhibition, and in the other 2 cells, the inhibitory effect of DA was reduced by $25\%$ as compared with DA application alone. In the remaining cells ($n = 5$), the DA-mediated inhibition was completely blocked by a GABA₂-R antagonist (paired t-test, adjusted $P = 0.003$; Fig. 2D).

Overall, for a majority of the tested cells ($n = 8$), DA still had an inhibitory action on interneuron firing when GABA₂ receptors were blocked. In the remaining cells ($n = 5$), the DA-induced reduction in firing rate was blocked.

**Effect of VTA Stimulation on PFC Interneuron Firing Rate**

The effect of VTA electrical stimulation was tested on 52 interneurons (Fig. 3A) and 2 patterns of response emerged. For a subgroup of cells ($n = 17$, 33%), VTA stimulation induced a short-latency excitatory response (latency: $7.5 \pm 3$ ms) consisting of 1 or 2 action potentials that was followed by an inhibitory period (latency: $17.6 \pm 8.5$ ms and duration: $90 \pm 56$ ms, Fig. 3B). For the majority of cells ($n = 35$, 67%), VTA

**Figure 7.** Cumulative response of interneurons to hippocampal stimulation (black line) and influence of the iontophoretic application of DA or VTA stimulation (gray area). In inset, each data point depicts the response probability of each individual interneuron to hippocampal stimulation during control plotted against DA application or VTA stimulation. (A) The iontophoretic application of DA during hippocampal stimulation ($n = 7$) significantly reduced the probability of an interneuron response to hippocampal stimulation. (B) VTA stimulation ($n = 12$) applied 5-35 ms before hippocampal stimulation also reduced the probability of an interneuron response to hippocampal stimulation. (C) Representative drawing of position of stimulating electrode in CA1/subicular region (gray dot). AP coordinate is given relative to bregma.
stimulation induced only an inhibition of interneuron activity (latency: 14.2 ± 6.6 ms and duration: 85 ± 47 ms, Fig. 3C). There was no significant difference in the latency or duration of the inhibition in both subgroups. To quantify the efficacy of the inhibition, the failure rate (number of sweeps for which an inhibition was absent as a percentage of the total number of sweeps, see Materials and Methods) was calculated for each cell. The mean failure rate of the inhibition for all the recorded interneurons was 5.1 ± 3.7%. For most of the cells in both subgroups (n = 41, 73%), the inhibitory period was followed by a long lasting increase in activity (latency: 135 ± 83 ms and duration: 174 ± 153 ms, Fig. 3A).
In 18 cases, an interneuron and a pyramidal cell were simultaneously recorded with the same electrode, and in each case, both the interneuron and the pyramidal cell presented an inhibitory component in their response to single-pulse VTA stimulation. The duration of the inhibition was significantly greater for the pyramidal cell than for the interneuron (139 ± 70 ms and 65 ± 26 ms, respectively; paired t-test, P < 0.001). In 8 out of the 18 recorded pairs, the pyramidal cell response included an antidromic action potential. Interestingly, the latency of the antidromic response (7.3 ± 2 ms) was in the same range as the latency of the interneuron early excitatory response (7.5 ± 3 ms), suggesting that the excitatory response resulted from the antidromic activation of recurrent collaterals of PFC pyramidal cells that project to the VTA (Fig. 4A).

**Pharmacological Analysis of the VTA-Induced Responses**

To test if the early excitatory responses had dopaminergic and/or glutamatergic components, D1-R (SCH 23390) and/or D2-R antagonists (raclopride or sulpiride) or a general glutamate receptor antagonist (kynurenic acid) was iontophoretically applied during VTA stimulation. The administration of a D1-R antagonist and of a D2-R antagonist in all cases did not significantly change the probability of an excitatory response to VTA stimulation (n = 6, paired t-test, adjusted P = 0.328; Fig. 4B). In contrast, the iontophoretic application of kynurenic acid considerably reduced (69%) the early excitatory response with low intensity (40–60 nA) and was able to block it with high-intensity (80–100 nA) ejection currents (n = 6, paired t-test, adjusted P < 0.001; Fig. 4C).

In sum, the application of a glutamate antagonist, but not that of DA antagonists, could completely block the early excitatory response of interneurons to VTA stimulation, suggesting that it is of glutamatergic origin.

Because the PFC receives both DAergic and GABAergic projections from the VTA, we tested the effect of DA-R antagonists, as well as of a GABA_A-R antagonist (bicuculline), on the inhibition induced by VTA stimulation. In the 1st series of experiments (n = 12), DA antagonists were administered intravenously. The mean failure rate after i.v. administration of (D1+D2)-R antagonists was significantly increased to 25.6 ± 15.8% (paired t-test, P = 0.003, Fig. 5). To determine the DA receptor subtypes implicated in the VTA-mediated inhibition of interneurons, D1-R and/or D2-R antagonists were iontophoresed during VTA stimulation. The iontophoretic application of a D1-R antagonist (n = 12) increased the mean failure rate of the inhibition to 11.4 ± 6.7% (paired t-test, adjusted P = 0.028, Fig. 6). The iontophoretic application of a D2-R antagonist (n = 14) increased the failure rate to 17.6 ± 13.6% (paired t-test, adjusted P = 0.028, Fig. 6). This effect was not significantly greater than that induced by the D1 antagonist (paired t-test, adjusted P = 0.7). The concomitant application of (D1+D2)-R antagonists (n = 12) increased the inhibition failure rate by 21.7 ± 8.1% (paired t-test, adjusted P = 0.006, Fig. 6), and this increase was significantly greater than that induced by either D1-R or D2-R antagonists (paired t-test, adjusted P = 0.007 and adjusted P = 0.021, respectively). The reduced efficacy of the inhibition induced by the combined or singular administration of either of the DA-R antagonists was not associated with a consistent effect on inhibition duration. The iontophoretic application of the GABA_A-R antagonist (n = 13) not only significantly increased the failure rate of the inhibition to 44.4 ± 15% (paired t-test, adjusted P = 0.007, Fig. 6) but also reduced the duration of the inhibition by 43% (Wilcoxon-signed rank test, P = 0.031). To confirm that the increase in failure rate was not simply due to a drug-induced increase in interneuron excitability, we also made comparisons after normalizing the data to account for elevated prestimulation levels of activity. The mean control condition inhibition efficacy normalized score was 0.80 ± 0.57, and this was significantly smaller than the mean score after GABA_A-R antagonist application of 1.7 ± 1.08 (paired t-test, P = 0.007).

In sum, the i.v. administration of D1-R and D2-R antagonists as well as their iontophoretic application consistently reduced the efficacy of the VTA-mediated inhibition, and this reduction could be mediated by either class of DA-Rs. The combined administration of both (D1+D2)-R antagonists had a more potent effect than the application of either antagonist alone. GABA_A-R blockade also consistently increased the failure rate and in addition reduced the duration of the inhibition (Fig. 6).

**Effect of DA Iontophoresis and VTA Stimulation on Interneuron Response Probability to Hippocampal Stimulation**

The probability of an excitatory response to hippocampal stimulation was defined as the number of sweeps for which the interneuron responded to hippocampal stimulation as a percentage of the total number of sweeps (see Materials and Methods). The mean probability of a PFC interneuron excitatory response to hippocampal stimulation was 63 ± 25% and was frequently composed of spike doublets, triplets, or bursts. When DA was iontophoresed during hippocampal stimulation, the mean response probability of the hippocampal-induced excitatory response was significantly reduced to 28 ± 25% (n = 6, paired t-test, adjusted P = 0.012, Fig. 7A) in 6 cells and in 1 cell DA had no effect on response probability. The mean reduction of response probability for the 6 cells was 29 ± 13%. Interestingly, for the cell where DA had no effect on response probability, it still reduced the overall magnitude of the response by significantly reducing the number of action potentials elicited by a stimulation pulse. When VTA stimulation was delivered 5–35 ms before hippocampal stimulation, the response probability of the hippocampal-induced excitatory response was significantly reduced to 43 ± 34% (n = 10, paired t-test, adjusted P = 0.001, Fig. 7B). The mean reduction of response probability was 20 ± 14%.

**Effect of DA Iontophoresis and VTA Stimulation on Temporal Variability of Interneuron Excitatory Response to Hippocampal Stimulation**

Iontophoresed DA not only reduced the magnitude but also reduced the temporal variability of the response to hippocampal stimulation (Fig. 8A,B). The mean variance for the latency of the 1st action potential in response to hippocampal stimulation was 13.46 ms when hippocampal stimulation was applied alone. In contrast, when DA was iontophoresed, the mean latency variance was significantly reduced by 44 ± 24% to 8.38 ms (paired t-test, adjusted P = 0.03). The variance data suggest that DA plays an important role in reducing the variability of the response latency of interneurons to hippocampal inputs. To test this possibility, we examined the coefficient of variation of the response (standard deviation of latencies/mean latency). The microiontophoresis of DA consistently reduced the coefficient
of variation, with a mean reduction of 31 ± 16% (paired t-test, adjusted \( P = 0.03 \)).

Stimulation of the VTA also reduced the temporal variability of the response (Fig. 8C). The mean variance for the latency of the first action potential in response to hippocampal stimulation was 3.02 ms when hippocampal stimulation was applied alone. In contrast, when the VTA was concurrently stimulated, the mean variance was significantly reduced by 70 ± 15% to 0.83 ms (paired t-test, adjusted \( P = 0.006 \)). VTA stimulation also consistently reduced the coefficient of variation of the interneuron response to hippocampal stimulation, with a mean reduction of 49 ± 15% (paired t-test, adjusted \( P = 0.003 \)). Because the VTA-induced reduction of the excitatory response to hippocampal stimulation could be due to DAergic as well as GABAergic inputs from the VTA, the action of an I.V. injection of \( \text{D}_1 + \text{D}_2 \) DA-R antagonists was tested on 6 of the 12 interneurons. As illustrated for the interneuron in Figure 8C, for all the tested cells the administration of DA-R antagonists partially reversed the VTA-induced reduction in response probability by 44 ± 22% (paired t-test, adjusted \( P = 0.002 \)) and the VTA-induced reduction in the coefficient of variability by 62 ± 18% (paired t-test, adjusted \( P = 0.002 \)), indicating that DA is involved in this effect.

**Discussion**

This is the 1st in vivo electrophysiological investigation of the effect of direct application of DA on PFC interneuron activity. The major results show that the iontophoretic application of DA consistently inhibited interneuron firing by acting on both \( \text{D}_1 \) and \( \text{D}_2 \) receptors. In the majority of cases, the iontophoretic application of GABA\(_{\text{A}}\)-R antagonist did not block the DA-mediated inhibition of interneuron activity. VTA stimulation induced an inhibition in all the recorded cells whose efficacy was reduced by \( \text{D}_1 \) and \( \text{D}_2 \)-R antagonists and was also decreased by a GABA\(_{\text{A}}\)-R antagonist. Both iontophoresed DA and VTA stimulation reduced the probability of an interneuron excitatory response to hippocampal stimulation. In the vast majority of cases, the inhibitory effect of DA resulted in a reduction of the variability of the latency of the excitatory response leading to a temporal focalization of hippocampus-evoked firing.

**Effect of DA on Spontaneous Activity**

It is generally assumed from in vitro recordings in PFC slices that DA potentiates the activity of local interneurons. In particular, DA increases interneuron excitability (Zhou and Hablitz 1999; Gao and Goldman-Rakic 2003) as well as the spontaneous release of GABA (Retaux et al. 1991) and the frequency of spontaneous inhibitory post-synaptic potential (IPSP) recorded from pyramidal cells (Penit-Soria et al. 1987; Zhou and Hablitz 1999; Gullodge and Jaffe 2001). Overall, the majority of in vitro studies find that DA increases spontaneous cortical inhibitory activity, and when tested, this increase is mediated by either a \( \text{D}_1 \)-R (Gorelova et al. 2002) or by a \( \text{D}_2 \)-R mechanism (Tseng and O’Donnell 2004). In some studies however, DA decreases interneuron signaling by reducing the amplitude of the evoked IPSP recorded from pyramidal cells or the evoked release of GABA. Here again, there is disagreement on whether the effect is mediated by a \( \text{D}_1 \)-R (Pralong and Jones 1993; Law-Tho et al. 1994; Gonzalez-Islas and Hablitz 2001) or a \( \text{D}_2 \)-R mechanism (Retaux et al. 1991; Seamans, Gorelova, et al. 2001; Trantham-Davidson et al. 2004; Wang et al. 2002). Our data showing that in vivo iontophoresed DA consistently reduced interneuron activity by \( \text{D}_1 \)- and \( \text{D}_2 \)-R mechanisms are consistent with these latter results reporting a decrease of evoked cortical inhibitory activity exerted by DA. In addition, the ability of DA to reduce interneuron firing, even in the presence of a GABA\(_{\text{A}}\)-R antagonist, demonstrates that DA, at least for a subpopulation of interneurons, can directly inhibit interneuron activity. For the remaining cells the effect of DA was blocked in the presence of GABA\(_{\text{A}}\)-R antagonists. This suggest that DA can also act indirectly, on a separate population of interneurons, presumably through presynaptic GABAergic terminals (Gonzalez-Islas and Hablitz 2001; Seamans, Gorelova, et al. 2001; Gao and Goldman-Rakic 2003).

In this study, VTA electrical stimulation consistently induced an inhibition of the activity of interneurons located in deep layers. The I.V. administration or the iontophoretic application of DAergic antagonists reduced the efficacy of this inhibition. These results are in agreement with anatomical studies that demonstrate appositions of dopaminergic terminals originating from the VTA on deep layer (V and VI) interneurons (Benes et al. 1993; Ohara et al. 2003). Furthermore, these synaptic contacts are symmetric and thus presumably inhibitory (Verney et al. 1990; Sesack et al. 1995). In addition to the DA component, our results show that the VTA-induced inhibition of interneurons also presents a GABAergic component that is very likely due to the activation of the GABAergic VTA–PFC pathway (Thierry et al. 1980; Sesack et al. 1995).

It is surprising that the effect of DA was similarly mediated by both \( \text{D}_1 \) and \( \text{D}_2 \)-Rs. The only observable difference was a tendency for a larger effect of \( \text{D}_2 \)-R antagonism in comparison to \( \text{D}_1 \)-R antagonism. This could potentially be explained by the higher affinity state of \( \text{D}_2 \)-R (Richfield et al. 1989). Furthermore, though both receptors are mediating similar qualitative effects, functionally they might be different based on whether modulation is occurring predominantly through tonic levels of DA acting on extrasynaptic low-affinity \( \text{D}_1 \)-Rs or phasic DA release acting primarily on synaptic high-affinity \( \text{D}_2 \)-Rs (Smiley et al. 1994; Yung et al. 1995; Caille et al. 1996). Finally, the presynaptic action of DA on interneurons may be acting preferentially on \( \text{D}_1 \)-Rs as is observed in studies on pyramidal cells (Momiyama and Sim 1996; Bourou and Reuter 1999; Behr et al. 2000; Gao et al. 2001; Seamans, Durstewitz et al. 2001).

Besides the inhibitory response, a short-latency fast excitatory post-synaptic potential (EPSP) can be evoked by VTA stimulation in PFC pyramidal cells (Mercuri et al. 1985; Lewis and O’Donnell 2000) and it is suggested to result from a corelease of glutamate from DAergic terminals (Sulzer et al. 1998; Lavin et al. 2005). Accordingly, we observed that VTA stimulation evoked a short-latency excitatory response in 33% of the recorded interneurons. This response is likely glutamatergic in origin because it is reduced or completely blocked by the application of a glutamate antagonist. The latency of this excitatory response strongly suggests that it results from afferents distinct from the DAergic pathway. Indeed, the short latency of the EPSP (8.5 ± 2.3 ms) is incompatible with the conduction time of the DAergic VTA–PFC pathway (19 ± 7 ms, Thierry et al. 1980). Considering that the latency of the excitatory response is in the same range of the PFC–VTA pathway (7.7 ± 5 ms, Thierry et al. 1980), this excitatory response could be due to the antidromic activation of PFC–VTA pyramidal cells that through recurrent collaterals activate the pyramidal cells that through recurrent collaterals activate the...
interneurons. Furthermore, though some VTA neurons that project to the mPFC express a vesicular glutamate transporter (Hur and Zaborszky 2005), it has also been shown that DA neurons can also express this same transporter (Dal Bo et al. 2004). Therefore, though we cannot exclude the possibility that electrical stimulation might be activating a nondopaminergic glutamatergic pathway, the existence of such a pathway remains to be demonstrated.

**Pyramidal Cell and Interneuron Interactions**

Previous electrophysiological studies have shown that stimulation of the VTA induces a monosynaptic EPSP--IPSP sequence in PFC pyramidal cells (Mercure et al. 1985). This sequence is very similar to that classically observed in any cortical region after electrical stimulation of its afferent pathways. Furthermore, activation of another monoaminergic (serotonin) pathway also induces excitatory and/or inhibitory responses (Puig et al. 2005). The IPSP induced in PFC pyramidal cells by the VTA stimulation does indeed contain a GABAergic component that primarily involves cortical interneurons and to a lesser degree GABAergic VTA afferents (Pirot et al. 1992). It was thus hypothesized that DA exerts an excitatory influence on interneurons. However, as demonstrated here, VTA stimulation as well as the direct application of DA induces a reduction of PFC interneuron activity. Consequently, in addition to DA's direct effect, the inhibition of pyramidal cells by VTA stimulation probably also involves the following 2 mechanisms: 1) the activation of interneurons via the antidromic activation of the recurrent collateral network of PFC--VTA projecting neurons; 2) the activation of the GABAergic VTA--PFC pathway. Accordingly, the VTA-induced glutamatergic activation of interneurons immediately preceded the inhibition of the pyramidal cells. In agreement with this proposed mechanism, the iontophoretic application of DA reduces the amplitude of the IPSP in pyramidal cells, and this reduction was hypothetically attributed to an inhibition of interneuron activity by DA (Bernardi et al. 1982). In addition, in favor of an inhibitory influence of DA on interneuron activity, it has also been observed that DA attenuates the PFC activation of putative interneurons in the amygdala (Rosenkranz and Grace 2001), and more recently the feedforward inhibition of PFC pyramidal cells by amygdala stimulation was attenuated by D2- and D4-R agonists (Floresco et al. 2002). This suggests that the VTA-induced glutamatergic activation of interneurons immediately precedes the inhibition of the pyramidal cells. In agreement with this proposed mechanism, the iontophoretic application of DA reduces the amplitude of the IPSP in pyramidal cells, and this reduction was hypothetically attributed to an inhibition of interneuron activity by DA (Bernardi et al. 1982). In addition, in favor of an inhibitory influence of DA on interneuron activity, it has also been observed that DA attenuates the PFC activation of putative interneurons in the amygdala (Rosenkranz and Grace 2001), and more recently the feedforward inhibition of PFC pyramidal cells by amygdala stimulation was attenuated by D2- and D4-R agonists (Floresco and Tse 2007). Overall, this suggests a key role for DA in gating inputs from limbic structures to the PFC and vice versa.

Altogether, the decreased firing of interneurons following DA application and VTA stimulation is consistent with a DA-induced decrease of PFC interneuron excitability. Interestingly, the reduction of hippocampal-evoked firing was accompanied by a reduction in the temporal variability of the response. Although this temporal effect was clearly induced by DA application, it was more efficient with VTA stimulation presumably because DA is being released synaptically in close proximity to glutamatergic inputs (Goldman-Rakic et al. 1989). Furthermore, because VTA stimulation activates both DAergic and GABAergic PFC afferents, the greater effect of VTA stimulation supports cooperation between these 2 components of the VTA--PFC pathway in defining the temporal window for integration of hippocampal inputs by PFC interneurons.

The direct activation of interneurons by hippocampus--PFC afferents leads to a strong feedforward inhibition of pyramidal cell activity such that only pyramidal cells with potent inputs fire action potentials (Tierney et al. 2004). Furthermore, the mesocortical DA system blocks hippocampus-evoked pyramidal cell excitatory responses (Jay et al. 1995), and this damping effect of DA would further increase the spatial selectivity of pyramidal cell firing. The DA-induced reduction in the temporal variability of the hippocampus-evoked interneuron excitatory response would result in a more temporally precise inhibition of pyramidal cells, and this would lead to a greater synchronization of the pyramidal cells that do fire action potentials. Overall, the depressive effect of DA on both cortical interneurons and pyramidal cells would on the 1 hand allow a spatial focialization of afferent excitatory inputs as well as on the other hand induce a higher synchronization of outputs from the PFC, ultimately leading to an improved signal to noise ratio.

**Functional Considerations**

Many studies to date have described the environmental conditions that lead to the burst firing of DA cells (Horvitz 2000; Hyland et al. 2002; Redgrave and Gurney 2006; Schultz 2006). However, there is a lack of information about the modulation of neuronal activity induced by focally increased levels of DA in the microcircuits targeted by the midbrain DA neurons (Day et al. 2007) and is important for learning and ultimately mediating goal-directed behaviors (Goto and Grace 2007). Specifically, prior studies have solely shown either increases or decreases in neuronal activity in response to DA application. In contrast, our results demonstrate that the inhibitory effect of DA on interneuron activity leads to a filtering of excitatory inputs that functionally results in a temporal focalization of interneuron responses. Studies in humans and primates reveal an essential role for neuronal synchronization in the transfer of information between specialized cortical regions during cognitive functions (Singer 1999). In particular, synchrony is associated with oscillatory firing in the beta (15–30 Hz) and gamma (30–80 Hz) frequency bands (Fries et al. 2001; Varela et al. 2001; Schnitzler and Gross 2005). However, with respect to psychiatric disorders, only recently have studies examined the relationship between neuronal synchrony and perturbed cognitive functioning (Uhlhaas and Singer 2006). Despite the frequent treatment of psychiatric disorders with drugs that target the various aminergic systems, very little is known on the role of monoaminergic neurotransmitters in the synchronization of neuronal firing. Our results are the 1st to demonstrate a role for DA in the temporal profiling of interneuron activity. This mechanism could precisely play a role in the synchronization of the higher frequency oscillations associated with cognitive processes. In the particular case of schizophrenia studies, observe a dysfunction of PFC inhibitory circuits (Benes et al. 1996), a hypodopaminergia of the PFC (Goldstein and Deutch 1992), and abnormal cortical activation patterns (Spencer et al. 2003; Uhlhaas et al. 2006). Interneurons are critically important for the temporal ordering of neuronal firing and a deficit in interneuron function, subsequent to a dysregulation of PFC DA, could contribute to the abnormal cortical activation patterns observed in schizophrenic individuals.

**Notes**

P.L.T. is the recipient of training grants from the Institut Servier and the Fondation Pour la Recherche Médicale. The authors would also like to thank A. M. Godicheu for her help with histology and P. O. Polack for advice on data analysis procedures. *Conflict of Interest*: None declared.
References


Dopamine Modulates Temporal Dynamics of Feedforward Inhibition


