

Ventral Tegmental Area Afferents to the Prefrontal Cortex Maintain Membrane Potential 'Up' States in Pyramidal Neurons via D₁ Dopamine Receptors

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The electrophysiological nature of dopamine actions has been controversial for years, with data supporting both inhibitory and excitatory actions. In this study, we tested whether stimulation of the ventral tegmental area (VTA), the source of the dopamine innervation of the prefrontal cortex, would exert different responses depending on the membrane potential states that pyramidal neurons exhibit when recorded *in vivo*, and whether VTA stimulation would have a role in controlling transitions between these states. Prefrontal cortical neurons have a very negative resting membrane potential (*down state*) interrupted by plateau depolarizations (*up state*). Although the *up state* had been shown to be dependent on hippocampal afferents in nucleus accumbens neurons, our results indicate that neither hippocampal nor thalamic inputs are sufficient to drive *up* events in prefrontal cortical neurons. Electrical VTA stimulation resulted in a variety of actions, in many cases depending on the neuron membrane potential state. Trains of stimuli resembling burst firing evoked a long-lasting transition to the *up state*, an effect blocked by a D₁ antagonist and mimicked by chemical VTA stimulation. These results indicate that projections from the VTA to the prefrontal cortex may be involved in controlling membrane potential states that define assemblies of activable pyramidal neurons in this region.

Introduction

Dopamine (DA) is a neurotransmitter involved in several neuropsychiatric disorders, including Parkinson's disease and schizophrenia. This transmitter has been extensively studied since its discovery by Arvid Carlsson (Carlsson *et al.*, 1958), with emphasis on forebrain DA systems (i.e. the nigrostriatal, mesoaccumbens and mesocortical projections). The mesocortical pathway originating in the ventral tegmental area (VTA) and targeting frontal cortical regions (Thierry *et al.*, 1973) participates in functions such as stress, reward, working memory and attention (Abercrombie *et al.*, 1989; Deutch *et al.*, 1990; Schultz, 1992; Schultz *et al.*, 1993; Williams and Goldman-Rakic, 1995). Despite intense efforts, the nature of DA actions on prefrontal cortical (PFC) and striatal neuron physiology remains controversial. Whenever this issue had been addressed, studies typically attempted to determine whether DA is inhibitory or excitatory, with data supporting either view. The notion of DA as a modulator attempts to overcome this problem; indeed, DA-dependent second messenger cascades may be involved in controlling the efficacy of fast synaptic responses. This is exemplified by D₁ receptor-dependent pathways regulating *N*-methyl-D-aspartate (NMDA) receptor phosphorylation (Snyder *et al.*, 1998). A modulatory role for DA on glutamate transmission is also supported by *in vitro* electrophysiological data (O'Donnell and Grace, 1994; Levine *et al.*, 1996b; Nicola *et al.*, 1996; Yang and Seamans, 1996; Hernández-López *et al.*, 1997).

DA can affect synaptic responses by controlling glutamate synaptic transmission (Brown and Arbuthnott, 1983; Pennartz *et al.*, 1992; O'Donnell and Grace, 1994; Nicola *et al.*, 1996), but

also by acting on ion currents in the postsynaptic neuron. The nature of this modulation depends on factors such as the receptor subtype involved and the membrane potential of the target cell. In an elegant series of studies, Michael Levine and colleagues have shown, for example, that D₁ receptors may enhance striatal neuron response to NMDA receptor activation, whereas D₂ receptors may decrease responses to non-NMDA receptors (Levine *et al.*, 1996a,b; Cepeda and Levine, 1998; Cepeda *et al.*, 1998). Furthermore, D₁ receptor activation has also been shown to prolong a persistent voltage-gated Na⁺ current ($I_{Na,p}$) in PFC neurons (Yang and Seamans, 1996) and L-type Ca²⁺ channels in striatal (Hernández-López *et al.*, 1997) and PFC (Yang *et al.*, 1998) neurons. Such modulation by DA may have an impact on transitions between membrane potential states that depend on such currents.

Neocortical pyramidal neurons exhibit *in vivo* a bistable membrane potential. A very negative resting membrane potential (*down state*) is interrupted by plateau depolarizations (*up state*) (Cowan *et al.*, 1994; Cowan and Wilson, 1994). *Up* and *down* membrane potential states have also been observed in PFC neurons (Branchereau *et al.*, 1996), as well as in medium spiny neurons in the striatum (Wilson and Groves, 1981; Wilson, 1993; Wilson and Kawaguchi, 1996) and nucleus accumbens (NAcc) (O'Donnell and Grace, 1995, 1998). *Up events* are driven by excitatory inputs (O'Donnell and Grace, 1995; Wilson and Kawaguchi, 1996), but they may be modulated by ion currents. The *down state* is maintained by a strong inwardly rectifying K⁺ current (Wilson, 1993). Once strong excitatory inputs depolarize the neuron to a point at which this current shuts down, the membrane potential can rapidly move to a more depolarized value, the *up state*. This depolarization can be maintained by Ca²⁺ currents and $I_{Na,p}$ (Wilson, 1993) and the extent of depolarization may be limited by another K⁺ current, the slow A-current (Gabel and Nisenbaum, 1998). Furthermore, at the very negative membrane potential of the *down state*, NMDA receptors are effectively blocked by Mg²⁺, and therefore a D₁-NMDA interaction would not be expressed. On the other hand, during the depolarized *up state*, the Mg²⁺ blockade of NMDA receptors would be partially removed. One prediction that can arise from *in vitro* studies on DA-NMDA interactions (Levine *et al.*, 1996a,b; Cepeda and Levine, 1998; Cepeda *et al.*, 1998) is that D₁ receptors may contribute to stabilizing the *up state* in neurons with a bistable membrane potential. This hypothesis was addressed in this study with *in vivo* intracellular recordings from PFC pyramidal neurons by stimulating the VTA. The role of D₁ receptors in mediating the responses was assessed by repeating the stimulation in the presence of a selective antagonist. Parts of this work have been presented in abstract form (Lewis and O'Donnell, 1999).

Materials and Methods

Animals

In vivo intracellular recordings were performed from 73 neurons in 51 Sprague-Dawley male adult rats (245–415 g). All experimental procedures were carried out according to the USPHS *Guide for the Care and Use of Laboratory Animals* and approved by the Albany Medical College Institutional Animal Care and Use Committee. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed on a stereotaxic apparatus. Supplemental anesthesia (chloral hydrate, 24–30 mg/h) was continuously delivered during the recording session via a cannula inserted i.p. and a minipump. Bupivacaine (0.25%) was applied s.c. before any skin incision was made. Burr holes were drilled in the skull for electrode placement; stimulating electrodes were located in the mediodorsal (MD) thalamic nucleus (rostrocaudal: bregma -2.8 mm, lateral: 0.5–1.0 mm; vertical: -5.3 mm from brain surface), VTA (bregma -5.8 mm; lateral: 0.5 mm; -8.3 mm from brain surface), and either the ventral subiculum (bregma -5.8 mm; lateral: 3.5 mm; -8.4 mm from surface) or the fimbria-fornix system (bregma -1.6 mm; lateral: 2.0 mm; -3.6 mm from surface), which carries the hippocampal afferents to the PFC.

Recordings

Recording electrodes were made of 1 mm o.d. Omegadot borosilicate glass tubing pulled with a P-97 Flaming-Brown puller (Sutter Instrument Co.). Electrodes were filled with 3 M potassium acetate and 2% Neurobiotin and had a resistance of 44–110 M Ω . Recording electrodes were lowered in the PFC (bregma +2.3 to +3.2 mm; lateral: 0.5–0.8 mm; recordings were attempted between 3 and 6 mm below brain surface). These electrodes were advanced using a hydraulic manipulator while monitoring activity on an oscilloscope. Signals were amplified using an IR-283 Neurodata amplifier (Cygnus Technology), filtered at 0.3–3 kHz with an eight-pole Bessel filter, and digitized with an interface board (DAP3215a, Microstar Labs) at 10 kHz and fed into a computer for data storage and offline analysis. All data handling was performed using custom-written software (Neuroscope). Once a stable cell was recorded for 5 min, the data were stored and stimulation protocols were carried out. Only neurons showing at least -55 mV membrane potential and 45 mV spike amplitude measured from threshold were analyzed and included in the study.

Electrical Stimulation

Concentric bipolar electrodes with 0.5 mm between tips were employed for electrical stimulation. Current pulses were generated by stimulus isolation units driven by a Master 8 Stimulator (AMPI, Jerusalem, Israel). Stimulation protocols were controlled by the computer using Neuroscope. Electrical stimulation of the VTA, MD and fimbria-fornix or ventral subiculum were performed by delivering current pulses 0.5 ms in duration and 0.1–1 mA in amplitude every 10 s. The VTA was also stimulated with trains of 5 pulses at 20 Hz or 10 pulses at 5 Hz to mimic DA cell burst firing (Chiodo and Bunney, 1983; White and Wang, 1983) [reviewed by White (White, 1996)]. In some cases, after evoked responses were recorded, the entire procedure was repeated following administration of the D₁ receptor antagonist SCH 23390 (0.3 mg/kg) via a cannula placed i.p.

In a subset of experiments the VTA was chemically, rather than electrically, stimulated ($n = 4$). Instead of the stimulating electrode, a 30-gauge cannula was lowered in the VTA. After recording baseline activity from a PFC neuron, 30 nl of a solution containing 100 μ M NMDA were injected in the VTA with the aid of a syringe pump. As a control, in three experiments 30 nl of 0.9% saline were injected instead.

Histology

After completion of the recordings, Neurobiotin was injected into the cell by passing positive current (1.0 nA, 200 ms pulses at 2 Hz) for at least 5 min. At the end of the experiment, the animals were given a lethal dose of pentobarbital, and transcardially perfused with ice-cold saline followed by 4% paraformaldehyde. Neurobiotin-filled neurons were evidenced by standard histochemical techniques. Brains were cryoprotected in 30% sucrose and sectioned using a freezing microtome. Serial 30 μ m thick

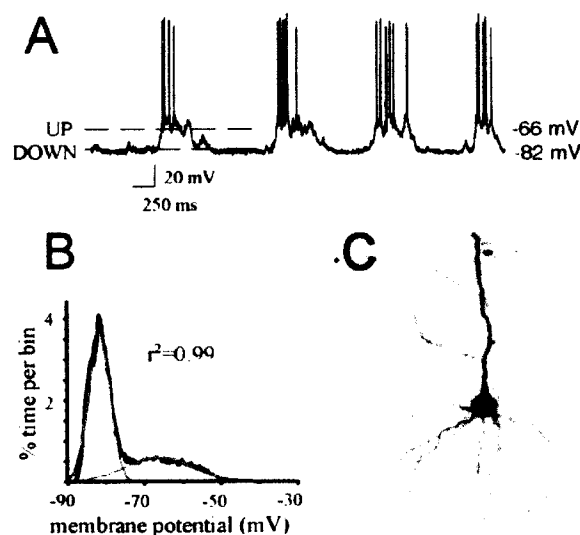


Figure 1. Most prefrontal pyramidal neurons exhibited a membrane potential alternating between up and down states. (A) Representative tracing from a neuron showing four up events during which action potential firing can be observed. (B) Proportion of points at different membrane potential values in the file from which the trace in (A) was obtained. The histogram shows a clear bimodal distribution, indicating a down state (most negative mode) of -82 mV and an up state (more depolarized mode) of -66 mV. The plot was fitted to a dual Gaussian distribution with a confidence of 0.99 using Origin 6.0 (Microcal Corp.); the green lines indicate each of the two normal distributions and the red line the two combined. (C) Neurobiotin injection revealed this as a layer V pyramidal neuron, with a thick apical dendrite extending toward the pial surface (beyond the top of this photograph).

sections were cut coronally through the medial PFC. Sections were incubated in 0.4% Triton X-100 in phosphate-buffered saline for 1–2 h, followed by 2 h in Vectastain Elite ABC reagent (Vector Laboratories). Following a series of rinses, sections were reacted with 3,3'-diaminobenzidine and urea-hydrogen peroxide (Sigma FAST DAB set). Sections were then rinsed, mounted on gelatin-coated slides, air-dried for 24 h, cleared in xylene, coverslipped in Permount and examined on an Olympus CH30 microscope. Neurons were identified morphologically and localized according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998).

Results

In vivo intracellular recordings were performed from neurons in the medial ($n = 53$) and orbital ($n = 20$) PFC. Since neurons from both prefrontal areas exhibited similar responses, their data were pooled. Forty-nine out of 73 neurons recorded exhibited up and down states in their membrane potential (Fig. 1A,B). The presence of a bistable membrane potential was determined when a histogram of membrane potential values over time exhibited a bimodal distribution that could be fitted to two Gaussian curves (Fig. 1B). The down state was -75.7 ± 7.2 mV (mean \pm SD) and the up state averaged -64.6 ± 7.6 mV. All recordings were analyzed with a software routine that determined frequency of transitions as well as average duration of up events. Up events were counted when the membrane potential crossed a threshold value set beyond the more negative (down) mode, typically at halfway between both steady-state membrane potentials, for at least 100 ms. Transitions to the up state occurred at 1.01 ± 0.35 Hz and lasted 362 ± 160 ms. These neurons had an input resistance of 39.6 ± 18.0 M Ω (measured

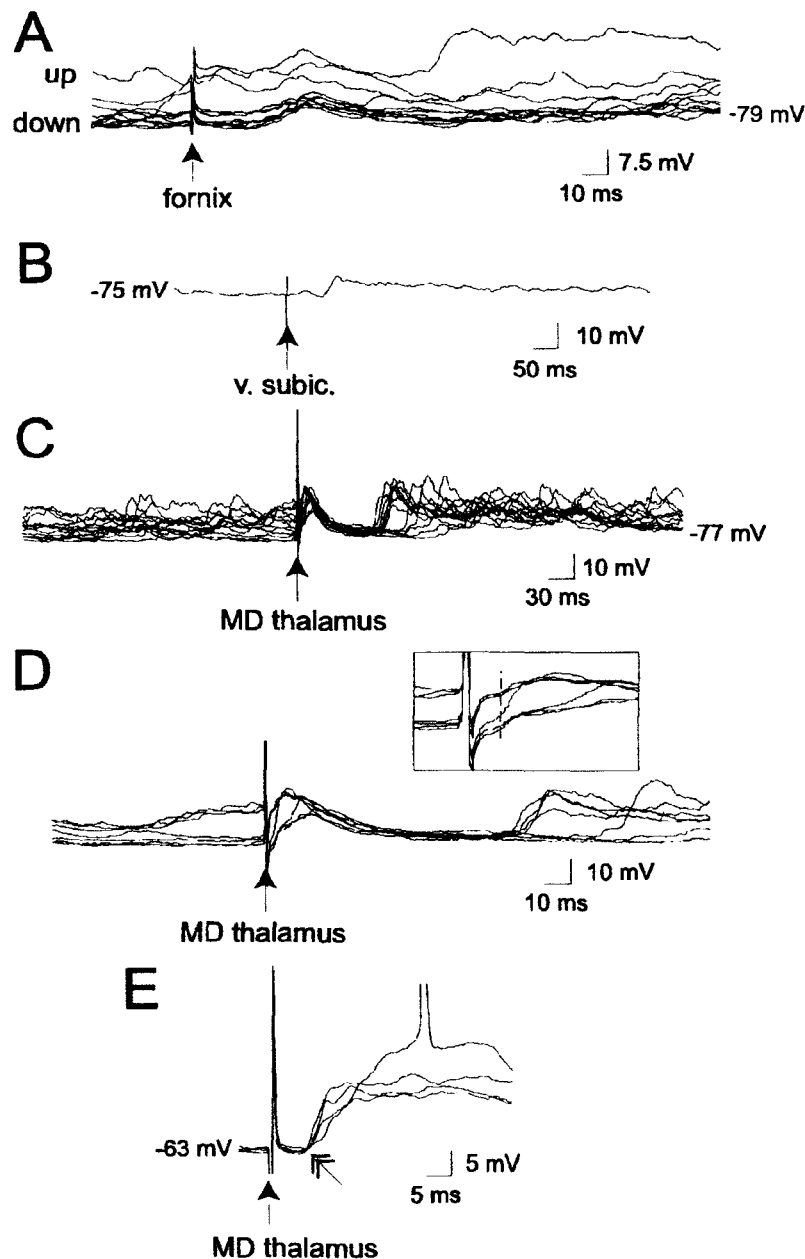


Figure 2. Excitatory responses obtained by glutamatergic afferent stimulation. (A) Stimulating the fimbria–fornix evoked EPSPs. Overlay of 10 traces from a neuron including responses obtained during both *up* (–72 mV) and *down* (–79 mV) states. (B) Stimulation of the ventral subiculum evoked an EPSP with a longer latency. Trace is an average of eight sweeps; note the time scale is 50 ms. (C) Stimulation of the MD thalamus evoked EPSPs. Overlay of 12 traces from the same cell shown in (B), evidencing an EPSP–IPSP sequence. (D) Six of the traces shown in (C) are displayed at a faster time base. Inset: The same six traces are displayed at a four times faster time base, in order to illustrate response onset (vertical dashed line). (E) Example of a PFC neuron response to MD stimulation with increasing stimulus intensity (0.4, 0.6, 0.7 and 0.9 mA) revealing a constant onset latency (double-headed arrow).

during the *down* state), a time constant of 6.4 ± 3.3 ms (measured as the time to reach 63% of a hyperpolarization evoked by a -0.2 nA intracellular current pulse), and an action potential amplitude of 56.2 ± 8.9 mV, measured from threshold. Spontaneous firing rate was 4.0 ± 4.9 Hz (range: 0–20 Hz). These action potentials were observed only during the small depolarizations observed during the *up* state, with an apparent threshold of -56.2 ± 8.9 mV. All neurons recorded and stained

with Neurobiotin exhibited morphological characteristics of pyramidal neurons (Fig. 1C) and were located in deep layers. A near-5 Hz oscillation in the membrane potential was occasionally observed in 10 neurons, independently of whether they exhibited a bistable membrane potential.

Activation of hippocampal afferents evoked synaptic responses in five of nine neurons tested. Stimulation of the fimbria–fornix system, which carries hippocampal afferents,

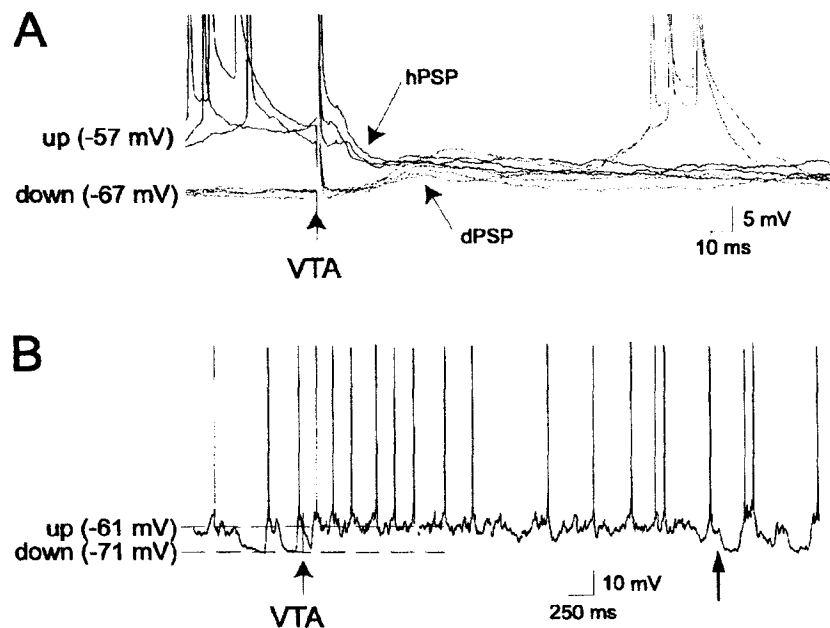


Figure 3. Diverse responses to VTA stimulation. (A) Example of dPSPs evoked during the *down* state and hPSPs evoked during the *up* state. Overlay of three traces recorded when stimulation occurred during the *up* state (-57 mV) with four traces recorded during the *down* state (-67 mV). (B) In some cases, VTA stimulation elicited a long-lasting depolarization resembling the *up* state. Representative trace of one of these responses, showing a return to *up-down* alternations (arrow) at the end of the trace, following the VTA-evoked depolarization that is much longer than 'spontaneous' *up* events. *Up* state: -61 mV; *down* state: -71 mV.

resulted in EPSPs in three of six cases (Fig. 2A) and stimulation of the ventral subiculum evoked EPSPs in two of three cells tested (Fig. 2B). EPSPs evoked by fornix stimulation occurred at 14.8 ± 11.6 ms latency, had 18.1 ± 9.8 mV maximum amplitude and decayed to half amplitude in 17.0 ± 5.2 ms. Stimulation of the MD evoked EPSPs in 20 of 31 neurons tested (Fig. 2C-E) and no response in the others. MD-evoked EPSPs had average maximum amplitudes of 7.2 ± 3.4 mV, latency of 12.3 ± 8.6 ms and duration to half amplitude of 9.9 ± 3.8 ms. In some neurons, the EPSP was followed by an inhibitory postsynaptic potential (IPSP). All synaptic responses exhibited a constant latency upon increasing stimulus amplitude, indicating a monosynaptic response (Fig. 2E). Neither hippocampal nor thalamic afferent activation resulted in transitions to the *up* state in neurons with a bistable membrane potential.

VTA stimulation with single pulses evoked some type of response in 21 out of 30 cases tested. Among the neurons with bistable membrane potential that showed synaptic responses to VTA stimulation (15 out of 20), seven exhibited depolarizing postsynaptic potentials (dPSPs), one had a hyperpolarizing postsynaptic potential (hPSP), four had dPSPs if they were in the *down* state or hPSPs if they were in the *up* state (Fig. 3A), and in three the response was a long-lasting transition to the *up* state (Fig. 3B). An additional neuron exhibited a fixed-latency action potential not preceded by a dPSP, a response probably reflecting antidromic activation of PFC-VTA fibers.

The VTA was also stimulated with trains mimicking burst firing, a pattern observed in DA neurons in the presence of behaviorally relevant stimuli (Schultz, 1992; Schultz *et al.*, 1993). Five pulses at 20 Hz or 10 pulses at 5 Hz elicited a long-lasting transition to the *up* state in 12 out of 14 neurons tested. The membrane potential of these VTA-evoked depolarizations was -64.6 ± 4.7 mV, not different from the spontaneous *up* state in

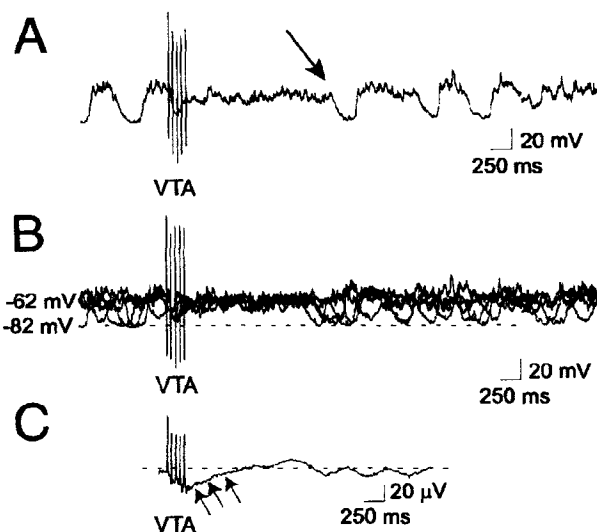


Figure 4. VTA stimulation with trains of pulses evoked a long depolarization resembling the *up* state in most cases. (A) Tracing showing VTA stimulation with five pulses at 20 Hz (vertical lines represent stimulus artifacts). The arrow indicates the return to *up-down* alternations. (B) Overlay of five repetitions showing the consistency of the response. (C) Example of a field potential recording (lowpass-filtered at 300 Hz) showing a negative DC shift (arrows) following VTA train stimulation.

those neurons (-66.3 ± 3.6 mV; $P = 0.45$). These depolarizations lasted between 200 ms and several seconds (Fig. 4A,B). Although the cells were depolarized, firing during this prolonged *up* state was reduced from 1.8 ± 1.5 to 0.6 ± 0.9 Hz (measured in active

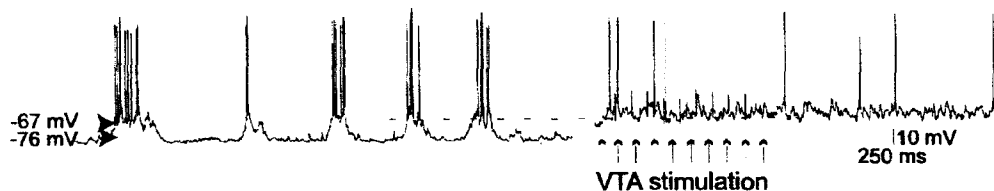


Figure 5. A train of stimuli delivered to the VTA elicited a prolonged depolarization accompanied with a decrease in cell firing. Left trace was recorded before stimulation; right trace was obtained during and after a train of stimuli was delivered (arrows), with a transition to the *up* state and reduced firing.

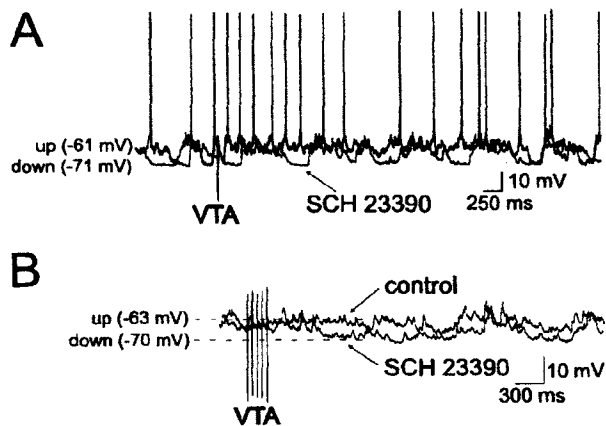


Figure 6. Systemic administration of a D_1 antagonist (SCH 23390, 0.3 mg/kg i.p.) reduced the prolonged depolarization evoked by VTA stimulation. (A) VTA stimulation with single pulses before (grayed trace; it is the same trace shown in Fig. 3B) and after SCH 23390 (black trace) showing a very short depolarization with the VTA stimulation and prompt return to *up-down* transitions following antagonist administration. *Up*: -61 mV; *down*: -71 mV. (B) Tracings showing a train stimulation of the VTA that also evoked a prolonged depolarization (control), but was reduced in duration after D_1 antagonist administration (SCH 23390). *Up*: -63 mV; *down*: -70 mV.

neurons with a depolarization lasting at least one second; $P = 0.02$, paired t -test; $n = 6$; Fig. 5). In some experiments, VTA stimulation evoked negative DC shifts recorded with extracellular electrodes (Fig. 4C; Y. Goto and P. O'Donnell, manuscript in preparation). These field potentials lasted 712 ± 157 ms and had amplitudes of 22.1 ± 7.7 μ V. In four neurons, VTA stimulation was repeated following systemic administration of the D_1 antagonist SCH 23390 (0.3 mg/kg i.p.). Although the transition to the *up* state by VTA stimulation remained, its duration was reduced from 600 ± 132 to 63.3 ± 75.2 ms ($P = 0.03$; paired t -test; Fig. 6).

To control for the possibility of current spread from the stimulation site, some experiments were conducted with chemical VTA stimulation. NMDA (100 μ M) was injected via a 30-gauge cannula and delivered with a syringe pump. In three out of four cases, NMDA injection into the VTA caused a long-lasting depolarization of PFC neurons to a membrane potential similar to the spontaneous *up* state (Fig. 7A), which lasted for 1–3 min. Firing was reduced from 3.6 ± 2.2 to 1.5 ± 1.5 Hz ($P = 0.02$, paired t -test; $n = 4$) during that depolarization. In three neurons tested, saline injection into the VTA did not alter *up-down* alternation or firing rate (Fig. 7B), ruling out non-specific factors in the NMDA-evoked depolarization.

Discussion

PFC pyramidal neurons exhibited a very negative membrane potential (*down state*) interrupted by plateau depolarizations (*up state*) lasting an average of 360 ms. This phenomenon had been reported previously (Branchereau *et al.*, 1996), and it had also been observed in other cortical regions (Cowan and Wilson, 1994) as well as in medium spiny neurons in the caudate-putamen (Wilson and Groves, 1981; Wilson and Kawaguchi, 1996) and NAcc (O'Donnell and Grace, 1995, 1998). During the *down state*, no action potential firing occurred, but the *up state* brought the membrane potential close to firing threshold. Thus, *up* events could be seen as 'enabled' periods in PFC neurons. Any input controlling transitions to the *up* state could allow for a gating mechanism like that reported in the NAcc (O'Donnell and Grace, 1995), with a strong impact on PFC function.

In the NAcc, *up* events are dependent on hippocampal input (O'Donnell and Grace, 1995). However, activation of hippocampal afferents failed to elicit transitions to the *up* state in PFC neurons. This may be due to differences in synaptic organization between these structures. In the NAcc, 5–10% of hippocampal terminals contact proximal dendrites and cell bodies (Meredith *et al.*, 1990), and therefore are positioned to exert a strong influence over NAcc neuron membrane potential. Such an arrangement has not been observed in PFC pyramidal neurons (Carr and Sesack, 1996). Furthermore, ongoing experiments in our laboratory indicate that a hippocampal lesion fails to alter the frequency or duration of *up* events in the PFC (O'Donnell *et al.*, 1999). Thus, neither hippocampal nor thalamic afferents alone may be sufficient to drive PFC neuron *up* states. The excitatory inputs responsible for these transitions may be a combined set of sources, probably including cortico-cortical projections.

VTA stimulation with train pulses mimicking DA cell burst firing evoked a prolonged depolarization resembling the *up* state. Its duration was reduced by a selective D_1 antagonist. The actual VTA-evoked transition had a short latency and was not blocked by SCH 23390. These results indicate that although the onset of *up* events may not involve DA receptor activation, their maintenance could depend on D_1 receptors. Thus, DA contribution to this response may be to maintain the depolarization via a state-stabilizing action (O'Donnell, 1999). Chemical VTA stimulation with NMDA also resulted in a prolonged *up* state. NMDA receptors are known to activate DA cells in the VTA (Mercuri *et al.*, 1992; Wang and French, 1993), evoking burst firing (Seutin *et al.*, 1994). Since this procedure has been shown to evoke DA release in the NAcc (Suaud-Chagny *et al.*, 1992), it is likely to also cause DA release in the PFC. The involvement of DA in the prolonged depolarization is also supported by *in vitro* studies showing that DA can maintain depolarization during tetanic stimulation of glutamate afferents in rat PFC slices (Otani *et al.*, 1998). An extracellular electrode located in the PFC was

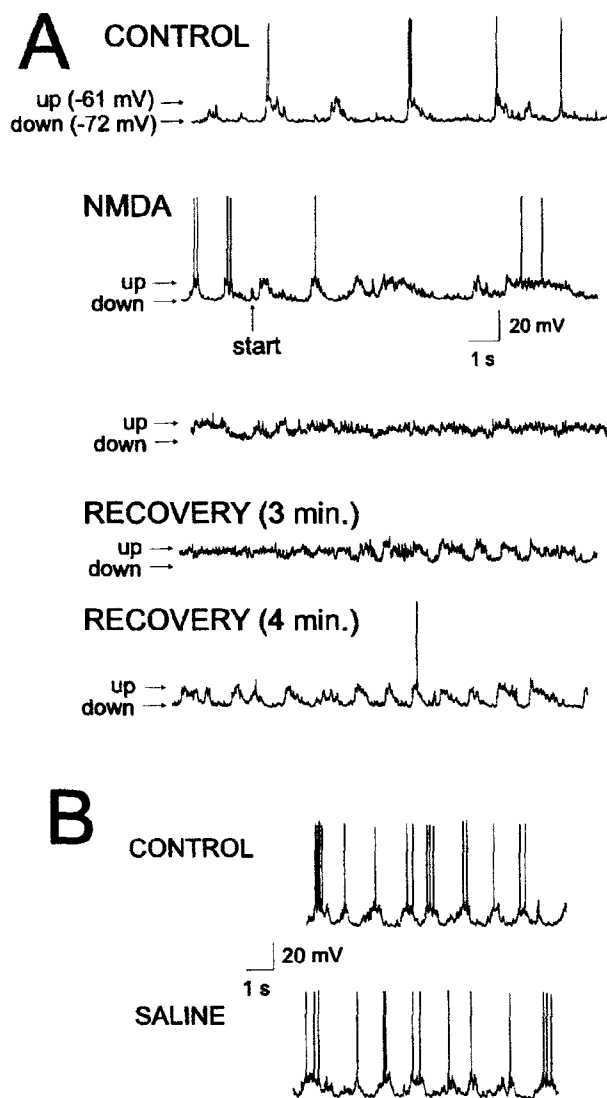


Figure 7. Chemical VTA stimulation with local application of NMDA also elicited a prolonged depolarization in PFC pyramidal neurons. (A) Five traces from the same neuron before (control), during (NMDA), immediately after (unlabeled), and 3 and 4 min after (recovery) intra-VTA NMDA injection, showing a maintained *up* state that reverses within 3 min. *Up*: -62 mV; *down*: -72 mV. (B) Intra-VTA injection of saline did not alter *up-down* membrane potential alternation. Two traces before (control) and immediately after (saline) administration of saline into the VTA.

able to detect a long-lasting field potential response. Since this was only a few microvolts in amplitude, it is unlikely that it would contribute significantly to the intracellular depolarization, which ranges between 8 and 22 mV. Rather, the negative DC shifts may result from synchronous depolarizations to the *up* state in a population of PFC neurons.

Along with the prolonged *up* state, VTA stimulation also decreased PFC neuron firing. This response had been previously observed with extracellular recordings (Ferron *et al.*, 1984; Jay *et al.*, 1995). Furthermore, early intracellular studies reported a reduction in firing along with depolarization in PFC neurons by DA iontophoresis (Bernardi *et al.*, 1978, 1982); membrane potential states, however, were not addressed in those studies.

Depolarization and decrease in firing may be due to independent mechanisms. Indeed, *in vitro* studies reported DA-mediated depolarizations that were not mimicked by combined D_1 and D_2 agonists in PFC (Shi *et al.*, 1997) and NAcc (O'Donnell and Grace, 1996) slices. On the other hand, DA may decrease firing rate via its action on voltage-gated Na^+ channels as demonstrated in the NAcc (Zhang *et al.*, 1998) and PFC (F.J. White, personal communication), or by uncoupling dendritic input zones in apical dendrites from basal dendritic-somatic areas (Yang *et al.*, 1999).

Some components of the responses observed may involve non-DA mechanisms. A few neurons responded with short-latency hPSPs to VTA stimulation. These could be due to activation of GABA projection cells (Steffensen *et al.*, 1998), which comprise a large proportion of VTA neurons. These responses have been typically observed during PFC neuron *up* states, bringing the membrane potential to a value around -70 mV. It is possible that the dPSPs observed with VTA stimulation during the *down* state were also GABA-mediated, since these depolarizations brought the membrane to a value similar to that of hPSPs evoked during the *up* state, which is at the presumed level of an *in vivo* Cl^- reversal potential. Alternatively, dPSPs may have a different source. DA cells have the capacity to release glutamate *in vitro* (Sulzer *et al.*, 1998); if this holds true for the *in vivo* condition, it could explain the VTA-evoked dPSPs in the PFC, particularly those observed at depolarized membrane potentials that cannot be accounted for by GABA. In any event, a fast component in the VTA-evoked response may be responsible for the transition to the *up* state, only to be maintained by the simultaneous release of DA.

A number of potential confounds need to be addressed. First, some of the responses could be due to antidromic electrical activation of PFC-VTA neurons that leave collaterals in the pyramidal cell being recorded. We believe this is unlikely because chemical activation of the VTA with local administration of NMDA (a procedure that would not result in antidromic activation) also evoked a prolonged depolarization. Second, anesthesia levels could affect membrane potential states. In a previous study, we reported that NAcc neurons would go into a prolonged *down* state at near-lethal doses of anesthesia (O'Donnell and Grace, 1995). To avoid changes in anesthesia levels, we used a continuous delivery with a syringe pump and an i.p. cannula. Neurons with a bistable membrane potential have been observed in the presence of a variety of anesthetic agents, including chloral hydrate (O'Donnell and Grace, 1995, 1998) and urethane (Wilson and Kawaguchi, 1996). Another issue derived from the use of an anesthetized preparation relates to whether *up* and *down* membrane potential states are expression of sleep patterns. In a recent study, a strongly periodical 5 Hz oscillation was observed in cortical and striatal neurons (Charpier *et al.*, 1999), similar to what we observed in this study and to what had been reported in the NAcc (O'Donnell and Grace, 1995). The strong periodicity of these oscillations is an indication that they may be related to the also very periodical sleep patterns. Indeed, the 5 Hz oscillation was in phase with EEG cortical spindles (Charpier *et al.*, 1999) in barbiturate-anesthetized animals. On the other hand, the alternation between *up* and *down* states should not be defined as an oscillation, given its very weak periodicity (Stern *et al.*, 1997). It is possible that during certain sleep stages *up-down* transitions may become synchronized and increase their periodicity, contributing to a slow (<1 Hz) EEG oscillation (Steriade *et al.*, 1993; Amzica and Steriade, 1998). However, intracellular

recordings from striatal neurons in unanesthetized or locally anesthetized animals have also shown *up* and *down* membrane potential states (Hull *et al.*, 1970; Wilson and Groves, 1981), even if sensory afferents were stimulated to ensure the animals were awake (Wilson and Groves, 1981). Given the correlation between cortical and striatal *up* states (Stern *et al.*, 1997), it is possible that cortical *up-down* transitions are not related to sleep. This issue will only be solved with intracellular recordings from awake animals.

Together, our results indicate that activation of VTA neurons depolarizes PFC neurons, bringing them to the *up* state (an effect probably not mediated by DA), which is then maintained by DA acting on D₁ receptors. Thus, DA cell burst firing may maintain the *up* state in a population of neurons. This could be an important mechanism involved in PFC function and plasticity. For example, D₁ DA receptors in the PFC are necessary for accurate performance in working memory tasks (Sawaguchi and Goldman-Rakic, 1994; Williams and Goldman-Rakic, 1995). Thérèse Jay has shown that it is easier to elicit long-term potentiation (LTP) in the PFC by hippocampal stimulation following a train of stimuli to the VTA (Jay *et al.*, 1996), even though PFC neurons decreased their firing rate and synaptic responses to hippocampal stimulation were reduced following VTA stimulation (Jay *et al.*, 1995). These findings, at first sight incongruent, could be explained by our observations. A long-lasting VTA-evoked *up* state may provide a PFC neuron depolarization that is sufficient to facilitate NMDA-dependent LTP (by virtue of a removal of the Mg²⁺ blockade of the NMDA channel), while at the same time PFC neuronal firing is reduced. Furthermore, the observed decrease in firing may actually be a mechanism filtering activity unrelated to ongoing behavior. It has been proposed that VTA cell firing may be related to attention and motivational mechanisms (Schultz, 1992). The involvement of DA in these functions may be achieved by its reinforcement of *up* events in an ensemble of PFC neurons, with ensembles defined as a distributed set of neurons in the *up* state (O'Donnell, 1999). Irrelevant activity would be filtered by the reduced firing, and strong or coincident excitatory inputs may be gated by the prolonged *up* state. In this way, VTA projections may reinforce behaviorally relevant assemblies in the PFC by this coincidence-detection mechanism. Although speculative, this could be a general operating principle of DA systems, and pathological conditions in which there is a decrease in DA, such as Parkinson's disease and perhaps negative symptoms of schizophrenia, may result in a poor ensemble-reinforcement with dramatic consequences in the motor and cognitive spheres respectively. On the other hand, an increased mesolimbic DA activity may result in inappropriate ensembles being reinforced, which is likely to result in positive symptoms of schizophrenia.

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