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

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 Arbuthnot, 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 (INa,p) in PFC neurons (Yang and Seamans, 1996) and i-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 INa,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 MΩ. Recording electrodes were lowered in the PFC (bregma +2.3 to +5.2 mm; lateral: 0.5–0.8 mm; recordings were achieved 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-285 Neurodata amplifier (Cygns 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 mM 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 μm thick 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 xylen, coverslipped in Permunt 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 ± 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 MΩ (measured...
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,
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 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
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 \( \mu \)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 \( \mu \)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...
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 D1 and D2 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-induced 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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