

Prefrontal Cortical Up States Are Synchronized With Ventral Tegmental Area Activity

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ABSTRACT The innervation of the prefrontal cortex (PFC) by the ventral tegmental area (VTA) has an important role in incentive-motivation and cognitive functions. Although this projection has been extensively studied, the precise actions of its transmitters, dopamine (DA) and GABA, on PFC pyramidal neurons remain to be determined. We have recently shown that VTA stimulation elicits a sustained depolarization in PFC pyramidal neurons resembling the periodic depolarizations (up states) these neurons exhibit. This response was shortened by a D₁ antagonist, suggesting that DA may sustain depolarized up states in PFC neurons. Here, we tested whether spontaneous PFC up states in vivo require spontaneous VTA activity. Intracellular recordings from PFC neurons conducted simultaneously with VTA local field potentials (LFPs) revealed PFC membrane potential fluctuations occurring synchronously with VTA field potential transitions. Extracellular PFC recordings performed simultaneously with VTA LFPs also indicated a high coherence between these two regions, with VTA oscillations trailing PFC oscillations by a few milliseconds. Furthermore, blockade of VTA activity with lidocaine transiently eliminated PFC LFPs, but not PFC cell up states; instead, up states became irregular during intra-VTA lidocaine administration. These results suggest that baseline levels of VTA activity are necessary for synchronizing PFC pyramidal neurons in the up–down oscillations observed in the anesthetized preparation, allowing the emergence of slow EEG components. **Synapse 52:143–152, 2004.**

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INTRODUCTION

Neurons in the ventral tegmental area (VTA) give rise to dopamine (DA) and GABA projections to the prefrontal cortex (PFC) and nucleus accumbens (Lindvall et al., 1974; Beckstead et al., 1979; Thierry et al., 1979). This innervation is important for cognitive functions such as working memory (Brozoski et al., 1979; Abi-Dargham et al., 2002). For example, sustained firing has been observed in the PFC during a delay in which animals have to hold information required to correctly perform a task (Kojima and Goldman-Rakic, 1982), and this sustained activity depends on a critical level of D₁ receptor activation (Sawaguchi and Goldman-Rakic, 1991, 1994). Although the precise mechanisms allowing persistent PFC activity remain to be determined, it is known that DA can affect activity states in the PFC. The membrane potential of PFC pyramidal neurons and striatal medium spiny neurons alternates between a resting down state and an active, glutamate-driven up state (Wilson and Kawaguchi,

1996; O'Donnell and Grace, 1995; Lewis and O'Donnell, 2000). It has been suggested that the effects of DA on cortical function involve modulation of up–down transitions. It is possible that persistent up states allow sustained activity in the appropriate ensemble of PFC neurons, and the mesocortical system contributes to sustain this activity by maintaining neural ensembles in the up state (O'Donnell, 2003).

According to that view, VTA cell firing would impact PFC electrical activity. DA cells exhibit two firing modes: single-spike and bursting (Grace and Bunney, 1984). Burst firing evokes phasic DA release, while

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single-spike firing results in tonic DA levels (Grace, 1991). Burst firing occurs typically in the presence of reward or reward-predicting stimuli (Schultz et al., 1997; but see Horvitz, 2000); the phasic DA release it causes may be important for persistent activity in the PFC, allowing plasticity mechanisms and working memory functions. Tonic DA release, on the other hand, yields baseline levels that are important for setting the gain of phasic DA release by adjusting DA autoreceptor tone (O'Donnell and Grace, 1998). Although physiological aspects of the PFC DA innervation have been extensively studied, the difference between phasic and tonic effects remains to be determined. We have recently shown that electrical VTA stimulation mimicking DA cell burst firing evokes a transition to the up state in PFC pyramidal neurons recorded in vivo (Lewis and O'Donnell, 2000). This response was shortened in the presence of a D₁ antagonist, indicating that D₁ DA receptors may contribute to sustaining up states. Since PFC neurons exhibit spontaneous up states, it is possible that these are also sustained by tonic VTA activity and baseline DA levels. We tested this possibility by performing intracellular recordings in the PFC simultaneously with field potential recordings in the VTA and by transiently inactivating the VTA with the local anesthetic lidocaine.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (275–350 g) were obtained from Taconic Farms, (Germantown, NY). All procedures involving animals were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and in agreement with the Albany Medical College Animal Institutional Care and Use Committee. Animals were anesthetized with 400 mg/kg chloral hydrate i.p. and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Supplemental chloral hydrate (20–30 mg/kg/h, i.p.) was continuously administered throughout the experiment with the aid of a minipump (Bioanalytical Systems, West Lafayette, IN) to keep anesthesia levels stable. Body temperature was assessed with a rectal probe and maintained at 37°C with a heating pad (Fine Science Tools, Foster City, CA). Bupivacaine (0.25%) was administered subcutaneously prior to skin incisions. Small burr holes were drilled into the skull to allow electrode placement.

Intracellular and extracellular recordings

Recording electrodes were pulled from 1 mm OD Omegadot borosilicate glass tubing (World Precision Instruments, Sarasota, FL) using a P-97 Flaming-Brown microelectrode puller (Sutter Instruments, Novato, CA). Intracellular electrodes were filled with 2M potassium acetate and 2% Neurobiotin, and advanced

into the PFC (2.7 mm rostral to bregma (A); 0.8 mm lateral (L); –3 to –6 mm from brain surface (V)) with the aid of a hydraulic manipulator (Trent Wells, Coulterville, CA). Extracellular electrodes were pulled similarly, but their tips were cut back to 1–10 μ m in diameter. These electrodes were filled with 2M NaCl and 2% Pontamine Sky Blue and lowered into the VTA (A: –5.8 mm, L: 0.5 mm, V: –8.3 mm). Both intracellular and extracellular traces were displayed on a Philips PM 3337 oscilloscope (Fluke, Everett, WA). The intracellular signal was amplified using an IR-283 Neurodata amplifier (Cygnus Technology, Delaware Water Gap, PA). Only neurons with a membrane potential of at least –50 mV, action potential amplitude of at least 40 mV measured from threshold, and a bimodal distribution of the membrane potential with amplitude difference >5 mV were included in this study. After completion of recordings, Neurobiotin was ejected by applying current pulses (1 nA, 200 ms at 2 Hz) for at least 10 min. VTA field potential activity was amplified 10,000 times with a high-gain AC-amplifier (Warner Instruments, Hamden, CT). Both intracellular and extracellular signals were digitized at 10 kHz using an interface board (Microstar Laboratories, Bellevue, WA) and fed to the computer for off-line analysis.

Statistical analysis

Spectral density analyses were conducted in both VTA and PFC recordings using Fast Fourier Transform (FFT) with Statistica (Tulsa, OK). Cross-spectral density was calculated and used to determine coherence between similar-frequency peaks. Squared cross-spectral density was divided by the product of spectral densities of each record. The result was taken as the squared correlation coefficient (r^2). High coherence values were indicators of slow oscillations being synchronized between both sites. In those experiments where lidocaine or saline were applied, before and after treatment comparisons were conducted using the nonparametric Wilcoxon matched pair test (WMPT) because the data were not normally distributed.

PFC extracellular recordings and VTA local lidocaine injection

Rats were anesthetized as described above. Extracellular electrodes were placed in the PFC (A: 2.7 mm, L: 0.8 mm, V: –3 to –6 mm) to record local field potentials (LFPs). A 30G cannula filled with 2% lidocaine in saline (Abbott Laboratories, Chicago, IL) was placed in the VTA (A: –5.8 mm, L: 0.5 mm, V: –7.5 mm) or in the substantia nigra, pars compacta (A: –5.8 mm, L: 2.4 mm, V: –7.4 mm). Upon completion of a 5-min baseline recording, lidocaine or saline (0.3 μ l) was infused at a rate of 0.1 μ l/10 sec using a microsyringe pump. Extracellular PFC activity was continuously monitored for modifications in LFPs (amplitude, frequency, or duration of spontaneous negative deflections).

PFC intracellular recordings and VTA lidocaine injections

In some experiments, intracellular recording electrodes were placed in the PFC while a microsyringe was lowered in the VTA. Lidocaine was injected (0.1 μ l at a rate of 21.6 μ l/h or 36.0 μ l/h) in the VTA while collecting intracellular signals from PFC cells exhibiting up and down membrane potential states.

Dual extracellular recordings

In some animals, two extracellular recording electrodes were used. They were lowered into the VTA and PFC to allow determining the phase lag between oscillatory activities that showed high coherence across both areas.

Histology

Following completion of the experiments, animals were given a lethal dose of sodium pentobarbital (100 mg/kg). Animals were then perfused with saline followed by 4% paraformaldehyde. All brains were removed from the skull and placed in 30% sucrose for cryoprotection. Serial 30–60 μ m coronal sections were cut using a freezing microtome. Sections containing Neurobiotin were further processed for exact identification of electrode placement. These sections were placed in 0.4% Triton X-100 (Sigma, St. Louis, MO) in PBS for 1–2 h followed by a 2-h incubation period with Vectastain Elite avidin-biotin complex reagent (Vector Laboratories, Burlingame, CA). Subsequently, sections went through a series of rinses before they were reacted with 3,3-diaminobenzidine (DAB) and urea-hydrogen peroxide (Sigma Fast DAB set). All other sections were mounted on gelatin-coated slides and Nissl-stained for identification of electrode tracks with an Olympus Optical CH30 microscope (Tokyo, Japan) using the atlas of Paxinos and Watson (1998) for reference.

RESULTS

PFC membrane potential oscillations are correlated with VTA field activity

Intracellular recordings from PFC pyramidal neurons exhibiting up and down membrane potential states were conducted simultaneously with VTA local field potentials. The resting membrane potential of PFC neurons (down state; -71.6 ± 12.5 mV; mean \pm SD) was periodically (0.77 ± 0.33 Hz) interrupted by plateau depolarizations (up state; -62.9 ± 9.7 mV; Fig. 1a). The average duration of up states was 345 ± 192 ms ($n = 13$). Histograms of time spent at different membrane potentials revealed a bimodal distribution that could be fitted to a dual Gaussian function (Fig. 1c). The values of up and down states were calculated from the peaks in these histograms. These characteristics of PFC neuron membrane potential are similar to

what has been previously reported (Lewis and O'Donnell, 2000).

VTA local field potentials exhibited a low-frequency oscillation at 0.65 ± 0.29 Hz ($n = 13$). These oscillations consisted of periodic small-amplitude negative field deflections (Fig. 1b). VTA LFPs occurred simultaneously with PFC neuron up states. FFT was used to determine the dominant frequency components of both the intracellular and extracellular waveforms. This continuous process analysis unveiled slow peaks that were similar in the intracellular and extracellular recordings in 11 out of 13 pairs (PFC intracellular: 0.67 ± 0.38 Hz; VTA fields: 0.60 ± 0.37 Hz; Fig. 1d). Co-spectral density analysis revealed a strong coherence between VTA and PFC signals ($r^2: 0.94 \pm 0.03$; Fig. 1d). In most cases ($n = 7$), spectral analyses revealed dominant slow peaks of identical frequency in both the PFC and VTA (Fig. 1d). In the remaining four cases showing cross-spectral peaks, the individual spectral plots revealed more than one peak in one or both electrodes. The frequency exhibiting high co-spectral density was not necessarily that of the dominant peak. Thus, a correlation between VTA population activity and PFC up states evaluated by coherence between signals was found in all pairs recorded when PFC neurons exhibited a bimodal membrane potential.

The spectral analyses revealed synchronized oscillatory activity in both the VTA and PFC. Although the onset time of intracellular up events in the PFC can be determined with accuracy, the high amplification necessary for extracellular VTA signals prevented measuring the onset times of negative shifts in LFPs with confidence. As an attempt to determine the temporal relationship between both signals, a phase lag for the frequency peak showing high coherence (therefore assumed as synchronized) was calculated using Statistica. VTA oscillations lagged behind PFC oscillations by 9.54 ± 18.88 ms. Although the mean value obtained could be considered consistent with a PFC to VTA drive, this should be taken with caution given the high variability of the results. Lags ranged from 49.2 ms in one direction to 10.0 ms in the opposite. Another factor that may have affected these results was the use of different amplifiers (that could impose different lags) for both signals. To solve this problem, additional experiments were conducted with dual extracellular recordings in the PFC and VTA. In five pairs, both PFC and VTA fields revealed identical 0.95 ± 0.27 Hz slow oscillations. Cross-spectral density analysis unveiled a high coherence between these oscillations ($r^2 = 0.84 \pm 0.09$). Phase-lag analysis revealed that PFC oscillatory activity preceded VTA oscillations by 7.63 ± 4.32 ms. In an additional case, the electrodes were located in the PFC and in the lateral substantia nigra; no correlation was observed in that pair of recordings. Thus, it is probable that PFC up states precede VTA negative shifts in local field potentials.

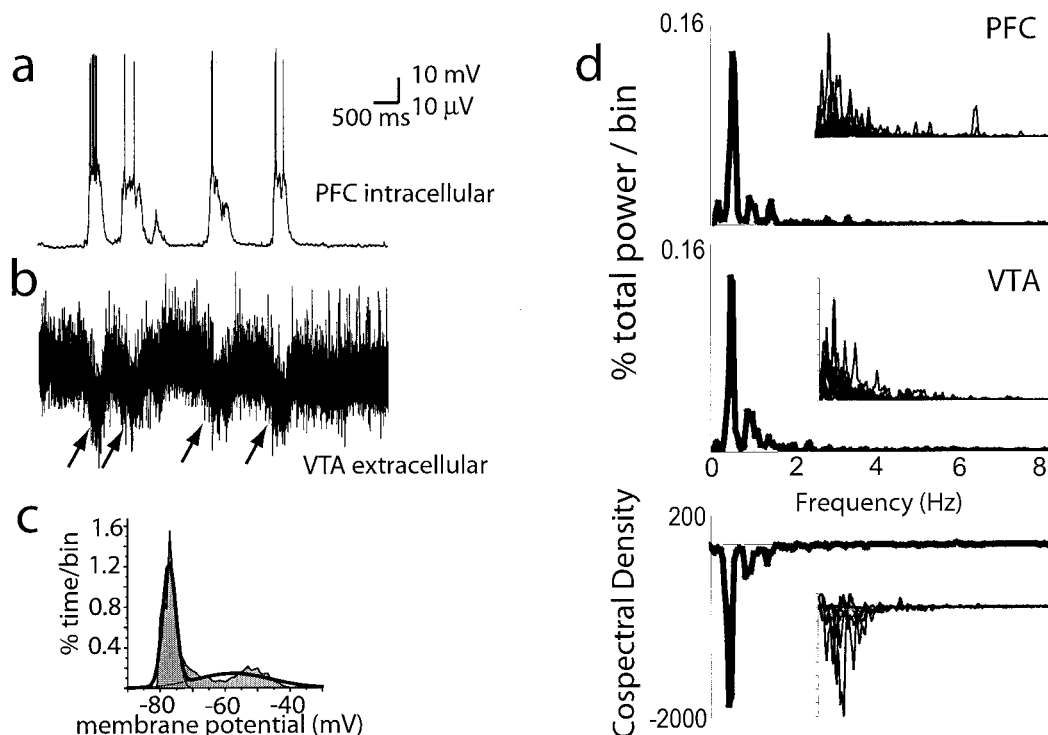


Fig. 1. Correlated activity between PFC and VTA. Representative traces obtained from simultaneous recordings. **a:** PFC pyramidal neuron fluctuating between up and down states, with action potential firing only during the up state. **b:** VTA field potential activity. Negative deflections in VTA LFPs (arrows) occur simultaneously with PFC up events. **c:** Membrane potential distribution histogram obtained from the trace shown in **a**. A bimodal distribution is evident, and it could be best fitted to a dual Gaussian function (thick line). **d:** Top. Fourier analysis of the PFC intracellular waveform shown in **a** reveals a 0.6 Hz peak. The plot has been normalized as the proportion

of the total power in the 0–40 Hz spectra per each bin (bins are 0.4 Hz). Inset is an overlay of spectral density plots from all PFC neurons, showing slow (<1 Hz) peaks. Middle. Fourier analysis of the simultaneously recorded VTA field potential indicating a peak around 0.6 Hz. This plot has also been normalized. Inset is an overlay of spectral densities from all VTA recordings. Bottom. Co-spectral density analysis indicating a high coherence between the intracellular and extracellular dominant frequencies. The data were plotted using their actual power value (μ V/s²). Inset shows an overlay of all co-spectral density plots.

Lidocaine injection in the VTA transiently abolishes cortical field potential oscillations

The correlation observed between VTA population activity and PFC pyramidal neuron up states could be explained by either PFC cells driving VTA activity or by VTA cells sustaining PFC up states. To test these possibilities, PFC recordings were conducted before and after transient VTA inactivation with the local anesthetic lidocaine. Extracellular PFC recordings were used initially, since it has been demonstrated that slow cortical LFP oscillations are correlated with membrane potential shifts in a population of neurons (Steriade et al., 1993). The frequency of LFP oscillations in the PFC was 0.8 ± 0.65 Hz prior to lidocaine administration (0.1 μ l over 10 sec). A few minutes after the injection of lidocaine in the VTA, the slow cortical field potential oscillations disappeared (Fig. 2). Cortical oscillations returned after lidocaine injection with a frequency of 0.82 ± 0.12 Hz. In some cases ($n = 2$), the oscillations returned in 10–15 min; in the rest, they reappeared in 25–40 min. In two cases, cortical field potentials exhibited an on–off pattern for several minutes following lidocaine, with short periods of oscilla-

tion alternating with epochs of flat activity. Saline injection in the VTA (0.1 μ l over 10 sec) did not alter cortical oscillations over a time period of 25 min in all four cases tested (Fig. 3). These results suggest that mesocortical neurons can modulate PFC LFP oscillations.

In an additional group of animals, LFP recordings from the PFC were conducted before and after inactivating electrical activity on a dopamine-containing region that does not project to the PFC. In three animals, lidocaine was injected in the lateral aspect of the substantia nigra, pars compacta. PFC LFPs were not altered in any of them (Fig. 4). This indicates that the loss of LFP oscillations in the PFC is selectively correlated with VTA population activity.

Intracellular recordings in the prefrontal cortex and VTA lidocaine injections

The suppression of PFC oscillations by intra-VTA lidocaine could be due to the elimination of up states or to desynchronization of up states in a local PFC network. To test for these possibilities, intracellular recordings were conducted from five medial PFC neurons

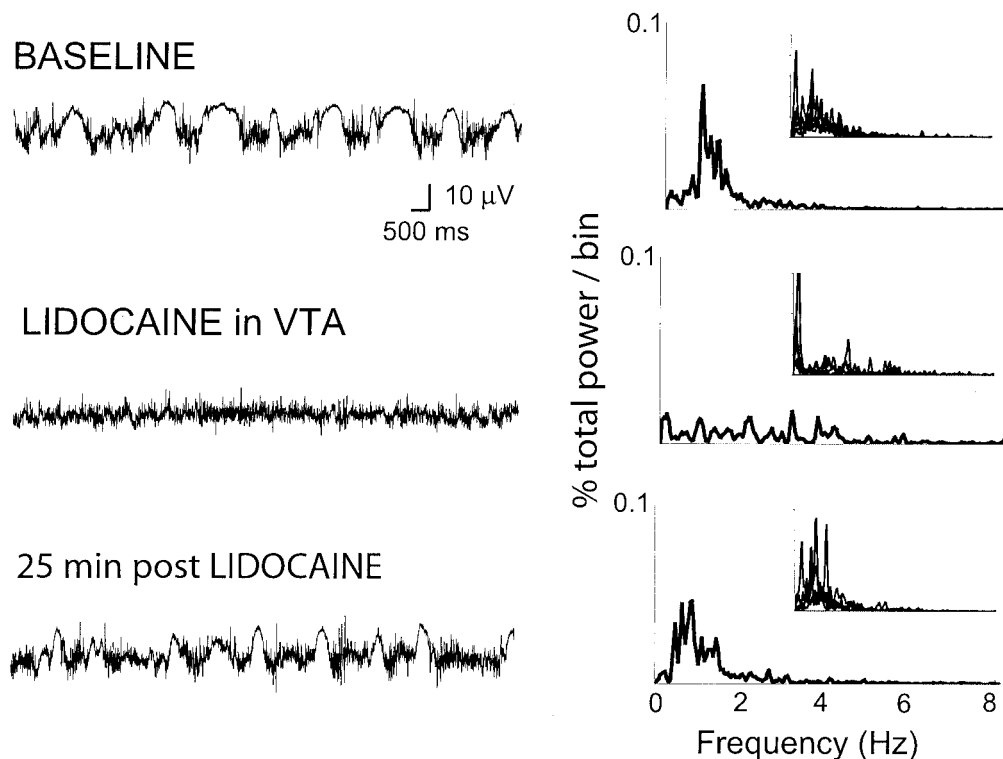


Fig. 2. Lidocaine injection in the VTA alters PFC field potentials. Top. Example of LFPs recorded from the PFC prior to the injection of lidocaine into the VTA (baseline). Right: Fourier analysis revealing a 0.8 Hz peak in the PFC activity. Plot is normalized to the proportion of total power in the 0–40 Hz range per 0.4 Hz bin. Inset: Overlay of similar plots for all PFC field potentials recorded. Middle. Field potential recording from the same PFC electrode immediately following

lidocaine injection in the VTA. Right: Fourier analysis showing disappearance of the slow peak. Inset: Overlay of similar plots for all PFC recordings immediately after intra-VTA lidocaine injection. Bottom. Field potential recorded 25 min following lidocaine injection and reappearance of the slow frequency peak in the spectral analysis (right). Inset: Overlay of all plots obtained from PFC recordings 25–45 min following lidocaine injection.

showing up–down transitions while injecting lidocaine in the VTA. These neurons oscillated between a down state at -75.3 ± 13.0 mV and an up state at -67.5 ± 12.0 mV. Up states lasted 515 ± 184 ms and occurred at 1.01 ± 0.22 Hz. After a stable baseline recording of 3–5 min, lidocaine was injected in the VTA. Following VTA inactivation, PFC pyramidal neurons continued to display a bimodal membrane potential distribution (Fig. 5). Resting membrane potential was -81.3 ± 8.0 mV after lidocaine, and the up state was -74.0 ± 7.4 mV; the down state was significantly hyperpolarized compared to control recordings ($P = 0.043$; WMPT), and the up state exhibited a trend towards hyperpolarization ($P = 0.08$, WMPT). Up state transitions occurred at similar frequency (0.91 ± 0.13 Hz; $P = 0.465$, WMPT) and exhibited similar duration (414 ± 96 ms; $P = 0.138$, WMPT) following transient VTA inactivation. Visual inspection of the traces revealed irregular up–down transitions following lidocaine administration, however (Fig. 5). This irregular pattern was reflected in increased variability of up state duration. The coefficient of variation (Cv) of 20 randomly selected consecutive up events increased from 0.40 ± 0.10 to 0.62 ± 0.15 following intra-VTA lidocaine injection ($P = 0.043$, WMPT). The irregular pattern was also

reflected in increased variability in the intervals between the onset of consecutive up events. The Cv of this measure was calculated from 20 consecutive intervals before and after VTA lidocaine injection. This index of up transition irregularity increased from 0.39 ± 0.11 to 0.58 ± 0.09 following VTA inactivation ($P = 0.043$, WMPT). The increased variability in up state duration seems related to the loss of the rapid return to the down state characteristic of up state termination. Instead, up states during VTA inactivation were terminated less abruptly. Firing rate of PFC neurons was reduced in all cases following lidocaine injection in the VTA. Average action potential firing was reduced from 5.6 ± 4.9 Hz to 0.6 ± 1.2 Hz following VTA inactivation ($P = 0.043$, WMPT; $n = 5$; Fig. 5). These intracellular recordings could not be held for long enough to obtain a reversal of the changes observed. This was likely due to the intrinsic difficulties on holding onto intracellular recordings while injecting small volumes into the brain. Although one could be concerned about the effects due to some sort of cell rundown, this is unlikely given the hyperpolarization observed; unhealthy neurons become depolarized when recorded with sharp electrodes. In any event, these results indicate that suppression of tonic VTA activity does not eliminate

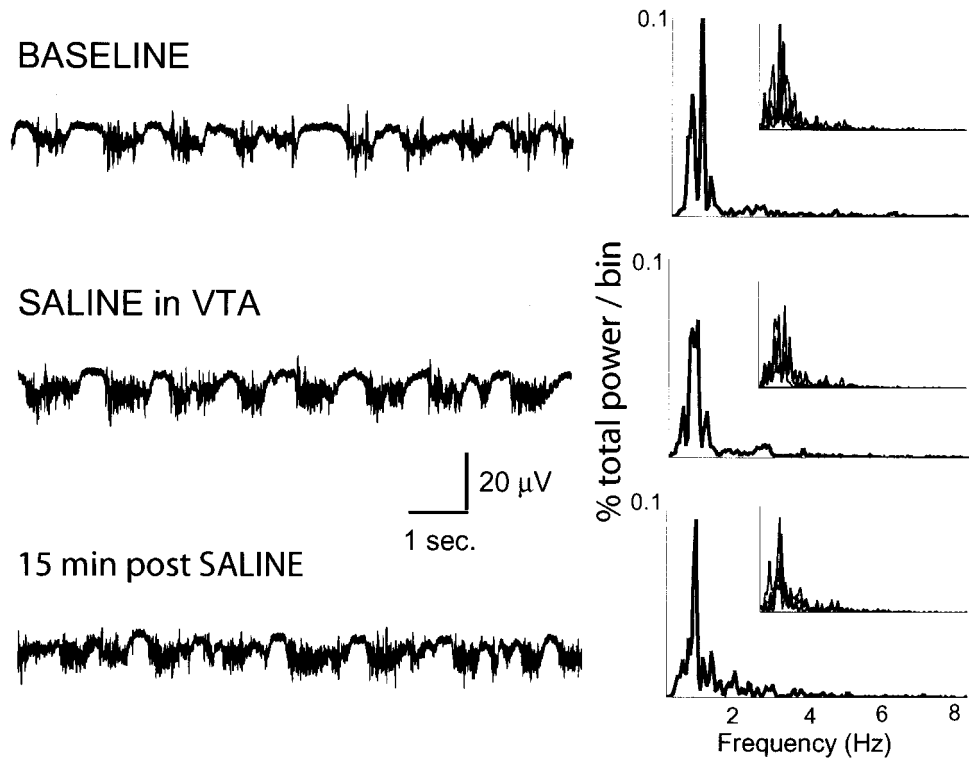


Fig. 3. Saline VTA injections do not affect PFC field potentials. Representative traces from a PFC recording before saline injection in the VTA (top), immediately following intra-VTA saline injection (middle), and 15 min after saline injection (bottom). No changes in slow oscillations were observed. Spectral density histograms (right) show absence of changes in the dominant frequency component over time. Insets show overlays of spectral density plots for all recordings.

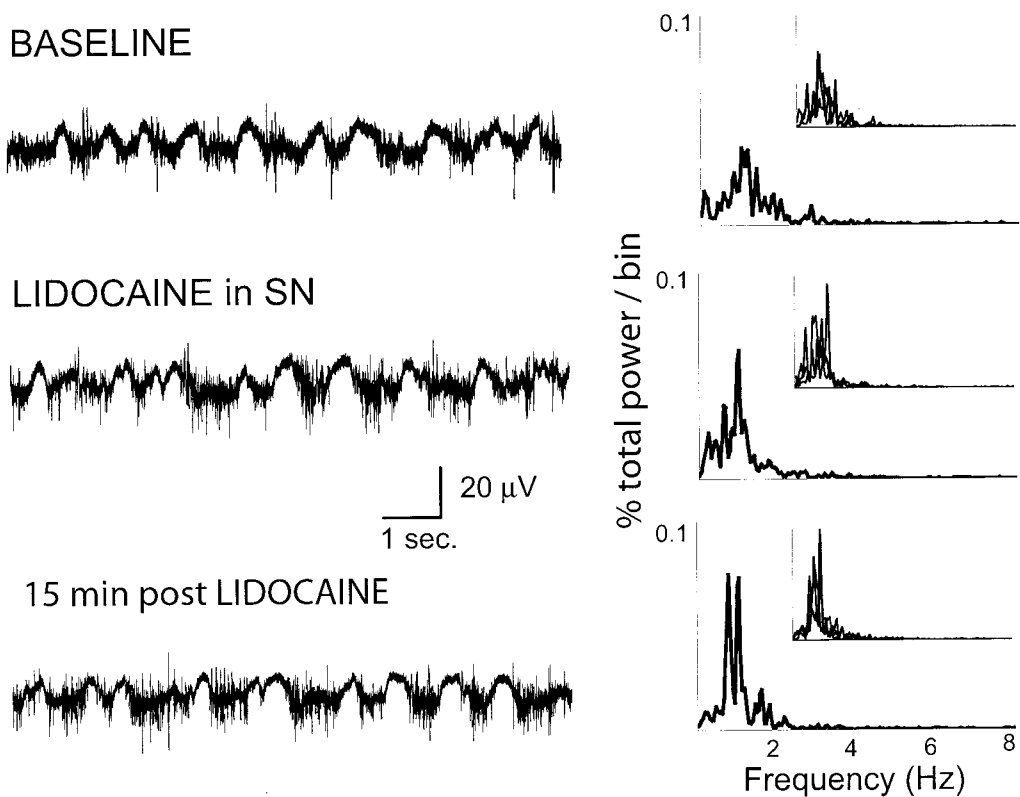


Fig. 4. Lidocaine injections in the substantia nigra do not alter PFC field potentials. Top. Representative trace (left) and spectral density histogram (right) of a PFC LFP revealing the characteristic near-1 Hz oscillation. Inset shows overlay of histograms for all recordings in this group. Middle. The oscillation persists shortly (2 min) after lidocaine injection in the substantia nigra, pars compacta. Bottom. Tracing and histogram from the same electrode 15 min following intra-nigra lidocaine injection.

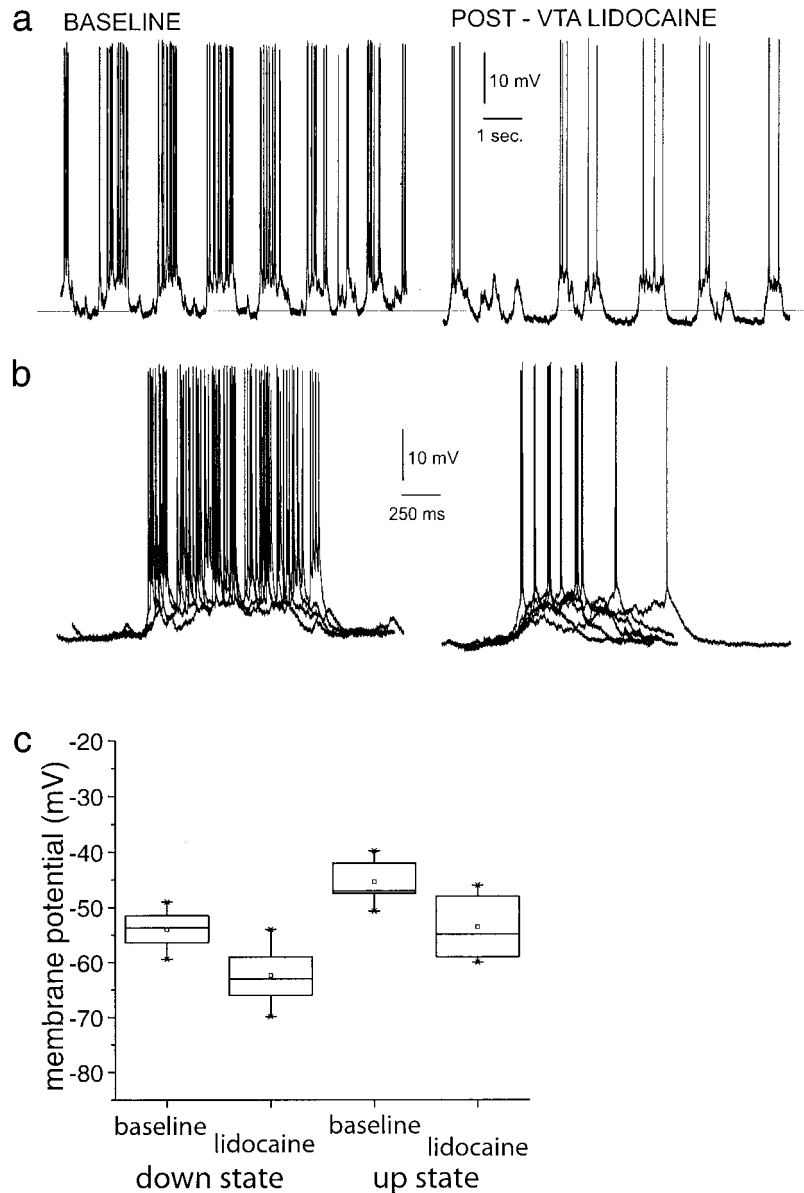


Fig. 5. PFC up states are not eliminated by VTA lidocaine injection. **a:** Left: Intracellular recording from a PFC pyramidal neuron showing up and down states. Right: Up and down states are still observed following intra-VTA lidocaine injection. Representative trace recorded 5 min postlidocaine injection showing persistence of up-down oscillations, albeit with a disorganized temporal pattern, and a slight membrane hyperpolarization. The thin horizontal line indicates the down state of the baseline recording. **b:** Overlay of up events in the baseline recording (left) showing a fairly regular duration. Right: Overlay of plateau depolarizations following intra-VTA lidocaine, revealing increased variability in their duration. **c:** Box plots illustrating changes in membrane potential of PFC neurons before and after VTA lidocaine injection.

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Figure 5

PFC pyramidal cell up states, but can disorganize their pattern.

DISCUSSION

Membrane potential fluctuations in PFC pyramidal neurons and PFC LFPs were correlated with local field potential oscillations in the VTA. Both signals exhibited similar <1 Hz peaks with high coherence and that frequency component was detected in the PFC a few milliseconds earlier than in the VTA. In addition, lidocaine injection in the VTA transiently eliminated PFC LFP oscillations, an effect that was not produced by

VTA saline injection or lidocaine injection in the substantia nigra. Up states exhibited increased variability in their duration and PFC neurons were more hyperpolarized and with a significant reduction in firing rate following VTA lidocaine injection. These results indicate a strong interdependence between VTA population activity and PFC up states.

To establish the correlation between the PFC and VTA, we calculated spectral densities in both recordings as well as their co-spectral densities; this allowed determining squared coherence and revealed a strong correlation between both signals. Although we did not

attempt to characterize VTA LFPs in this study, this type of signal can be assumed to reflect a population of neurons depolarizing simultaneously in the vicinity of the recording electrode without necessarily reflecting synchronous action potential firing. The high coherence observed between VTA LFPs and PFC up states could be due to VTA activity sustaining ongoing up states, PFC cell firing driving synaptic events in the VTA, or a third structure driving both.

The dependence of PFC up states on VTA activity is evident in the transient suppression of PFC LFPs after lidocaine injection in the VTA. Studies using simultaneous intracellular and field potential recordings in neocortical areas have revealed that up states are correlated with negative deflections in cortical LFPs when they are recorded from deep cortical layers (Steriade et al., 1993). This suggests that negative LFP shifts are the expression of a large population of pyramidal neurons entering the up state simultaneously (Steriade et al., 1993). The lidocaine-induced suppression of <1 Hz oscillations in the PFC could reflect a suppression of spontaneous up states. However, intracellular recordings revealed persistence of up states following the temporary suppression of VTA activity with lidocaine, with the only difference being their disorganized pattern. Up events may be occurring almost randomly and canceling each other in a large number of neurons, since there was no evidence of slow activity in the cortical LFP. Furthermore, in a previous study we observed persistence of PFC up states with systemic administration of a D₁ antagonist (Lewis and O'Donnell, 2000), suggesting that D₁ receptor activation may not be necessary to drive up states. The flat LFP signal in the PFC with persistent but disorganized up states indicates that a transient inactivation of the mesocortical system may desynchronize up states in PFC neuronal populations. One possible explanation is that tonic VTA firing (including both DA and GABA cell activity) synchronizes a local cortical network (O'Donnell, 2003).

Although VTA cell firing can affect PFC activity states, PFC neurons also exert a strong influence on VTA firing. The PFC is an important source of glutamatergic projections to the VTA (Sesack and Pickel, 1992; Carr and Sesack, 2000). DA cell burst firing depends on NMDA receptor activation, likely derived from cortical inputs (Gariano and Groves, 1988; Tong et al., 1996). Phase-lag analysis revealed that slow PFC oscillations precede VTA oscillations by less than 10 ms. Thus, the correlation observed here may involve PFC driving VTA neurons, but it may also involve VTA sustaining PFC activity, as discussed above. In fact, PFC neurons send projections to VTA DA neurons that in turn project back to the PFC and to VTA GABA neurons that project to the nucleus accumbens (Carr and Sesack, 2000). It is therefore conceivable that a continuous reverberation exists between a glutamater-

gic drive of VTA cell firing by the PFC and VTA support of PFC up states.

The possibility that a third structure influences the activity of both the PFC and VTA cannot yet be discarded. Potential sites include the pedunclopontine nucleus (PPN) and laterodorsal tegmental nucleus. The PPN has been implicated in driving VTA DA cell burst firing (Floresco et al., 2003) and projects also to the PFC. It is possible that this projection may be sufficient to elicit and sustain transitions to the up state in the PFC and perhaps the VTA gating such inputs.

Although the data reported here do not provide direct evidence for a role of DA in VTA-PFC synchrony, previous findings may allow speculating that it could be the case. We have previously shown that VTA stimulation can evoke a prolonged depolarization resembling the up state in PFC pyramidal neurons (Lewis and O'Donnell, 2000). This depolarization was shortened by systemic administration of a D₁ antagonist. However, blockade of DA receptors failed to completely suppress VTA-evoked up states in the PFC (Lewis and O'Donnell, 2000), suggesting that a non-DA component is responsible for initiating the depolarization in response to VTA stimulation. It is likely that glutamatergic inputs are responsible for bringing cortical pyramidal neurons to the up state, since a ventral hippocampal lesion locked PFC pyramidal neurons in the down state (O'Donnell et al., 2002). The rapid transition from down to up state could be elicited by glutamate and the up state maintained by DA via D₁ receptors. Recent *in vitro* data obtained in our laboratory suggests that glutamate may not be sufficient to induce cortical oscillations, requiring combined administration of a D₁ agonist and NMDA (Tseng and O'Donnell, unpubl. obs.). Thus, it is possible that the sustained depolarizations constituting the spontaneous up states are maintained by a D₁ enhancement of NMDA actions (Wang and O'Donnell, 2001). A dependence of PFC up states on DA released from VTA projections is further indicated by recent findings showing that selective activation of DA neurons by intra-VTA administration of the μ opioid agonist DAMGO (which disinhibits DA neurons by reducing GABA cell activity) resulted in prolonged up states in PFC pyramidal neurons (Lewis and O'Donnell, 2002). The correlation observed between PFC and VTA activity may involve not just VTA DA neurons. The VTA includes both GABA and DA neurons projecting to the PFC (Steffensen et al., 1998; Carr and Sesack, 2000) that target both pyramidal cells and interneurons. GABA neurons may inhibit DA neurons within the VTA, preventing them from exerting their full depolarizing action on PFC pyramidal neurons. In addition, GABA release in the PFC may contribute to bringing the membrane potential of PFC pyramidal neurons to values close to the up state. However, GABA-A receptor-evoked currents are short in

duration and may not have the prolonged effects that DA has via second-messenger pathways. Thus, it is likely that the observed PFC-VTA synchrony involves activation of DA projections acting on D₁ receptors on pyramidal neurons, as well as GABA neurons projecting to the PFC and their return pathways.

An intriguing set of findings is the significant hyperpolarization of the down state and corresponding reduction in action potential firing following VTA inactivation with lidocaine. Although this procedure may inactivate both DA and GABA projecting cells, the hyperpolarization and reduced firing are not likely to result from GABA inactivation. The decreased firing is also unlikely the result of removal of a direct effect of DA, since iontophoretically applied DA in the PFC has been shown to reduce cell firing (Bernardi et al., 1982). However, in the same study a DA-induced depolarization was also observed in PFC neurons. Therefore, inactivating the VTA may affect PFC cell firing by a network mechanism. It is possible that tonic DA provides a sufficient drive for activation in a network of neurons that could result in synchronized up states and basal levels of action potential firing. Removing VTA inputs may reduce the levels of activity in this PFC network, thereby reducing action potential firing and desynchronizing up states.

Reciprocal connections between the VTA and PFC are crucial elements in working memory, reward, and attention. VTA neurons are activated by reward or reward-predicting stimuli (Schultz et al., 1997; Hollerman and Schultz, 1998; Hollerman et al., 1998; Steffensen et al., 2001). They can also be driven into burst firing by salient stimuli (Hyland et al., 2002). At rest, the low (tonic) levels of DA released in the cortex may allow for some clusters of neurons to oscillate in phase, resulting in slow EEG components characteristic of sleep or inattentive awake conditions. This action renders those neural ensembles in a "ready" state, and they could be set in a persistent up state whenever phasic DA arises in conditions that demand the animal's attention (O'Donnell, 2003). Silencing VTA cells would impair activation of appropriate cortical ensembles, yielding inappropriate or out of context cortical activation, as seen in a variety of neuropsychiatric disorders such as schizophrenia and autism. Thus, the close interaction between VTA cell activity and PFC up states reported here may be an important player in the interaction of animals with their environment.

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