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Slow Oscillatory Firing: A Major Firing Pattern of Dopamine Neurons in the Ventral Tegmental Area

Wei-Xing Shi

Neuropsychopharmacological Research Unit, Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut

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Shi, Wei-Xing. Slow oscillatory firing: A major firing pattern of dopamine neurons in the ventral tegmental area. *J Neurophysiol* 94: 3516–3522, 2005; doi:10.1152/jn.00317.2005. Using spectral analysis and in vivo single-unit recording in rats, the present study revealed a pronounced slow oscillation (SO) in the firing activity of about half the dopamine (DA) neurons recorded in the ventral tegmental area. DA neurons in this group tended to fire repetitive spike clusters, making them appear to be rhythmic bursting cells. However, only some of these burst-like events met the traditional "80/160 ms" burst criteria entirely. The observation that the SO could be found in nonbursting DA cells, occurred at frequencies different from those of bursts, and persisted after bursts were digitally removed from spike trains further supports the suggestion that the SO is different from the traditionally defined bursting. Interspike intervals (ISIs) had been thought to be bimodally distributed in bursting DA neurons. This study found that some nonbursting DA cells also had a bimodal ISI distribution and a significant number of bursting cells did not. In the majority of cells where less than half the spikes occurred in bursts, a bimodal ISI distribution was highly predictive of the presence of the SO. Results further showed that the generation of the SO required forebrain inputs to DA neurons but not the adrenergic α1 receptor activation responsible for psychostimulant-induced increases in the SO. Taken together, these results suggest that the SO is distinct from the traditionally defined bursting and represents a major firing pattern of DA neurons in the ventral tegmental area.

INTRODUCTION

Midbrain dopamine (DA) neurons receive inputs from widely distributed brain areas. Information from these areas is integrated at the DA cell soma and then transmitted, in the form of spike trains, to DA terminals. While it remains elusive how exactly the information is encoded in the spike trains in DA neurons, evidence suggests that both firing rate and firing pattern play a role in carrying the information.

Two firing patterns have been recognized in DA neurons: single spiking and bursting (Grace and Bunney 1984a,b). Most spontaneously active DA cells recorded in vivo exhibit both forms of activity. The level of bursting, however, varies considerably from cell to cell. For a given DA neuron, it also alters in response to sensory stimuli and to drugs acting on a variety of different mechanisms (e.g., Freeman and Bunney 1987; Horvitz et al. 1997; Hyland et al. 2002). These observations led to the suggestion that "switching from a single spiking to a bursting mode of firing is one means that these neurons have of changing their influence on the postsynaptic neurons" (Freeman and Bunney 1987). Supporting the suggestion, stimulation of DA neurons mimicking bursting induces more DA release than when mimicking single spiking (Gonon 1988).

We have previously reported that psychostimulants, including d-amphetamine and cocaine, have two opposing effects on DA neurons: a DA-mediated inhibition and a non-DA-mediated excitation (Shi et al. 2000, 2004). Spectral analysis further shows that the excitation is associated with an increase in not only firing rate and bursting, but also a slow oscillation (SO, 0.5–1.5 Hz) in firing rate (Shi et al. 2004). Preliminary observation suggests that in some DA neurons the SO is already present under baseline conditions and is further enhanced by psychostimulants. A previous study carried out in locally anesthetized, paralyzed rats suggests that 19% of DA neurons in the substantia nigra also exhibit a SO (Wilson et al. 1977). Thus the first goal of the present study was to determine how widely the SO is present in DA neurons in the ventral tegmental area (VTA). Because DA neurons exhibiting the SO often appeared to fire in a repetitive-bursting mode, the second goal of this study was to examine how the SO and the traditionally defined burst firing may be related to each other. Third goal of this study was to investigate whether the SO observed under baseline conditions, like that induced by psychostimulants, is synaptically mediated and depends on adrenergic α_1 receptor activation (Shi et al. 2004).

METHODS

Electrophysiological recording

All procedures were performed in accordance with protocols approved by the Yale Animal Care and Use Committee. Male Sprague-Dawley rats, weighing between 250 and 300 g, were anesthetized with chloral hydrate (400 mg/kg ip, with supplemental doses administered via a lateral tail vein). Single-unit activities of VTA DA neurons were recorded extracellularly using techniques described previously (Shi et al. 2000, 2004). DA neurons were identified by well-established electrophysiological criteria (Bunney et al. 1973; Grace and Bunney 1983; Ungless et al. 2004), which include a biphasic (positive-negative) or triphasic (positive-negative-positive) spike waveform with a duration of >3.0 ms, a broad initial positive phase (>1 ms, measured from the start of action potential to the negative trough) (Ungless et al. 2004), an initial segment-somatodendritic break in the initial positive phase, and a slow firing rate (<10 spikes/s). In addition, DA neurons can be reliably distinguished from non-DA neurons by the characteristic low-pitched sound they produce on an audio monitor. To assess the prevalence of the SO in DA neurons, the "cells per track" technique (Bunney and Grace 1978; Chiodo and Bunney 1983) was used to record all spontaneously active DA cells encountered while passing an electrode through the VTA. In each

Address for reprint requests and other correspondence: W.-X. Shi, Neuropsychopharmacological Research Unit, Yale University School of Medicine, 300 George St., Rm. 8300C, New Haven, CT 06511 (E-mail: wei-xing.shi@yale.edu).

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animal, 12 electrode tracks were made in the right side of the VTA (from lamda: AP 2.7–3.3 mm, ML: 0.5–0.9 mm). Each track was separated by 200 μm.

To determine whether the SO depends on forebrain inputs, ipsilateral forebrain hemisections were made in a separate group of rats. A slit was drilled in the skull 4.0 mm posterior to bregma and perpendicular to the midline. A blunted, flattened 25-gauge needle was inserted to the base of the skull and moved from side to side along the slit to completely interrupt connections between the VTA and forebrain.

DA neurons were also recorded in brain slices using methods described previously (Shi and Zhang 2003). Briefly, rats weighing 60–100 g were anesthetized with chloral hydrate (400 mg/kg ip) and decapitated. The brains were quickly removed. Horizontal slices (300 μm) containing the VTA were cut and transferred to an incubating chamber where they were held at 35°C for ≥1 h before recording. DA cells were recorded extracellularly using methods identical to those used in vivo.

Drug

Prazosin (Sigma-RBI, St. Louis, MO) was dissolved at 2.5 mg/ml in 25–30% poly(ethylene glycol; average molecular weight: 200). Before injection, the solution was diluted with distilled water so that the final volume of injection was 0.05 or 0.1 ml.

Data analysis

All analyses were performed using in-house programs written in Visual Basic for Applications in Microsoft Excel. Bursts were identified according to the "80/160 ms" definition proposed by Grace and Bunney. Thus the onset of a burst was identified as the concurrence of two spikes with an interspike interval (ISI) <80 ms and the termination of a burst was defined as an ISI >160 ms (Grace and Bunney 1984a). Firing periodicity was examined using methods similar to those described previously (Shi et al. 2004). Briefly, rate histograms (binwidth: 50 ms) and autocorrelograms (using autocovariance function with a binwidth of 50 ms over 2,048 bins) were constructed based on 2-min recordings selected from each cell. Following tapering using the Hanning-Tukey window function (15 windows with 50% overlap) and removal of the linear trend, the fast Fourier transform (FFT) was performed on both the rate histograms and autocorrelograms, yielding spectra with a resolution of 0.078 Hz. Spectra obtained from rate histograms and autocorrelograms were qualitatively identical. For simplicity, this study reports only the results from the FFT of autocorrelograms. To help visualize slow changes, rate histograms and autocorrelograms were smoothed in Fig. 1 (Shi et al. 2004). All spectral results, however, were obtained from unsmoothed data.

Differences between cell groups were determined using ANOVA followed by a post hoc Tukey test. Effects of drugs were determined by comparing measures before and after drug injection using ANCOVA followed by a post hoc Tukey test. The covariate was the baseline value prior to drug injection. Spectral data were log-transformed before being subjected to statistical comparison. All numerical results were expressed as means ± SE.

RESULTS

Traditional measures

Recordings were made from 206 spontaneously active VTA DA neurons in 15 rats. Firing rates of these neurons ranged from 0.6 to 9.1 spikes/s (4.0 ± 0.1). The percent of spikes in bursts ranged from 0 to 92% (33.4 ± 1.8%). The average number of spikes per burst was 2.8 ± 0.1 (from 2 to 7.7). The frequency of bursting varied from 0 to 1.97 per second (0.48 ± 0.03). The mean coefficient of variation (CV) of ISIs was 75.5 ± 1.9% (ranging from 22 to 179% of the mean ISI). A modest correlation was found between the degree of bursting and CV (r = 0.64) and between firing rate and bursting (r = 0.57). No correlation was found between firing rate and CV (r = 0.09). These results were similar to those reported previously (e.g., Floresco et al. 2003; Grace and Bunney 1984a,b; Grenhoff et al. 1988).

Slow oscillatory firing and "rhythmic bursting"

Confirming our preliminary observation, a subset of VTA DA neurons exhibited slow oscillatory firing under baseline

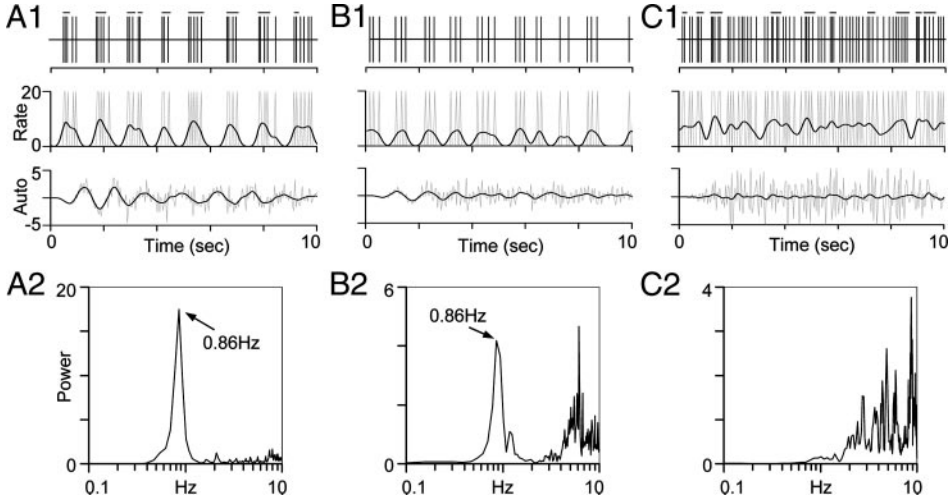

FIG. 1. Firing patterns of dopamine (DA) neurons. *A1–C1, top*: segments of spike trains from 3 different DA neurons recorded in the ventral tegmental area (VTA). Both *cells A* and *B* exhibited a firing pattern resembling rhythmic bursting. However, most burst-like events in *cell A* were different from the traditionally defined burst (indicated by the bars above the spike train, see text for details). *Cell B,* on the other hand, was a nonbursting cell. *Cell C* was a bursting DA cell, but exhibited no discernable slow rhythmicity. *Middle*: portions of rate histograms from the same cells (binwidth: 50 ms; ordinate unit: spike/s). To help visualize slow changes, histograms were smoothed (Shi et al. 2004). The original and smoothed histograms are shown in thin and thick lines, respectively. *Bottom*: autocorrelograms with linear trend removed and data at the beginning tapered (binwidth: 50 ms; ordinate unit: spike^2/s). Thin and thick lines are the original and smoothed autocorrelograms, respectively. *Cells A* and *B,* but not *C,* exhibited a slow oscillation in both the rate histograms and autocorrelograms. *A2–C2*: power spectra obtained from the FFT of the autocorrelograms of the 3 cells. Spectra from both *cells A* and *B,* but not *C,* showed major spectral peaks (arrow) between 0.5 and 1.5 Hz. In this and following figures, the power of a spectral peak is expressed as a percent of the total power so that the sum of all peaks equals 100.

conditions. These cells were often misidentified as repetitive-bursting neurons because of the rhythmic sound that they produced on an audio-monitor. Visual inspection confirmed the presence of repetitive spike clusters in their spike trains. Detailed examination showed, however, that only some of these burst-like events met the "80/160 ms" burst criteria entirely (see METHODS) (Grace and Bunney 1984a). Figure 1, *A* and *B*, show two examples of such cells (top traces).

Most spike clusters in the two cells failed to qualify as bursts because of one of the following reasons. First, all ISIs within the cluster were >80 ms (all spike clusters in *cell B*). Second, the cluster consisted of more than one so-called burst (the 3rd cluster in *cell A*). Third, the cluster contained both single spikes and bursts (the 1st 2 and the last 2 clusters in *cell A*). Thus based on the current "80/160 ms" definition of bursting for DA neurons, it would be inaccurate to describe the activity of *cells A* and *B* in Fig. 1 as rhythmic bursting. According to the definition, *cell B* was in fact a nonbursting cell. The following results suggest that the pattern of the activity in both cells can be described as slow oscillatory firing.

A SO in firing rate was first visible when the rate histogram was constructed with a binwidth of 50 ms instead of 10 s as used in most previous studies. The SO became more evident after smoothing of the histogram (Fig. 1, *A1* and *B1, middle traces,* thick lines), and was further confirmed by autocorrelograms (Fig. 1, *A1* and *B1, bottom traces*) and by the power spectra of both rate histograms (not shown) and autocorrelograms (Fig. 1, *A2* and *B2*). Spectral results further showed that the frequency of the SO was between 0.5 and 1.5 Hz (Fig. 1, *A2* and *B2*, arrows).

Figure 1*C* shows data from another VTA DA neuron, which like *cell A,* was a bursting DA cell. Different from both *cells A* and *B,* however, *cell C* exhibited no obvious slow rhythmicities in either its rate histogram or autocorrelogram (Fig. 1, *C1*). Its power spectrum showed no significant peaks between 0.5 and 1.5 Hz (Fig. 1, *C2*). The observation that both *cells A* and *C* were classified as bursting DA neurons but showed very different firing patterns further suggests that the current "80/160 ms" definition of bursting is insufficient for a complete description of different firing patterns seen in DA neurons.

Identification of the SO and its prevalence in VTA DA neurons

The *left chart* in Fig. 2 is an average spectrum of all 206 cells recorded. The elevated power between 0.5 and 1.5 Hz suggests that oscillations in this low-frequency range are the most dominant rhythmic activity in VTA DA neurons. Consistent with this finding, ~39% of cells had their highest spectral peaks located at frequencies <1.5 Hz (Fig. 2, *middle*). The actual number of cells displaying the SO must be higher because spectra of some DA neurons were bimodal, with one major peak found at <3 Hz and one >3 Hz, and the low-frequency peak was sometimes not the highest in the spectrum (e.g., Fig. 1, *B2*).

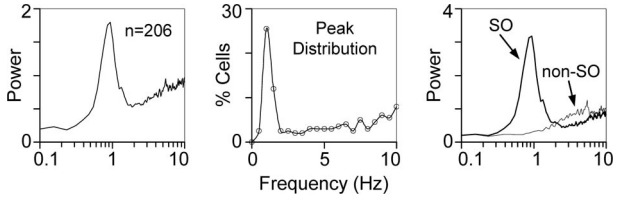

FIG. 2. Prevalence of the slow oscillation (SO) in VTA DA neurons. *Left*: average spectrum of all 206 VTA DA neurons recorded. The elevated power between 0.5 and 1.5 Hz suggests that oscillations in this low-frequency range are the most dominant oscillatory activity in VTA DA neurons. *Middle*: frequency distribution of the highest spectral peaks. Of all VTA DA cells examined, 39% had their highest spectral peaks located at ≤1.5 Hz. *Right*: average spectra of all SO (thick line) and non-SO cells (thin line), respectively. The power between 0.5 and 1.5 Hz was elevated in the spectrum of SO cells, but not that of non-SO cells.

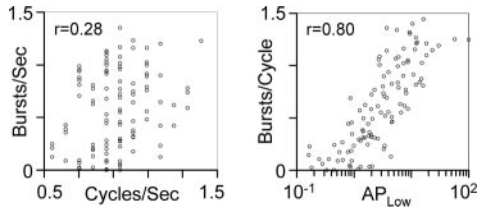

FIG. 3. Correlations between the SO and bursting in SO cells. *Left*: frequency of bursting (bursts/s) and the frequency of the SO (cycles/s) were not significantly correlated. *Right*: number of bursts per oscillation cycle (bursts/cycle) was highly correlated with the amplitude of the SO (AP_{Low}, absolute power between 0.5 and 1.5 Hz).

To identify all cells with prominent SO between 0.5 and 1.5 Hz, the mean power between 0.5 and 1.5 Hz ($P_{0.5–1.5Hz}$) was compared with the mean power between 0 and 3 Hz (P_{0-3Hz}). A cell was considered to be slowly oscillating (SO cell) if $P_{0.5–1.5Hz}$ was greater than the 95% confidence level of P_{0-3Hz}. Based on this criterion, 107/206 cells were identified as SO cells. In the remaining cells, $P_{0.5–1.5Hz}$ was not different from or smaller than P_{0-3Hz}. For convenience, these cells were collectively referred as non-slowly oscillating (non-SO) cells. Most non-SO cells had major spectral peaks at >1.5 Hz. A few ($n = 4$) had significant peaks at <0.5 Hz, indicating the presence of ultra slow oscillations. Previous studies have shown that a small subset of nigra DA neurons (3/14) also display ultra slow oscillations (Ruskin et al. 1999).

The *right chart* in Fig. 2 shows two overlapping spectra: one is the average spectrum of all SO cells (thick line) and one is that of non-SO cells (thin line). As might be expected, the power between 0.5 and 1.5 Hz is elevated in the spectrum from SO cells but not that from non-SO cells. Statistically, $P_{0.5–1.5Hz}$ was significantly higher in SO cells (1.84 ± 0.11, $n = 107$) than in non-SO cells [0.31 ± 0.03, $n = 99$, $F(1,204) = 258.39$, $P < 0.001$]. Oscillations at high frequencies (3–10 Hz, P_{3-10Hz}), however, were higher in non-SO (0.92 ± 0.01, $n = 99$) than in SO cells [0.71 ± 0.02, $n = 107$, $F(1,204) = 68.46$, $P < 0.001$].

Relations between the so and bursting

The differences between the spike clusters seen in SO cells and the current ISI definition of bursting (Fig. 1, *A* and *B*) suggest that the SO is not an epiphenomenon of repetitive bursting. Supporting this suggestion, the SO was observed in cells that had no bursts ($n = 2$, Fig. 1*B*) or only a few bursts (<10% spikes in bursts, $n = 9$), and was absent in >24% of high-bursting DA cells (>40% spikes in bursts; e.g., Fig. 1*C*). The frequency of bursting and the frequency of the SO were only weakly correlated ($r = 0.28$, Fig. 3, *left*). The ratio of the two measures ranged from 0 to 1.44, indicating that each oscillation cycle might contain none, one, or more than one burst depending on the cell examined. The ratio was correlated

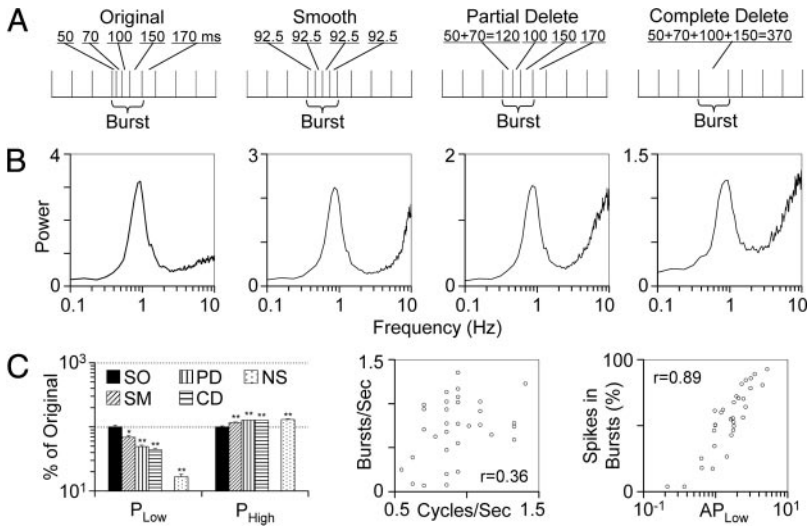

FIG. 4. Effects of burst removal on the SO. *A*: schematic illustration of 3 different methods used for digitally removing bursts from spike trains (see text for details). Numbers above the spike train are ISIs (in ms) within the burst. *B*: average spectra of SO cells ($n = 107$) obtained before and after burst removal using the method shown above each spectrum. Note that the power between 0.5 and 1.5 Hz remained elevated after burst removal. *C, left*: effects of burst removal on low- and high-frequency oscillations (P_{Low}: mean power between 0.5 and 1.5 Hz; P_{High}: mean power between 3 and 10 Hz). For comparison, measures of non-SO cells are also shown on the *far right* (NS). All values are expressed as percent of the original value of SO cells (SO). P_{Low} was decreased after removal of bursts but remained higher than that of non-SO cells. P_{High} was increased after burst removal. SM, PD, and CD denote, respectively, the data after smoothing, partial deleting, and complete deleting of spikes in bursts. $*P < 0.005$, $**P < 0.001$ compared with values before burst removal (SO). *Middle*: a subset of SO cells (30%) was converted to non-SO cells after deleting all spikes in bursts. Even in this subset of SO cells, the frequency of bursting (bursts/s) were only weakly correlated with the frequency of SO (cycles/s). *Right*: in the same cells, the level of bursting (spikes in bursts) was significantly correlated with the amplitude of the SO (AP_{Low}).

significantly with the amplitude of the SO ($AP_{0.5–1.5Hz}$, the absolute mean power between 0.5 and 1.5 Hz, Fig. 3), suggesting that the SO, when large enough, may trigger bursts. Consistent with this possibility, the amplitude of the SO was also correlated with the percent of spikes in bursts ($r = 0.81$) and the frequency of bursts ($r = 0.8$). Also consistent with this possibility, the average level of bursting was much higher in cells with the SO (43.8 ± 2.5% spikes in bursts, $n = 107$) than those without the SO [22.2 ± 2.2% spikes in bursts, $n = 99$; $F(1,204) = 42.2$, $P < 0.001$]. The mean firing rate of the two groups of cells, however, was not significantly different [SO: 4.18 ± 0.18 spikes/s; non-SO: 3.89 ± 0.17 spikes/s; $F(1,204) = 1.44$, $P = 0.23$].

To confirm further that the observed SO is not secondary to bursting, bursts were digitally removed from spike trains using three different methods illustrated in Fig. 4*A*. In the smoothing method, each ISI <80 ms was averaged with the next ISI. For each burst, this was repeated until all ISIs within the burst were >80 ms. If the average of all ISIs was still <80 ms, spikes within the burst were removed one by one until the average ISI was >80 ms. In the partial-deleting method, spikes with an ISI <80 ms were removed. In the complete-deleting method, all spikes in bursts were deleted.

All three methods completely eliminated bursts. None, however, eliminated the SO (Fig. 4*B*). Compared with the original value, $P_{0.5–1.5Hz}$ was decreased after burst removal but remained higher than that of non-SO cells ($P < 0.001$, Fig. 4*C, left*).

A small percent of SO cells was converted to non-SO cells after smoothing or partial-deleting of spikes in bursts (5 and 12%, respectively). The percentage was higher (30%) when all spikes in bursts were removed. Even in this 30% of cells, the frequency of bursting was only weakly correlated with the frequency of the SO (Fig. 4*C*, *middle*). In the same cells, the amplitude of the SO and the level of bursting were highly correlated (Fig. 4*C, right*).

ISI distribution and firing pattern

ISIs are often thought to be bimodally distributed in bursting DA neurons (e.g., Robinson et al. 2004; Shepard and Bunney 1988) and non-DA neurons of other brain areas (e.g., Bennett and Wilson 1999; Nowak et al. 2003). In a bimodally distributed ISI histogram, the first peak is believed to represent the average ISI within bursts, and the second corresponds to the mean ISI between bursts. Results of this study suggest that burst firing is not always associated with a bimodal ISI distribution and that in low-bursting DA neurons a bimodal ISI distribution is highly predictive of the presence of the SO.

To determine whether a bimodal ISI distribution is selectively associated with burst firing, DA cells were divided into subgroups based on their levels of bursting (% spikes fired in bursts). In each group, the percent of cells showing a bimodal ISI distribution was calculated. As shown in Fig. 5*A* (*left*), there was an increased tendency for high-bursting DA cells to show a bimodal ISI distribution. However, many low-bursting (<40% of spikes in bursts) DA cells (35/126) also showed a

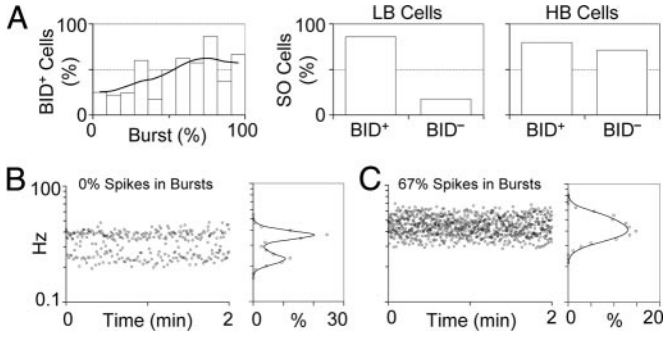

FIG. 5. ISI distribution and firing pattern. *A, left*: histogram showing percent of neurons with bimodal ISI distribution (BID^+ cells) in different cell groups divided based on their levels of bursting (percent spikes in bursts). A significant percent of low-bursting cells exhibited a typical bimodal ISI distribution (*far left bars*), whereas many high-bursting cells lacked a typical biomodal ISI distribution (*far right bars*). *Middle*: histogram showing that in DA neurons with <40% spikes occurred in bursts (LH cells), 86% of BID^+ cells were also SO cells, and 83% of cells that lacked a typical bimodal ISI distribution (BID^- cells) also lacked the SO. *Right*: histogram showing that in DA neurons with >40% spikes in bursts (HB cells), the percent of cells showing the SO was about equal in BID^+ and BID^- groups. *B* and *C*: plots of 1/ISIs against time (*left*) and corresponding ISI distribution curves (*right*) of 2 different VTA DA neurons. *Cell B* was the same nonbursting SO neuron shown in Fig. 1*B,* and *cell C* was the same bursting non-SO cell shown in Fig. 1*C.* ISIs in *cell B* had a typical bimodal distribution, whereas those in *cell C* were unimodally distributed.

bimodal ISI distribution, which included both nonbursting cells ($n = 2$, Fig. 5*B*) and cells that had <10% spikes in bursts ($n = 9$). A significant number of high-bursting cells (31/80), on the other hand, did not show a bimodal ISI distribution (e.g., Fig. 5*C*).

In low-bursting DA cells, a bimodal ISI distribution was highly predictive of the presence of the SO (Fig. 5*A, middle*); of the 35 cells showing a bimodal ISI distribution, 86% were SO cells (e.g., Fig. 5*B*), whereas 82% of cells lacking a bimodal ISI distribution ($n = 91$) also lacked a significant SO (Fig. 5*A, middle*). In high-bursting DA neurons, the occurrence of the SO was about equal in cells with and without a bimodal ISI distribution (79 and 71% of cells, respectively, Fig. 5*A, right*).

Figure 5, *B* and *C*, shows results from two DA neurons, the data of which are also shown in Fig. 1, *B* and *C*. *Cell B* was identified as a nonbursting SO DA cell (Fig. 1*B*) and had a typical bimodal ISI distribution (Fig. 5*B*). *Cell C* was a high-bursting non-SO DA cell (Fig. 1*C*) lacking a typical bimodal ISI distribution (Fig. 5*C*).

Role of afferent inputs

We have previously shown that when the D2-mediated feedback inhibition is blocked, psychostimulants increase the SO. Our data further suggest that the effect of psychostimulants requires afferent inputs to DA neurons (Shi et al. 2004). To test whether the SO observed under baseline conditions shares the same property, DA cells were recorded in animals with forebrain inputs interrupted by hemisections or in brain slices. To avoid potential false positive results, only cells with firing rates >1.5 Hz were included in the analysis.

In rats with forebrain hemisections, DA cells fired in a highly regular manner with a CV of 18.9 ± 2.1% ($n = 12$) as opposed to 74.8 ± 1.8% in intact animals ($n = 209$, see preceding text). In all cells tested, no significant spectral peaks were observed between 0.5 and 1.5 Hz (Fig. 6*A, left* and Fig. 6*B*).

In brain slices, DA neurons also exhibited a highly regular firing pattern with a CV of only 9.7 ± 1.0% ($n = 14$). A similar result has been reported previously (e.g., Grace and Onn 1989; Shepard and Bunney 1988). Spectral analysis, again, showed no significant SO in any of the cells examined (Fig. 6*A, right* and Fig. 6*B*).

Effects of prazosin

To determine whether the SO observed under baseline conditions, like that induced by psychostimulants (Shi et al. 2004), depends on α_1 activation, the α_1 antagonist prazosin was administered (0.5–1 mg/kg iv). The drug increased the SO in 10/18 cells (14–612% of baseline) and decreased it in the remaining cells (9–80%). On average, $P_{0.5–1.5Hz}$ was not significantly changed [57 ± 37%, $n = 18$, $F(1,33) = 0.35$, $P = 0.56$, Fig. 6*C*]. Prazosin also produced no significant effects on firing rate, spikes in bursts, and $P_{3\text{-}10Hz}$ (Fig. 6*D*).

DISCUSSION

This study shows that the SO is widely present in VTA DA neurons and represents a new firing pattern distinct from the previously defined bursting. Results further suggest that the generation of the SO depends on forebrain inputs but does not require activation of adrenergic α1 receptors.

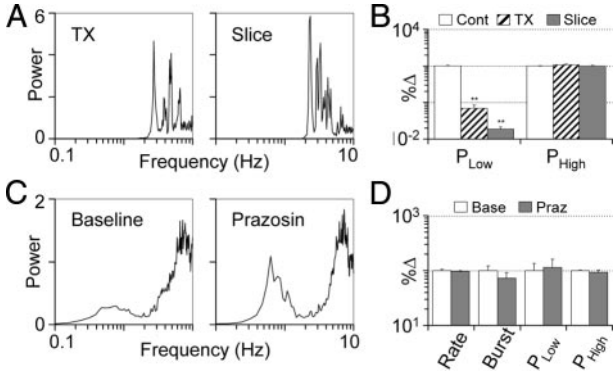

FIG. 6. Role of afferent inputs and α_1 receptors in the generation of the SO. *A*: average spectra of cells recorded in rats with forebrain hemisections (TX, *left*) and in brain slices (slice, *right*). *B*: summary bar graph showing differences between cells recorded in intact control animals (cont), animals with forebrain hemisections (TX) and brain slices (slice). Of the latter 2 groups, the mean power between 0.5 and 1.5 Hz (P_{Low}) was significantly less than that of control group. The mean power between 3 and 10 Hz (P_{High}) was similar between groups. Values are expressed as percent of control. $^{**}P < 0.001$ compared with control values. *C*: average spectra from 18 DA cells recorded under baseline conditions (*left*) and after administration of prazosin (*right*). Prazosin produced a statistically insignificant increase in the SO. *D*: summary bar graph showing the lack of a significant effect of prazosin (praz) on firing rate, spikes in bursts, P_{Low}, and P_{High}. Values are expressed as percent of baseline (base).

SO and burst firing

Based on numerous recordings, Grace and Bunney proposed that bursts in DA neurons can be identified by pairs of spikes with an ISI <80 ms. Subsequent spikes are considered to be part of the initial burst until an ISI >160 ms is encountered (Grace and Bunney 1984a). In the past two decades, this "80/160 ms" definition of bursting has been widely used in characterizing the firing pattern of DA neurons. The present study suggests that this definition is insufficient for a complete description of different firing patterns seen in DA neurons and shows that activities of DA neurons can also be characterized based on their oscillatory properties.

Like neurons of most other brain areas examined, VTA DA neurons exhibited robust oscillatory activities. Of the frequencies examined (0–10 Hz), the oscillation between 0.5 and 1.5 Hz was most dominant. Neurons exhibiting the SO constituted ~50% of all VTA DA neurons examined. The remaining neurons, collectively referred as non-SO cells, may be further divided into subgroups according to their dominant oscillation frequencies. The distribution curve shown in Fig. 2 (*middle*) suggests that non-SO cells are distributed relatively evenly in different frequency bands. The similar firing rate between SO and non-SO cells suggests that their differences in the oscillatory properties are unlikely to be caused by different levels of depolarization. The suggestion that the SO is a network-mediated event (see following text) further points to the possibility that the presence or the absence of the SO reflects the properties of the neuronal network that a DA neuron resides in.

Possible mechanisms underlying the SO

Results from brain slices and from rats with forebrain hemisections suggest that forebrain inputs play an important

role in the generation of the SO. Consistent with this possibility, a similar SO has been observed in the prefrontal cortex (PFC) (Cowan et al. 1994; Lewis and O'Donnell 2000) and the nucleus accumbens (NAc) (Goto and O'Donnell 2001a,b; Leung and Yim 1993; O'Donnell and Grace 1995). Both structures are located in the forebrain and both project to VTA DA neurons. More recently, Peters et al. (2004) showed that local field potentials recorded in the VTA also displayed a SO and that the activity was highly synchronized with oscillations seen in the PFC. VTA local field potential may reflect synchronized activities of DA neurons. If this proves to be true, findings by Peters et al. would suggest that the SO in VTA DA neurons are related to activities in the PFC. Studies have shown that cortically generated SO can transmit to the basal ganglia and related nuclei under certain conditions (Magill et al. 2000; Tseng et al. 2001).

Psychostimulants are known to inhibit DA neurons by increasing DA release and consequent activation of feedback mechanisms. When this feedback inhibition is blocked by a D2 antagonist, psychostimulants excite DA neurons and increase the SO (Shi et al. 2004). Both effects require intact forebrain inputs and activation of adrenergic α_1 receptors (Shi et al. 2004). This study suggests that the SO observed under baseline conditions also depends on forebrain inputs but does not require α_1 activation. Thus α_1 activation may enhance but is not required for the operation of the network responsible for the generation of the SO. Alternatively, the SO may be generated by different mechanisms under different conditions.

Potential significances of the SO

The frequency of the SO is in the low delta band. At the electroencephalographic (EEG) level, delta rhythms are most apparent in slow wave sleep and in general anesthesia. However, they can also be detected in the awake state. In the human, for example, cortical delta oscillations are significantly increased while performing the Wisconsin Card Sorting Test (Gonzalez-Hernandez et al. 2003). Studies with visual and auditory evoked potentials suggest that the delta response is related to signal detection and decision making (Basar-Eroglu et al. 1992). In freely moving rats, delta rhythms in the NAc are highest in amplitude and regularity during awake immobility and face washing (Leung and Yim 1993). In locally anesthetized, paralyzed rats, 19% of putative DA neurons (initial prolonged trough cells) in the substantia nigra exhibited a SO (Wilson et al. 1977). Whether the SO in the VTA is present in freely moving animals and how it may be related to behavior remain to be investigated.

Oscillatory firing may lead to an oscillatory release, which, in turn, may allow DA terminals to differentially interact with other inputs to the same target neurons in a frequency-dependent manner. In the NAc, DA is suggested to modulate the response of medium spiny neurons to glutamate inputs (O'Donnell 2003). When released in an oscillatory manner, DA may selectively modulate inputs that coincide with DA release. Oscillatory firing may also lead to an increased release. Gonon (1988) showed that stimulation mimicking bursting was more effective in increasing DA release than when mimicking single-spike firing. The burst stimulation protocols Gonon used also mimic slow oscillatory firing; the frequency of the SO produced by these protocols is exactly 1 Hz.

Oscillations may also play a role in information processing at the soma and dendrites. As recently reviewed by Buzsaki and Draguhn (2004), neuronal oscillations may serve as a critical "middle ground" that links single-neuron activity to behavior. Functions of neuronal oscillations include input selection, facilitation of synaptic plasticity, and promotion of synchrony between functionally related neurons (Buzsaki and Draguhn 2004).

The potential significance of understanding the SO is also highlighted by the fact that it can be differentially affected by different drugs (Shi et al. 2004). In the presence of D2 blockade, psychostimulants increase the SO. Under the same conditions, the nonpsychostimulant DA agonist l-dopa produces no effect. The $GABA_A$ agonist muscimol, on the other hand, suppresses the SO. Interestingly, although all these drugs are capable of increasing DA release, only psychostimulants are addictive. It would be interesting to further determine whether the lack of reinforcing properties of l-dopa and muscimol is related to their lack of the ability to activate the network underlying the SO.

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