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Impulse activity of midbrain dopamine neurons modulates drug-seeking behavior

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Abstract *Rationale:* Withdrawal from non-contingent exposure to psychostimulants increases the activity of midbrain dopamine cells and impairs the function of impulse-regulating dopamine autoreceptors. It is unclear whether these neuroadaptations play an important role in withdrawal-associated drug seeking. *Objectives:* We determined whether cocaine self-administration modifies the impulse activity of midbrain dopamine neurons and dopamine autoreceptor function, and whether experimentally induced reduction in dopamine cell activity (by autoreceptor activation) could influence drug-seeking behavior. *Methods:* Animals were trained to self-administer saline or cocaine (500 µg/kg per infusion) for 7 days. At different withdrawal periods, we used single-unit extracellular recordings to measure impulse activity of dopamine cells and administered the D2/D3 dopamine receptor agonist quinpirole to determine autoreceptor sensitivity. In a separate set of experiments, we determined the effects of autoreceptor-selective doses of quinpirole on drug-seeking behavior (non-reinforced responding in the absence of cocaine) during an extinction/reinstatement task. *Results:* Cocaine self-administration induced a short-lived increase in the mean firing rate and bursting activity of midbrain dopamine cells. This effect was greatest at early withdrawal and was paralleled by decreased ability of quinpirole to inhibit dopamine cell firing rate and drug-seeking behavior. Changes in dopamine cell activity dissipated over time; at late withdrawal, when both impulse activity and autoreceptor sensitivity returned to control values, quinpirole dramatically de-

creased drug-seeking behavior. *Conclusions:* These results show that inhibiting dopamine cell impulse activity, by activation of dopamine autoreceptors, reduces drug-seeking behavior. This suggests that the impulse activity of midbrain dopamine cells could be an important factor contributing to relapse.

Keywords Cocaine · Self-administration · Dopamine · Ventral tegmental area · Relapse · Electrophysiology

Introduction

Drug addiction is associated with compulsive drug seeking and vulnerability to relapse. Research on the neurobiological substrates of this disorder has focused on the dopamine system, which plays an important role in mediating the reinforcing and psychomotor-stimulant effects of drugs of abuse (Wise 1998; White and Kalivas 1998; Robinson and Berridge 2000; Koob and Le Moal 2001; Everitt and Wolf 2002). The impulse activity of dopamine cells originating in the ventral tegmental area (VTA) is of particular interest, given its role in influencing learning, motivation and reward (Grace 2000; Cooper 2002). Phasic increases in cell activity occur in response to appetitive stimuli and could serve as a signal that facilitates the learning of reward-related events (Schultz 1994, 1997, 2001), or that allows the switching of attention and behavior towards salient stimuli (Redgrave et al. 1999). In addition, heightened firing and bursting activity of midbrain dopamine cells is also a predictor of enhanced propensity to develop psychostimulant self-administration; conversely, decreased cell activity is associated with resistance to develop self-administration (Marinelli and White 2000; Marinelli et al. 2001).

Increases in the impulse activity of midbrain dopamine cells are not only associated with enhanced learning and development of drug self-administration, but are also observed following exposure to psychostimulants. In particular, repeated injections of cocaine or amphetamine cause a short-lived enhancement of dopamine cell activity

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that lasts for less than 5 days after discontinuing the drug treatment (Henry et al. 1989; White and Kalivas 1998). This transient increase in cell activity is related to subsensitivity of impulse-regulating dopamine autoreceptors, which play a critical role in regulating cell activity (Grace and Bunney 1984b; Bunney et al. 1987; White 1996). However, these studies were performed using non-contingent (experimenter delivered) drug injections, leaving it debatable whether these neuroadaptations can arise from voluntary drug consumption.

Given that repeated injections of psychostimulants cause a progressive increase in drug effects (i.e., behavioral sensitization; for review see Robinson and Berridge 1993, 2000) and enhance the propensity to acquire psychostimulant self-administration (Piazza et al. 1989, 1990; Horger et al. 1992; Pierre and Vezina 1997), these neuroadaptations in dopamine cell functioning may be critical for the initiation of behavioral sensitization and increased addiction susceptibility (Wolf et al. 1993, 1994; Henry et al. 1998). In addition, it is also possible that these changes in dopamine cell plasticity could play a significant role in relapse and drug-seeking behavior.

To address these issues, we determined whether voluntary cocaine intake modifies the activity of midbrain dopamine cells and whether drug-induced neuroadaptations in dopamine cell functioning might influence drug-seeking behavior. Rats were allowed to self-administer cocaine for 7 days. Then, at various withdrawal times from cocaine self-administration, impulse activity of VTA dopamine cells and sensitivity of impulse-regulating autoreceptors were determined. In parallel, we studied the effects of reducing dopamine cell activity (by autoreceptor activation) on drug-seeking behavior during an extinction/reinstatement task.

Materials and methods

Subjects

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 200–225 g upon arrival were used. Animals were housed individually in a temperature- and humidity-controlled colony room, under a 12-h/12-h dark/light cycle (lights on at 0700 hours). Food and water were available ad libitum throughout all experiments, at all times. The animals were allowed at least 1 week to acclimate to the animal room before any experimental manipulation. Rats were handled according to the "Guide for the Care and Use of Laboratory Animals" (1996) and the Institutional Animal Care and Use Committee of The Chicago Medical School approved all procedures.

Intravenous self-administration

Rats were anesthetized with a ketamine/xylazine solution (65 mg/kg and 20 mg/kg, respectively, in a 1-ml/kg volume). A silastic catheter (10- μ l dead volume) was inserted in the right auricle through the external jugular vein, passed under the skin and fixed in the mid scapular region. After surgery, all rats received an infusion of the antibiotic gentamicin for three consecutive days (2 mg/kg, i.v.). Thereafter, catheters were flushed daily with a sterile solution of heparin (100 μ l, 10 IU) to prevent clogging. One week after

surgery, animals were allowed to self-administer cocaine (500 μ g/kg per infusion) or saline over 7 days. Rats were submitted daily to 1-h sessions between 1500 hours and 1800 hours. Before the start of each session, the catheter was flushed with 50 μ l 0.9% NaCl and its external end connected to a pump-driven syringe. One priming infusion was given at the start of each self-administration session. The self-administration cage (41 \times 24-cm floor area, 21 cm high, MED Associates, St. Albans, VT) was equipped with two holes located 2 cm above the floor and placed on one of the 24-cm-wide sides. Nose poking in one of the holes (designated active), resulted in a 30- μ l infusion of the cocaine or saline solution over a period of 3 s. In addition to delivering cocaine, nose poking in the active hole illuminated the active hole for 30 s. In order to avoid overdosing, a time out period of 10 s was used for the first two self-administration days. This was increased to 20 s for the next 2–3 days and to 30 s for the remaining days. During the time-out period, nose poking in the active hole was recorded but had no consequences. Nose poking in the other hole (designated inactive) never had any consequences. The number of nose pokes in both holes and the number of infusions were recorded throughout the experiments using commercially available software (MED Associates Instrumentation Software for Research, St. Albans, VT). Animals were considered to perform self-administration when the number of nose pokes in the active hole was significantly higher than the number of nose pokes in the inactive one (95% confidence limit). Occasionally, some rats self-administering cocaine displayed very high levels of nose-poking behavior, possibly due to stereotypies or to computer-related problems. When the number of nose pokes in one of the holes was greater than ten standard deviations from the group mean (e.g., values generally >1000), then nose pokes in that hole for that particular day and rat were excluded from the statistics and graphs. This occurred in less than 0.3% and less than 3% of observations for the active and inactive holes, respectively.

In animals undergoing extracellular recordings, catheter patency was determined by the ability of sampling blood through the catheter on the last day of the experiment; inability to pull back blood excluded rats from the study. In animals undergoing the cocaine-seeking-behavior experiment, catheter patency was confirmed on the last day of the cocaine-seeking-behavior experiment by delivering 200 μ l of the ketamine/xylazine solution through the catheters; rats that did not succumb to the anesthetic within 3–5 s were eliminated from the study ($n=2$).

Cocaine-seeking behavior

Cocaine-seeking behavior was tested in different groups of animals either on withdrawal day (WD) one or ten following the cocaine self-administration procedure using a between-within protocol of extinction/reinstatement (Tran-Nguyen et al. 1999; Shalev et al. 2002). Rats were placed in the self-administration chambers in the absence of cocaine so that nose poking in both active and inactive holes was without consequences. Every 3 min for 2 h, rats were presented with the cocaine-paired stimulus light (Fuchs et al. 1998). The first hour served as an extinction period; the second hour served to measure seeking behavior following an injection of saline or cocaine (i.e., reinstatement). Rats were brought to the testing room in their home cages; they received an injection of quinpirole (0, 10, 50 μ g/kg, s.c.) and were placed back in their home cage. After 3–5 min, they were placed in the self-administration chamber to determine seeking behavior during the extinction phase. After the first hour of extinction, rats were removed from the self-administration cage and received a second injection of quinpirole (same dose as the previous injection); they were placed back in their home cage for 3–5 min, after which they received an injection of saline or cocaine (20 mg/kg, i.p.) and were immediately replaced in the self-administration cage to test for seeking behavior during the reinstatement phase. During both phases (extinction and reinstatement), in addition to recording nose-poking behavior, we also monitored locomotor activity via two photo beams located on the long axis of the self-administration cages.

Extracellular single-unit recording

Impulse activity of VTA dopamine cells was determined using previously described methods (Henry et al. 1989; Marinelli and White 2000). Briefly, recordings were made from naive control rats or saline or cocaine self-administration groups at four withdrawal times from 1 day to 34 days: WD1, WD3, WD10 (WDs 10–12) and WD30 (WDs 28–34). Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (Activational Systems Inc., Warren, MI). A lateral tail vein was catheterized to administer additional anesthetic or drugs. Body temperature was monitored using a rectal thermometer (Poly Medica Healthcare Inc., Golden, CO) and maintained at $37.0 \pm 0.3^\circ\text{C}$ with a thermostatically controlled heating pad (Fintronics Inc., Orange, CT). A burr hole was drilled in the skull, and the dura matter was retracted from the area overlying the VTA. A glass electrode with a tip diameter of 1–2 μm , and filled with a 2-M NaCl solution saturated with 1% fast green dye (Fisher Scientific Co., St Louis, MO), was slowly advanced with a hydraulic microdrive (David Kopf Instruments, Tujunga, CA) to the dopamine cell region. The coordinates for the VTA were 3.0–3.8 mm anterior to lambda, 0.3–0.7 mm lateral from the midline and 7.5–8.5 mm ventral from the cortical surface (Paxinos and Watson 1986).

During extracellular recording, electrical signals were detected by a high-impedance amplifier (Fintronics Inc., Orange, CT), and monitored by an oscilloscope (Tektronic R5110, Chicago, IL), using a window discriminator and an audio-amplifier to detect single-unit events (Grass AM8, Quincy, MA). Dopamine cells were identified by anatomical location in the VTA and according to standard physiological criteria (White 1996). These neurons had (1) a characteristic triphasic (+/-/+) waveform with a wide duration action potential of 2.5–3.5 ms, (2) low spontaneous firing rates of 0.5–10 Hz, and (3) either a slow irregular firing pattern or a slow bursting pattern with decreasing spike amplitude and increasing interspike interval within the burst.

Neuronal activity of VTA dopamine neurons was determined on 3–4 cells per rat (no more than 6 cells). Cells were recorded between 3 min and 5 min to establish a mean baseline-firing rate. Only cells with stable activity over at least 3 min (<5% variation) were included in the study.

For some experiments, an analog-to-digital interface digitized the signal (Digidata 1200 series, Axon Instruments Inc., Foster City, CA) to a PC running AxoScope software (Axon Instruments Inc., Foster City, CA) that measured impulse activity on-line and stored all data for future analysis. Stored data were then analyzed with a custom-made program from our laboratory (BURST c, M. Marinelli) that calculated firing and bursting activity. Firing rate was calculated as the total number of spikes over time. Instantaneous interspike intervals were calculated for each cell in bins of 20 ms and reported as the percentage of observations for each bin (from 0–20 ms to 2120–2140 ms; total of 107 bins). To determine the variability of individual interspike intervals and the precision of spike timing, we calculated the coefficient of variation (CV). This was determined by dividing the standard deviation of the interspike interval distribution (fitted to a Gaussian function) by the average interspike interval and expressed as percentage (Wolfart et al. 2001; Wolfart and Roeper 2002). We also determined bursting activity; bursts are clusters of spikes occurring at high frequencies and were calculated according to previously established criteria (Grace and Bunney 1983, 1984a). Briefly, a burst initiates with a pair of spikes having an interspike interval of 80 ms or less and terminates when interspike intervals are 160 ms or greater. We evaluated the amount of bursting activity by calculating the percentage of spikes emitted in bursts and the burst event frequency (number of burst events over time). To determine the characteristics of the bursts, we evaluated the burst size (number of spikes within each burst), the duration of each burst, and the average spike frequency within bursts. All cells (except for two, i.e., <2%), displayed some level of bursting activity during the 3–5 min of recording. Bursting activity was, however, observed to different degrees, ranging from as low as 0.5% of bursting spikes to as high as 96%. Cells with lowest

bursting activity generally displayed numerous single spikes (i.e., protracted periods of non-bursting activity) and a low proportion of two-spike bursts. Cells with high bursting activity, displayed a small number of single spikes and numerous bursts, either two-spiked or larger.

Autoreceptor-mediated inhibition of VTA dopamine cells

Following at least 3 min of stable basal firing, the D2/D3 dopamine receptor agonist quinpirole was administered through the tail vein using a cumulative dosing regimen in which each dose doubled the previous one at 60- to 90-s intervals. The D2 receptor selective antagonist eticlopride (0.1 mg/kg, i.v.) was administered to the reverse agonist-induced inhibition. For this experiment, only one cell was recorded from each rat.

Histology

The position of the electrode tip was marked by passing a 28- μA cathodal current through the electrode for 20 min at the end of the recording. Rats were then deeply anesthetized with additional chloral hydrate and perfused transcardially with 0.9% NaCl followed by 10% formalin. Brains, stored in 10% formalin, were then cut on a freezing microtome (American Optical Corporation, Buffalo, NY). Coronal sections (30 μm) were then mounted and electrode placement was verified using light microscopy.

Drugs

Quinpirole HCl and eticlopride HCl were obtained from Research Biochemicals Inc. (Natick, MA). Chloral hydrate, ketamine and heparin were from Sigma Chemical Co. (St. Louis, MO). Gentamicin was from ICN Biomedicals Inc (Costa Mesa, CA). Xylazine was from Phoenix Scientific Inc (St. Joseph, MO). Cocaine HCl was obtained from the National Institute on Drug Abuse (Rockville, MD).

Statistical analyses

All data were analyzed with an analysis of variance (ANOVA) considering the different experimental groups as between factors. Post-hoc comparisons were made using *t*-tests. To compare saline self-administration with cocaine self-administration, we performed an ANOVA considering drug (saline vs cocaine) as the first between factor and WDs (WD1, 3, 10, 30) as the second between factor. For each group, we also determined the active and inactive hole discrimination by performing repeated-measures ANOVAs or paired *t*-tests (where appropriate). All correlation analyses were performed using Pearson's Correlation tests. The dose-response effects of quinpirole on cell firing rate were analyzed with repeated-measures ANOVA using the different experimental groups as between factor and the doses of quinpirole as the within factor. The effects of quinpirole were also analyzed with repeated-measures analysis of co-variance (ANCOVA) using basal firing rate (i.e., quinpirole dose 0) as the co-variate and the doses of quinpirole as within factor. This analysis was performed to determine whether any differences between groups were due to differences in basal firing rates (White and Wang 1984a). The dose of quinpirole required to produce inhibition of firing rate was calculated as the dose that did not statistically differ from maximal effect using a paired *t*-test.

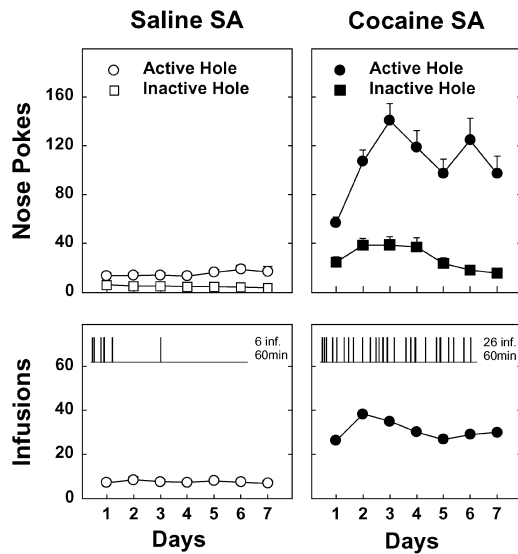


Fig. 1 Self-administration (SA) of saline or cocaine (500 µg/kg per infusion) over 7 days. Nose pokes in active and inactive holes are depicted in the *top panels*, infusions in the *bottom panels*. Each *point* represents the mean \pm SEM of each group ($n=22$ for saline, $n=134$ for cocaine). *Insets* show representative infusion records from two rats during the last self-administration session; each *vertical line* represents an infusion (*inf.*) and the *x-axis* represents time (60 min)

Results

Cocaine self-administration

As Fig. 1 shows, all rats acquired self-administration behavior of saline and cocaine (500 µg/kg per infusion). Throughout the self-administration training, nose poking in the active hole (associated with cocaine and/or the light cue) was significantly greater than in the inactive hole (hole effect: $F_{1,21}=42.2$, $P<0.001$ and $F_{1,133}=20.5$, $P<0.001$ for saline and cocaine, respectively). As expected, drug intake (number of self-infusions) and responding (number of nose pokes in the active hole) were greater for cocaine than for saline (drug effect: $F_{1,154}=105.2$ and 23.1 , $P<0.001$ for infusions and active hole nose pokes, respectively). Rats self-administering saline took an average of 6–9 infusions per sessions, whereas rats self-administering cocaine took an average of about 30 infusions per session (i.e., 15 mg/kg i.v. per day for 7 days). Self-administration behavior was constant across the last four self-administration sessions (days effect $F_{3,399}=2.28$ and 1.93 , n.s. for infusions and active hole, respectively); see Fig. 1 insets for representative infusion records during the last self-administration session.

Groups were normalized by assigning rats to different experimental groups according to their self-administration behavior, so that animals in each group had a similar history of drug responding and intake (F values=0.05–1.64, n.s. for all group comparisons).

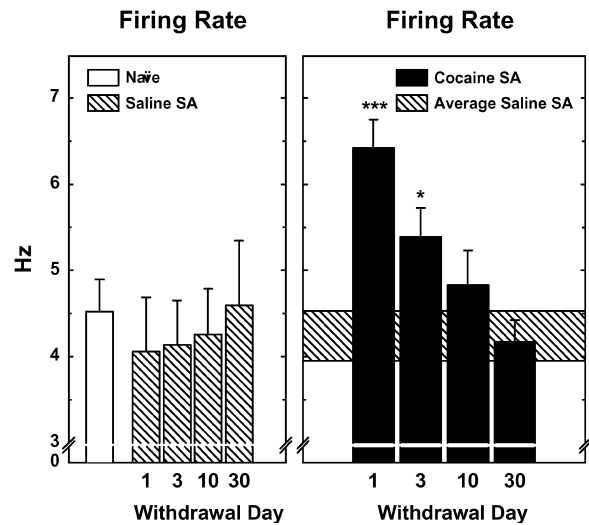


Fig. 2 Firing rate of ventral tegmental area dopamine cells in naive rats, and in rats recorded at different withdrawal days from saline or cocaine self-administration (SA). *Vertical bar* represents the mean \pm SEM of each group. Naive rats: $n=39$ cells and 18 rats; saline SA: $n=10$ –16 cells per group and 4–6 rats per group; cocaine SA: $n=26$ –40 cells per group and 8–11 rats per group. The *horizontal bar* represents the mean \pm SEM of the pooled saline self-administration groups (average saline SA group; $n=55$ cells and 22 rats). * $P<0.05$, *** $P<0.01$ compared with the average saline SA group

Impulse activity of dopamine cells following cocaine self-administration

In these experiments, we compared impulse activity of VTA dopamine neurons in naive control rats, control rats that self-administered saline and rats that self-administered cocaine. Recordings were performed in separate groups of animals at different WDs from self-administration: WD1, WD3, WD10, WD30.

For all parameters, there were no differences between WDs across the saline self-administration groups (WD effect F values=0.08–0.34, n.s., see Fig. 2 for firing rate; other data not shown). Thus, data from animals that self-administered saline were pooled across WDs (average saline self-administration group). There were also no differences between rats that self-administered saline and naive rats (t values=–0.86–0.61, n.s., see Fig. 2 for firing rate; other data not shown).

Firing rate

After cocaine self-administration, there was a short-lived increase in dopamine cell impulse activity (group effect: $F_{4,179}=9.13$, $P<0.001$). The firing rate of rats recorded on WD1 from cocaine self-administration was approximately 2 Hz higher than that observed in control rats (>50% increase). Firing rate was still elevated on WD3; baseline values were recovered by WD10 and WD30 (Fig. 2). Cocaine self-administration also modified the distribution of firing rates across VTA dopamine cells. In naive rats

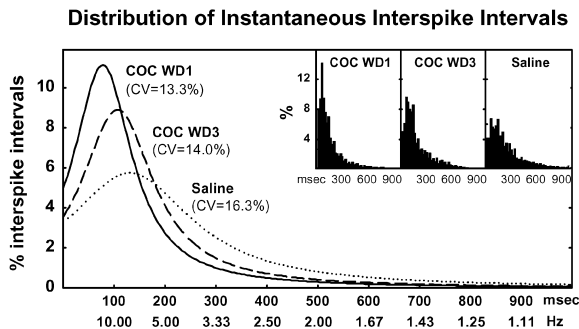


Fig. 3 Peak and distribution of interspike intervals in ventral tegmental area dopamine cells following saline self-administration or at different withdrawal times from cocaine self-administration. Instantaneous interspike intervals are binned in 107 intervals of 20 ms each (from 0–20 ms to 2120–2140 ms) and plotted as a percentage of observations occurring at each particular bin (expressed in ms and Hz on the *x*-axis; axis truncated at 1000 ms in the above figures). *Insets* represent the data used to generate the Gaussian curves from animals recorded on WD1 or WD3 from cocaine self-administration (COC WD1, $n=34$ cells and 9 rats; COC WD3, $n=24$ cells and 9 rats, respectively), or after saline self-administration (saline, $n=54$ cells and 22 rats). The coefficient of variation (CV) was determined by dividing the standard deviation of the Gaussian curve by the average interspike interval and expressed as a percentage

and rats that self-administered saline, only a small subpopulation of neurons exhibited high (>5 Hz) firing rates (28.21% and 34.55% of cells, respectively). The proportion of fast-firing cells was doubled on WD1 from cocaine self-administration (70.00% of cells), and gradually returned to control levels in a time-dependent manner (54.84, 38.46 and 31.25% of cells for WDs 3, 10 and 30, respectively).

Interspike intervals

Changes in impulse activity paralleled changes in interspike intervals. As Fig. 3 shows, cocaine self-administration and withdrawal modified both the peak and the distribution of the interspike intervals. Control rats had an interspike interval peak at 131.2 ± 5.1 ms and a widespread distribution of interspike intervals (CV=16.3%). Instead, rats recorded on WD1 from cocaine self-administration had a peak at 77.8 ± 2.3 ms, and a more narrow distribution of interspike intervals (CV=13.3%); thus, most spikes were emitted at short interspike intervals and a minority at longer intervals. On WD3 from cocaine self-administration, interspike interval peak (106.8 ± 2.9 ms) and distribution (CV=14.0%) were still modified. They gradually returned to control values over time (data not shown; for WD10 and WD30, respectively: interspike interval peak 111.8 ± 3.4 ms and 149.3 ± 4.9 ms; CVs 15.7% and 17.4%).

Amount of bursting

Cocaine self-administration and withdrawal modified the percentage of spikes emitted in bursts (group effect $F_{4,145}=6.35$, $P<0.001$). Approximately 30% of spikes were emitted in bursts in the saline self-administration group. The amount of bursting was doubled in animals recorded 1 day after the end of the cocaine self-administration procedure and gradually returned to control values over time (Fig. 4a). Self-administration of cocaine also modified the distribution of bursting activity across VTA dopamine cells. In saline self-administration control rats, only a minority of VTA dopamine cells (25.93%) fired more than 50% of their spikes in bursts. Instead, on WD1 from cocaine self-administration, the majority of cells (67.65%) fired more than 50% of their spikes in bursts. The proportion of cells showing high levels of bursting activity gradually returned to control levels over time (41.67, 27.27 and 29.63% of cells on WDs 3, 10 and 30, respectively). See Fig. 4c for traces depicting different levels of bursting activity.

Cocaine self-administration and withdrawal modified the amount of burst events emitted over time (group effect $F_{4,145}=5.30$, $P<0.001$). In saline self-administering rats, burst events occurred at low frequencies; instead, the number of burst events was almost twice as high in rats recorded 1 day after the end of the cocaine self-administration experience and gradually returned to control values as the withdrawal time from cocaine self-administration increased (Fig. 4b). The proportion of cells exhibiting high levels of burst events also changed after cocaine self-administration. In animals that self-administered saline, only a minority of cells (16.67%) exhibited more than 1 burst event/s. Instead, in animals recorded on WD1 from cocaine self-administration, over one-half of the cell population exhibited this frequency of bursting (52.94% of cells). The increased frequency of burst events progressively returned to control levels over time (33.33, 18.18 and 14.81% of cells on WDs 3, 10 and 30, respectively).

Characteristics of the bursts

Cocaine self-administration induced a short-lived increase in the number of spikes per burst (group effect $F_{4,143}=8.65$, $P<0.001$). Within each burst event, rats that self-administered saline generally displayed several two-spike bursts, and a smaller proportion of three or more spikes per burst. On WD1 from cocaine self-administration, the average number of spikes per burst was about 50% higher. This increase in spikes per burst induced by cocaine self-administration gradually dissipated over time (Fig. 5a). Cocaine self-administration also modified the distribution of spikes per burst in VTA dopamine cells. In animals that self-administered saline, only a minority of cells (33.33%) displayed more than 3 spikes per burst. Instead, on WD1 from cocaine self-administration, a majority of cells exhibited more than 3 spikes per burst

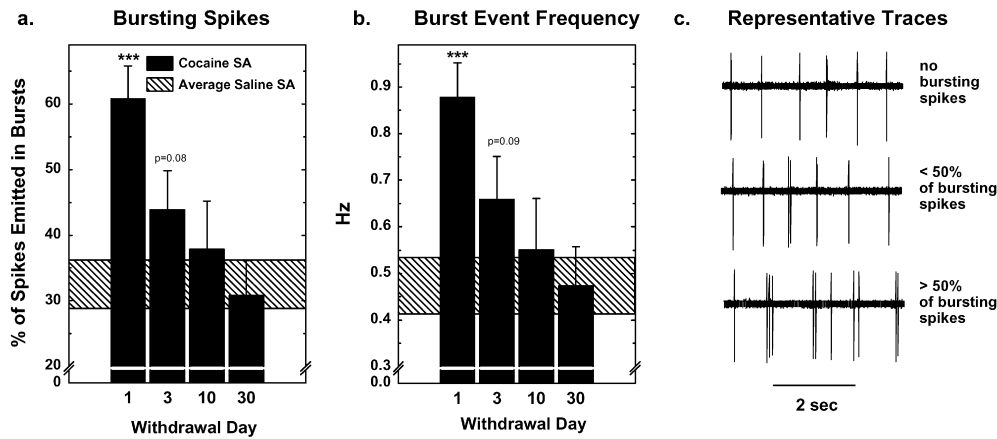


Fig. 4a–c Amount of bursting activity in ventral tegmental area dopamine cells following saline self-administration, or at different withdrawal times from cocaine self-administration. **a** Bursting spikes, calculated as a percentage of bursting spikes over the total number of spikes. **b** Burst event frequency, calculated as the number of burst events/s. Vertical bars represent the mean \pm SEM of the cocaine self-administration groups (cocaine SA; $n=11$ – 34 cells and $n=5$ – 9 rats per group). The horizontal bar represents the mean \pm SEM of the pooled saline self-administration groups (average saline SA group; $n=54$ cells and $n=22$ rats). $P=0.09$, $P=0.08$,

*** $P<0.001$ compared with the average saline SA group. **c** Representative excerpts from traces illustrating different levels of bursting activity: no bursting (no spikes emitted in bursts), low rate of bursting (<50% of spikes emitted in bursts), high rate of bursting (>50% of spikes emitted in bursts). Portions of traces showing no bursting or low rate of bursting were typically observed in naive animals or rats that self-administered saline. Instead, a majority of cells exhibited high rate of bursting on WD1 from cocaine self-administration

(73.53% of cells), and many cells displayed several burst events with averages of 10 spikes per burst (Fig. 5c). The proportion of cells exhibiting this strong bursting decreased in a time-dependent manner (50.00, 36.30 and 29.63% of cells on WDs 3, 10 and 30, respectively).

Cocaine self-administration and withdrawal modified the duration of the bursts (group effect: $F_{4,143}=5.78$, $P<0.001$). Compared with control animals, the average burst duration was increased by more than 100 ms in rats recorded on WD1 from cocaine self-administration. This increase in burst duration induced by cocaine dissipated over time, and there was a tendency for decreased burst duration on WD30 from cocaine self-administration (Fig. 5b).

There were no changes in firing rates within the bursts (group effect $F_{4,143}=1.56$, n.s.), reflecting the parallel changes in burst duration and spikes per burst that were observed across groups (Fig. 5). The intra-burst spike frequency was 18.10 ± 0.99 , 16.40 ± 0.98 , 16.25 ± 0.77 , 15.02 ± 0.68 and 19.34 ± 1.56 for the saline and WD1–30 groups, respectively.

Correlation between firing and bursting activity

Given that only two cells exhibited no bursting activity and only a small number of cells in the WD1 group showed low levels of bursting activity, it is difficult to determine whether the increase in firing rate observed following cocaine self-administration was specific to bursting versus non-bursting cells. To address this issue, we analyzed whether the level of firing was correlated with the level of bursting in control animals and in groups

whose impulse activity was increased after cocaine self-administration (WD1, WD3). As Fig. 6 shows, correlation between firing and bursting activity was similar across groups, suggesting that cells with both low and high levels of bursting activity were equally affected by cocaine self-administration ($r=0.66$, $P<0.001$; $r=0.76$, $P<0.001$, $r=0.74$, $P<0.001$, for animals in the saline, WD1 or WD3 groups, respectively; comparison between regression slopes: saline vs WD1, $P>0.36$, saline vs WD3, $P>0.51$). Had only elevated-bursting cells been affected, we would have observed different slopes in the cocaine self-administration groups.

Correlation between cocaine self-administration and impulse activity

Although animals that self-administered cocaine showed increased impulse activity of VTA dopamine cells, there was no correlation between this increase and prior self-administration behavior. Thus, there was no relationship between the amount of cocaine each rat took and its subsequent dopamine cell firing rate (Table 1) or bursting activity (data not shown). For example, on WD1, an 8-Hz firing rate was observed both in an animal that took an average of 18 infusions of cocaine and one that took 50. On the same line, on WD3, two different rats, both having taken an average of 35 infusions of cocaine, exhibited firing rates of 3.8 Hz and 7.5 Hz. Similarly, impulse activity was not correlated with drug responding (number of nose pokes in the active hole) or with active/inactive hole ratio. Finally, there was no correlation between impulse activity and saline self-administration behavior.

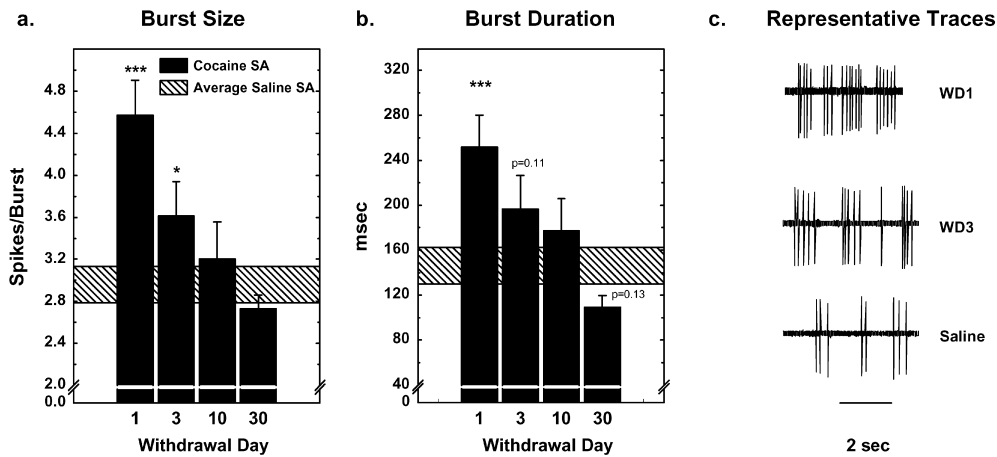


Fig. 5a–c Characteristics of bursts in ventral tegmental area dopamine cells following saline self-administration or at different withdrawal times from cocaine self-administration. **a** Number of burst events/s. **b** Burst size (number of spikes per burst). Each vertical bar represents the mean \pm SEM of the cocaine self-administration groups. The horizontal bar represents the mean \pm SEM of the pooled saline self-administration groups. The number of rats and cells per group are reported in legend to Fig. 4. $P=0.13$,

$P=0.11$, $***P<0.001$, compared with the average saline SA group. **c** Representative traces illustrating the high level bursting that was observed at early withdrawal times from cocaine self-administration. The top trace was obtained from a rat recorded on WD1 from cocaine self-administration, the middle trace from one recorded on WD3, and the bottom trace from a rat that was recorded after saline self-administration (note the difference in burst size and duration across groups)

Table 1 Correlation analyses between impulse activity of ventral tegmental area dopamine and prior self-administration behavior. For each rat, we correlated the average firing rate of dopamine cells with previous intake of cocaine (average over the 7 days of testing),

responding in the active hole (AH) or the ratio between active and inactive hole (AH/IH). Values represent the number of animals (n) and the Pearson's correlation coefficients for each group

Group	n	Firing rate vs infusions	Firing rate vs AH	Firing rate vs AH/IH
Saline	$n=22$	$r=0.027$, $P=0.75$	$r=-0.039$, $P=0.86$	$r=0.028$, $P=0.90$
WD1	$n=11$	$r=0.006$, $P=0.98$	$r=0.012$, $P=0.97$	$r=0.329$, $P=0.32$
WD3	$n=10$	$r=-0.038$, $P=0.91$	$r=0.031$, $P=0.93$	$r=-0.340$, $P=0.34$
WD10	$n=11$	$r=0.457$, $P=0.16$	$r=0.187$, $P=0.58$	$r=-0.428$, $P=0.19$
WD30	$n=8$	$r=0.148$, $P=0.73$	$r=0.376$, $P=0.36$	$r=-0.346$, $P=0.40$

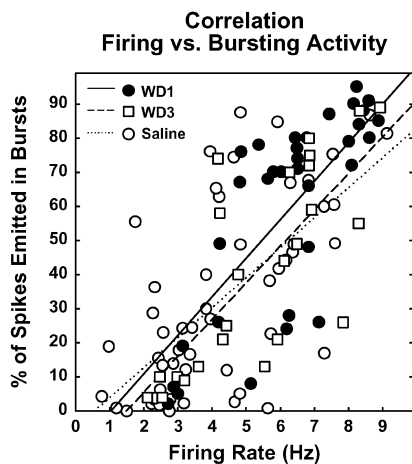


Fig. 6 Correlation between firing and bursting activity in ventral tegmental area dopamine cells. Points represent firing rate and bursting activity of individual cells recorded from rats at different withdrawal times from cocaine self-administration (WD1 or WD3), or after saline self-administration (Saline)

Autoreceptor-mediated decrease in impulse activity of dopamine cells following cocaine self-administration

In this experiment, we tested the ability of VTA dopamine cell autoreceptors to inhibit impulse activity in control conditions (following saline self-administration), or at different withdrawal times from cocaine self-administration (WD1 or WD10). These withdrawal times were chosen on the basis of the previous experiment, which showed that impulse activity was modified after 1 day from the end of the cocaine self-administration experience, but it was recovered by WD10. The response of dopamine neurons to intravenous administration of the D2/D3 receptor agonist quinpirole was used as a measure of autoreceptor sensitivity (for review see White 1996).

In all groups of animals, quinpirole caused a dose-dependent decrease in the firing rate of VTA dopamine cells (dose effect $F_{11,198}=130.08$, $P<0.001$). However, this effect differed across experimental groups (group effect $F_{2,18}=6.86$, $P<0.01$; group \times dose interaction $F_{22,198}=5.81$, $P<0.001$). Thus, while quinpirole-induced inhibition was similar between animals that self-administered saline and

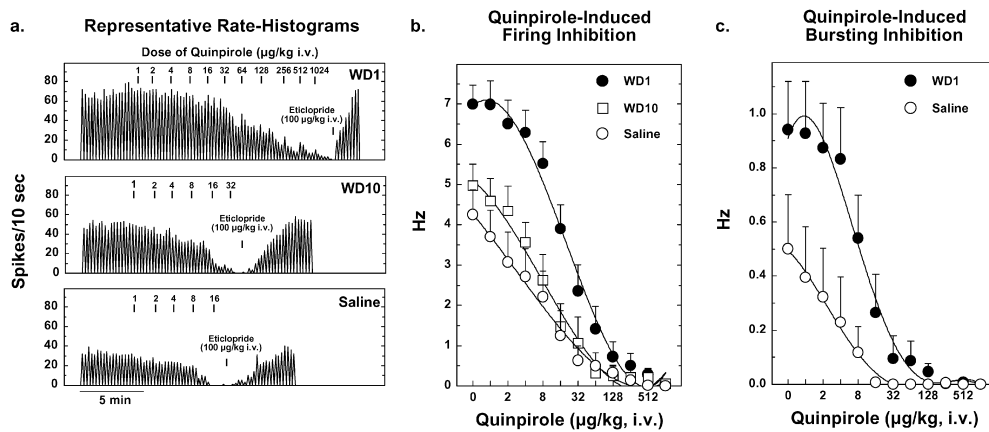


Fig. 7a–c Autoreceptor-mediated inhibition of firing and bursting in ventral tegmental area dopamine cells. **a** Representative rate-histograms illustrating quinpirole-induced inhibition of firing rate in a rat recorded on WD1 from cocaine self-administration, on WD10, or after self-administration of saline. In all cells, the effects of quinpirole were reversed by the D2-class receptor antagonist eticlopride (100 $\mu\text{g/kg}$, i.v.). Vertical lines overlapped by a number respectively indicate the time points and doses at which drugs were administered. **b** Effects of cumulative doses of quinpirole on the

firing rate of cells recorded at 1 or 10 WDs from cocaine self-administration (WD1 and WD10 groups), or after self-administration of saline (Saline). Each point represents the mean \pm SEM of the saline self-administration group ($n=8$), the WD1 group ($n=7$) or the WD10 group ($n=6$). **c** Effects of cumulative doses of quinpirole on the burst event frequency of cells recorded on WD1 after cocaine self-administration or after self-administration of saline. Each point represents the mean \pm SEM of each group ($n=7$ per group)

those recorded on WD10 from cocaine self-administration ($P>0.47$), it was attenuated in animals recorded on WD1 from cocaine self-administration ($P<0.01$ and $P<0.02$ compared with animals in the saline self-administration or WD10 groups, respectively) (Fig. 7a, b).

Because the sensitivity of dopamine cells to autoreceptor agonists is negatively correlated with basal firing rate (White and Wang 1984a), the response to quinpirole was also analyzed using an analysis that controls for these differences in baseline activity (ANCOVA). When this initial difference in baseline (group effect for baseline firing rate $F_{2,18}=6.63$, $P<0.01$; WD1 vs saline, $P<0.05$; WD1 vs WD10, $P<0.05$; saline vs WD10, $P>0.70$) was statistically controlled, there were no longer any overall group differences (group effect $F_{2,17}=0.43$, n.s.), although we still observed a group \times dose interaction (group \times dose interaction $F_{20,180}=6.24$, $P<0.001$). This suggests that the higher basal firing rate of animals recorded on WD1 from cocaine self-administration partly contributed to the lower sensitivity to quinpirole.

Regardless of this, animals in the WD1 group required a fourfold higher dose of quinpirole to produce complete inhibition of neuronal firing (128 $\mu\text{g/kg}$, paired t -test with the maximal dose of quinpirole, $P>0.10$) compared with animals in the other two groups (32 $\mu\text{g/kg}$, $P>0.12$ and $P>0.16$ for WD10 and saline groups, respectively). In addition, whereas a very low dose of quinpirole was sufficient to produce a significant decrease in basal firing rate in the saline self-administration group (1 $\mu\text{g/kg}$, paired t -test with baseline, $P<0.05$), higher doses were required to produce a similar effect in animals from the WD1 group (4 $\mu\text{g/kg}$, $P<0.05$). The first dose to differ from baseline in the WD10 group was 2 $\mu\text{g/kg}$ ($P<0.05$), and the 1- $\mu\text{g/kg}$ dose almost reached statistical signifi-

cance ($P=0.08$). This indicates that changes in firing rate of VTA dopamine cells could not be produced by low doses of quinpirole at an early withdrawal time (WD1), whereas they could at a later withdrawal time (WD10).

Similar effects were observed on bursting activity. Thus, compared with control rats, animals recorded on WD1 from cocaine self-administration showed reduced quinpirole-induced inhibition of burst event frequency (Fig. 7c; group effect $F_{1,12}=5.1$, $P<0.05$; group \times dose interaction $F_{11,132}=3.40$, $P<0.001$) and percentage of bursting spikes (data not shown, group effect $F_{1,12}=7.57$, $P<0.02$; group \times dose interaction $F_{11,132}=3.85$, $P<0.001$).

Cocaine-seeking behavior

In these experiments, we tested the effects of low (autoreceptor-selective) doses of quinpirole on cocaine-seeking behavior. For this, animals were placed in the self-administration cage in the absence of cocaine; nose poking in the previously active hole was considered as an index of seeking behavior (Shalev et al. 2000, 2002; Stewart 2000). Seeking behavior was tested over 2 h; the first hour served as an extinction session (extinction); the second hour tested the ability of saline or cocaine (20 mg/kg, i.p.) to reinstate seeking behavior (reinstatement). Quinpirole (0, 10, 50 $\mu\text{g/kg}$, s.c.) was administered both before the extinction and the reinstatement session.

Separate groups of rats were tested at different withdrawal times from cocaine self-administration: on WD1 or on WD10. These withdrawal times were chosen on the basis of the previous experiment, which showed that sensitivity to quinpirole-induced firing inhibition of VTA dopamine cells was functionally decreased on WD1,

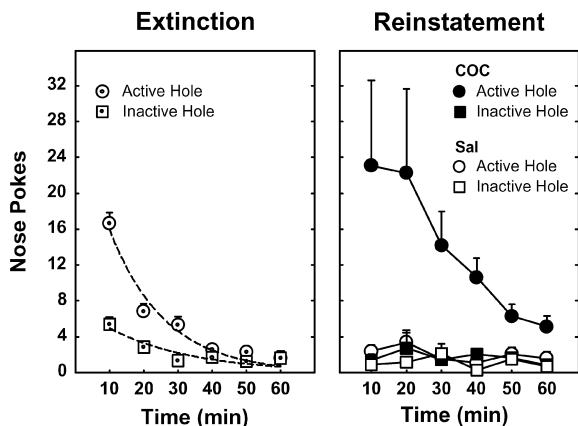


Fig. 8 Drug-seeking behavior in control animals. Nose poking in the previously active or inactive hole during the extinction (*left panel*) or reinstatement (*right panel*) phase. During the reinstatement phase, animals received either cocaine (COC, 20 mg/kg, i.p.) or saline (Sal) as the reinstatement drug. Points represent the mean \pm SEM of each group. For the extinction phase: $n=41$ rats; for the reinstatement phase: $n=28$ or $n=13$, respectively, for groups receiving cocaine or saline as the reinstatement drug. Dotted lines during the extinction phase represent the exponential decay fit for active or inactive holes

and was recovered by WD10. To determine the effects of quinpirole on seeking behavior, for each WD, data were expressed as a percentage of the response obtained in their respective control group (quinpirole dose 0 μ g/kg).

Comparison between WD1 and WD10 and analysis of seeking behavior

In a first set of analyses, we compared behavioral responses obtained on WD1 with those observed on WD10 in control animals (rats treated with 0 μ g/kg quinpirole). Responding was similar across WDs, during both the extinction and reinstatement phases (data not shown; WD effect: $F_{1,37}$ values=0.01–2.75, n.s. for active hole, inactive hole, and locomotor activity during the extinction or reinstatement phases). For rats tested on WD1 or WD10, respectively, nose pokes in the active hole were 31.4 ± 3.2 and 41.3 ± 4.7 during the extinction phase and 81.2 ± 33.9 and 82.1 ± 22.1 for cocaine-induced responding.

Given the similarity between WDs in control animals, we pooled groups to determine the validity of our extinction/reinstatement paradigm. As Fig. 8 shows, during the first hour of extinction, responding decreased exponentially over time (for previously active hole: exponential decay function fit $r=0.98$, $P<0.001$, decay time 16 min; for inactive hole $r=0.91$, $P<0.02$, decay time 25 min). The number of nose pokes in the previously active hole was higher than in the inactive hole (hole effect $F_{1,40}=72.3$, $P<0.001$), but this difference was only present during the first half of the session (time 0–30 min: hole effect $F_{1,40}=92.0$, $P<0.001$), and not during the second half (time 40–60 min: hole effect $F_{1,40}=2.79$, n.s.);

this indicates that by the end of the session, seeking behavior had extinguished.

Responding at the end of the extinction phase did not increase significantly after an injection of saline (from 1.54 ± 0.53 to 2.38 ± 0.73 ; $t_{12}=-0.966$, n.s.); animals receiving saline during the second hour maintained very low levels of responding throughout the session, indicating the absence of spontaneous recovery of seeking behavior. Instead, responding increased from 1.68 ± 0.37 at the end of the extinction session to 23.07 ± 9.50 after the injection of cocaine, revealing reinstatement of previously extinguished seeking behavior ($t_{27}=-2.25$, $P<0.04$). Throughout the reinstatement phase, animals receiving cocaine showed higher levels of responding in the previously active hole compared with animals receiving saline (reinstatement-drug effect $F_{1,39}=4.20$, $P<0.05$). No differences were observed for the inactive hole (reinstatement-drug effect $F_{1,39}=0.43$, n.s.).

Effects of autoreceptor doses of quinpirole on WD1

On WD1, seeking behavior was only moderately modified by quinpirole (Fig. 9a). During the extinction phase, the low dose of quinpirole did not modify responding in the previously active hole, and the high dose of quinpirole decreased responding by approximately 50% (group effect $F_{2,51}=6.70$, $P<0.003$). A slight decrease in the number of responses in the inactive hole were also observed (data not shown, group effect $F_{2,51}=3.63$, $P<0.05$). Quinpirole had similar effects during the reinstatement phase, when animals were tested for seeking behavior after an injection of cocaine or saline (group effect $F_{4,49}=2.50$, $P<0.05$). The low dose of quinpirole did not modify cocaine-induced responding, and the high dose decreased it by 50%, but this effect did not reach statistical significance. Quinpirole had no effects on saline-induced responding. These effects were specific to the previously active hole, as no significant group differences were seen in the number of nose pokes in the inactive hole (data not shown; group effect $F_{4,49}=1.12$, n.s.).

Similar findings were observed for locomotor activity (Fig. 9b). During the extinction phase, the low dose of quinpirole slightly (about 20%) decreased locomotor activity, while the higher dose of quinpirole decreased motor activity by about 50% (group effect $F_{2,51}=14.00$, $P<0.001$). During the reinstatement phase (group effect $F_{4,49}=3.75$, $P<0.01$), the low dose of quinpirole did not modify locomotor activity following the injection of cocaine, and the high dose decreased it by about 40%. Quinpirole had no effects on saline-induced locomotor activity.

Effects of autoreceptor doses of quinpirole on WD10

On WD10, seeking behavior was greatly modified by quinpirole (Fig. 10a). During the extinction phase, both

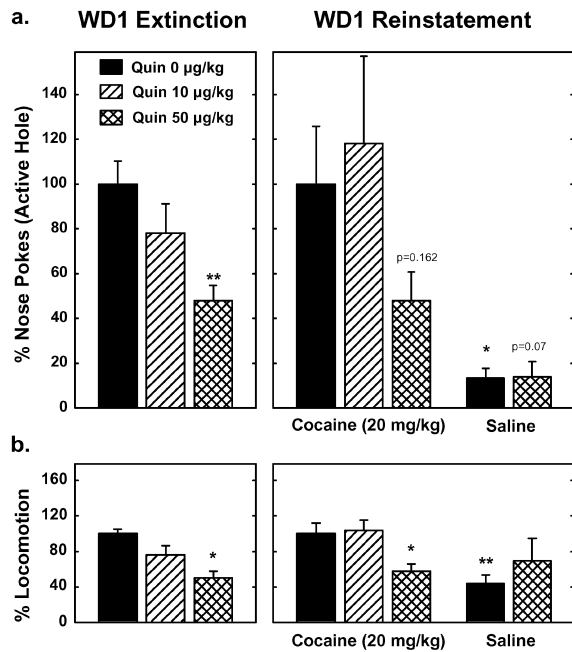


Fig. 9a,b Drug-seeking behavior and locomotor activity on WD1 from cocaine self-administration. Effects of quinpirole on (a) nose-poking behavior in the previously active hole or (b) locomotor activity. Behavior was recorded during the extinction (*left panels*) or reinstatement (*right panels*) phases. During the reinstatement session, animals received either cocaine (COC, 20 mg/kg, i.p.) or saline. Bars represent mean +SEM of each group (reinstatement drug cocaine: $n=10-18$ per group; reinstatement drug saline: $n=6-7$ per group). Data are expressed as a percentage of the response obtained in animals receiving quinpirole dose 0 µg/kg; this group served for all post-hoc comparisons ($P=0.162$, $P=0.07$, $*P<0.05$, $**P<0.01$)

the low and the high doses of quinpirole dramatically decreased nose poking in the previously active hole (dose-dependent effects of 60% and 80% decreases, respectively). The decreases were mainly restricted to this hole (group effect $F_{2,37}=20.92$, $P<0.001$), as quinpirole only slightly, but not significantly, decreased responding in the inactive hole (data not shown, group effect $F_{2,37}=2.41$, $P=0.07$). Similar effects on seeking behavior were observed during the reinstatement phase (group effect $F_{4,35}=5.99$, $P<0.001$). Thus, nose poking in the previously active hole was 70% and 80% lower in animals receiving the low and high doses of quinpirole, respectively. These effects were specific to cocaine-induced reinstatement because quinpirole did not modify saline-induced reinstatement or responding in the inactive hole (data not shown; group effect $F_{4,35}=0.85$, n.s.).

Concerning locomotor activity (Fig. 10b), both doses of quinpirole dramatically decreased locomotor activity during the extinction phase (group effect $F_{2,37}=48.27$, $P<0.001$). During the reinstatement phase, locomotor activity differed across the five groups (group effect $F_{4,35}=2.88$, $P<0.05$). Only the high dose of quinpirole decreased cocaine-induced locomotion, whereas the low dose had no effects. Thus, on WD10, the low dose of

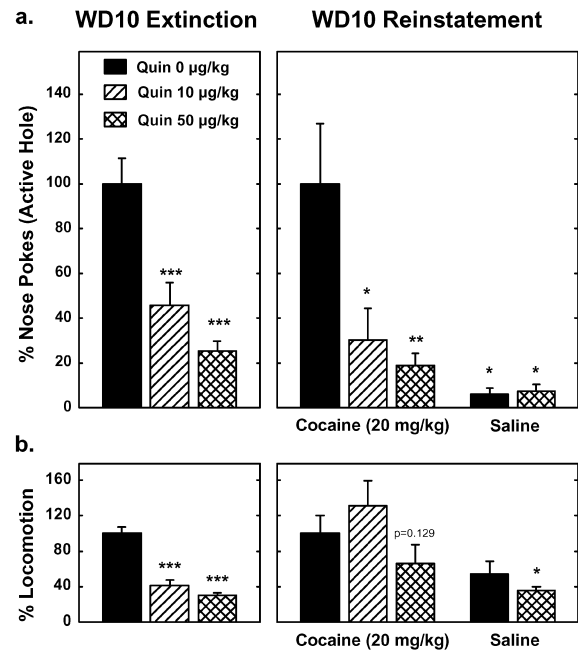


Fig. 10a,b Drug-seeking behavior and locomotor activity on WD10 from cocaine self-administration. Effects of quinpirole on (a) nose-poking behavior in the previously active hole or (b) locomotor activity. Behavior was recorded during the extinction (*left panels*) or reinstatement (*right panels*) phases. During the reinstatement session, animals received either cocaine (COC, 20 mg/kg, i.p.) or saline. Bars represent mean +SEM of each group (reinstatement drug cocaine: $n=8-10$ per group; reinstatement drug saline: $n=6-7$ per group). Data are expressed as a percentage of the response obtained in animals receiving quinpirole dose 0 µg/kg; this group served for all post-hoc comparisons ($*P<0.05$, $**P<0.01$, $***P<0.001$)

quinpirole decreased cocaine-induced seeking behavior without affecting cocaine-induced locomotion.

Discussion

Our results show that cocaine self-administration induces a short-lived increase in the firing and bursting activity of midbrain dopamine cells. This effect was greatest on WD1 and was paralleled by a functional subsensitivity of impulse-regulating dopamine autoreceptors. Changes in dopamine cell activity dissipated over time; both impulse activity and autoreceptor sensitivity returned to control values by WD10. On WD1 (when dopamine autoreceptors were functionally subsensitive), autoreceptor activation only modestly modified drug-seeking behavior; whereas, on WD10, (when dopamine autoreceptors were normo-sensitive), it dramatically decreased drug-seeking behavior. These results suggest that drug-seeking behavior can be reduced by an autoreceptor-mediated decrease in dopamine cell activity.

Cocaine self-administration and impulse activity of midbrain dopamine cells

Enhanced dopamine cell activity was greatest on WD1 and diminished in a time-dependent manner on subsequent days. Impulse activity was still elevated on WD3 for firing rate and burst size, and there was a trend toward an increased burst duration, burst event frequency and percentage of relative bursting. By WD10, all parameters had returned to control levels. Interestingly, there was no correlation between increased impulse activity and prior drug intake or drug responding. This suggests that a low threshold quantity of cocaine is sufficient to switch dopamine cells to a state of enhanced excitability. These effects were not the consequence of being engaged in an operant behavior alone, as animals that actively self-administered saline showed similar impulse activity as naive animals.

Cocaine self-administration and withdrawal also increased the proportion of cells showing high levels of bursting activity. In addition, the quality of bursting was also changed as evidenced by the fact that rats recorded on WD1 from cocaine self-administration showed greater burst duration, burst event frequency and numbers of spikes per burst. These results, together with the fact that we observed a similar correlation between firing and bursting activity across all groups, suggest that cocaine exposure induced a global increase in impulse activity and bursting.

Interestingly, cocaine self-administration and withdrawal also produced a leftward shift in the interspike interval peak and narrowed the distribution of interspike intervals, suggesting an increase in the precision of spike timing. This association between decreased variability of interspike intervals and increased bursting observed *in vivo* after cocaine self-administration is unlike *in vitro* bursting, which is manifested as an increase in irregular firing and a reduction in the degree of pacemaker precision (Wolfart et al. 2001; Wolfart and Roeper 2002).

Our findings on dopamine cell firing are consistent with previous results showing increased cell excitability/activity at early, but not late, withdrawal times following repeated injections of psychostimulants, such as cocaine or amphetamine (White and Wang 1984b; Henry et al. 1989; Wolf et al. 1993; Zhang et al. 1997). However, other drugs of abuse, such as alcohol, cannabinoids or opiates, have been shown to decrease cell activity following withdrawal from their repeated administration (Diana et al. 1993, 1995, 1998; Bailey et al. 2001). Interestingly, whereas these other drugs acutely increase dopamine cell activity (Gysling and Wang 1983; Matthews and German 1984; Mereu et al. 1984; Gessa et al. 1998; Brodie et al. 1999), psychostimulants decrease it (Bunney et al. 1973, 2001; Wang 1981; Einhorn et al. 1988; Lacey et al. 1990). Thus, it is possible that these neuroadaptations represent a compensatory response such that repeated administration of drugs that reduce impulse activity produces rebound increase in cell activity upon

discontinuation, whereas repeated administration of drugs that increase impulse activity has opposite effects.

Dopamine impulse activity is positively correlated with dopamine release, and burst firing in particular produces a supra-linear increase in synaptic dopamine (Gonon 1988; Suaud-Chagny et al. 1992; Chergui et al. 1994). Bursting is known to increase the spread of depolarization into the dendrites (Miles and Wong 1986), which may result in increased somatodendritic dopamine release during short-term withdrawal from cocaine. In fact, it has been reported that basal dopamine levels in the VTA are increased at early withdrawal times from repeated exposure to cocaine (Kalivas and Duffy 1993). However, our findings may or may not reflect terminal dopamine levels in the nucleus accumbens (NAc), as measured by microdialysis. These studies have reported inconsistent basal extracellular dopamine concentrations at short withdrawal periods from repeated psychostimulant exposure (for review see Kuhar and Pilotte 1996; Pierce and Kalivas 1997; Vanderschuren and Kalivas 2000). Dopamine levels, as measured with microdialysis, do not reflect dopamine release, but are the result of release, degradation and re-uptake; so it is possible that different cocaine withdrawal-induced neuroadaptations occur in the terminal regions to differentially affect the release, degradation or re-uptake of dopamine (Wilson et al. 1994; Hitri et al. 1996; Lu and Wolf 1997; Maggos et al. 1997; Pilotte 1997).

Cocaine self-administration and impulse-regulating autoreceptors

Several mechanisms could underlie increased dopamine cell excitability. For example, cocaine exposure and withdrawal could modify synaptic and/or intrinsic plasticity of midbrain dopamine cells by altering synaptic signaling, voltage-gated channels, neuron morphology, or dopamine autoreceptor function (for review see Grace and Bunney 1984a, 1984b; Overton and Clark 1997; Kitai et al. 1999; Grillner and Mercuri 2002; Cooper 2002).

Evidence for synaptic plasticity following psychostimulant exposure has been demonstrated (Overton et al. 1999; Jones et al. 2000; Thomas et al. 2000; Ungless et al. 2001) and could be the result of increased excitatory tone or decreased inhibitory activity. Intrinsic changes in dopamine cell excitability are also likely because we observed enhanced neuronal activity under reduced sensory input, in the anesthetized condition. Concerning these intrinsic properties, dopamine cells exhibit membrane potential oscillations (Kitai et al. 1999) that depend on a balance between inward voltage-gated Na^+ and Ca^{++} currents and outward K^+ currents; therefore, repeated exposure to cocaine could modify the balance of these currents, which are known to regulate dopamine cell activity (Seutin et al. 1993; Overton and Clark 1997; Canavier 1999; Kitai et al. 1999; Wolfart and Roeper 2002).

Neuronal morphology, and in particular dendritic structure, is also an important determinant of neuronal firing patterns. Modeling experiments of dopamine cells have shown that membrane potential oscillations are faster in small diameter dendrites than in large somatic compartments, which can generate out-of-phase inter-compartmental oscillations leading to burst firing (Wilson and Callaway 2000). To our knowledge, psychostimulant-induced changes in dopamine cell structure have not been examined; however, long-term changes have been reported for medium spiny neurons in the NAc and for pyramidal cells in the cortex (Robinson and Kolb 1997, 1999; Robinson et al. 2001). In addition, short-term withdrawal from morphine treatment has been shown to modify the morphology of midbrain dopamine cells (Sklair-Tavron et al. 1996; Spiga et al. 2003).

Finally, somatodendritic autoreceptors inhibit dopamine cell firing and therefore play a critical role in regulating the basal activity of dopamine cells (Bunney et al. 1987; Clark and Chiodo 1988; Mercuri et al. 1992; White 1996). We tested their functional state by determining the effects of quinpirole, a direct D2/D3 receptor agonist primarily acting on somatodendritic dopamine D2 autoreceptors (White and Wang 1984a, 1984c; Williams and Lacey 1988; Pucak and Grace 1994; Koeltzow et al. 1998), on the impulse activity of VTA dopamine cells. We found that at an early withdrawal time from cocaine self-administration (WD1) of low doses of quinpirole did not produce significant decreases in dopamine firing and bursting activity and very high doses were required to produce complete neuronal inhibition. However, by WD10 from cocaine self-administration, quinpirole-induced inhibition was greater and was similar to that observed after saline self-administration. This suggests that functional subsensitivity of impulse-regulating autoreceptors may contribute to the increased activity of midbrain dopamine cells observed at early withdrawal times from cocaine self-administration.

Our results showing decreased reactivity of dopamine cells to autoreceptor regulation following cocaine self-administration are consistent with those following repeated non-contingent injections of cocaine or amphetamine (White and Wang 1984b; Henry et al. 1989; Ackerman and White 1990; Wolf et al. 1993; Pierce et al. 1995; Zhang et al. 1997; Gao et al. 1998) and extend them by showing that changes in dopamine cell function are also relevant for voluntary drug intake. This allowed us to study the potential role of autoreceptor regulation on drug-seeking behavior.

Impulse-regulating autoreceptors and drug-seeking behavior

Low doses of quinpirole minimally reduced seeking behavior on WD1 but dramatically decreased it on WD10. These effects were dose dependent and were similar across the extinction and reinstatement phases.

The effects of quinpirole are likely due to activation of impulse-regulating dopamine autoreceptors because the doses of quinpirole we used are selective for pre-synaptic dopamine autoreceptors (White and Wang 1984c). Even our highest dose of quinpirole decreased seeking behavior suggesting that, under our conditions, post-synaptic activation of dopamine D2 receptors is unlikely, as this induces drug-seeking behavior (Self et al. 1996; De Vries et al. 1999, 2002). It is possible that quinpirole-induced decrease in seeking was due to a generalized decrease in locomotor activity or nose-poking behavior; however, this is unlikely because the low dose of quinpirole preferentially decreased cocaine-induced seeking without affecting cocaine-induced locomotion; in addition, quinpirole-induced changes were preferential to the active versus inactive hole.

In our studies, control animals self-administering cocaine showed similar seeking behavior across WDs; this is in apparent contrast with previous findings that reported a time-dependent increase in drug-seeking behavior (Grimm et al. 2001). Differences in the self-administration paradigm can probably account for this difference, as our animals were only exposed to 1 h of cocaine/day, whereas in the aforementioned study animals were exposed to 6 h of cocaine/day. It should also be noted, however, that increased drug-seeking over time is most evident at later withdrawal periods from cocaine self-administration; only small increases are expected around WD10 (Grimm et al. 2001).

Functional implications

The ability of quinpirole to suppress dopamine cell impulse activity paralleled its ability to suppress drug-seeking behavior. On WD1, the autoreceptor agonist moderately reduced both parameters, whereas it greatly reduced them on WD10. This suggests that quinpirole-induced inhibition of dopamine firing and bursting was responsible for the observed decrease in drug-seeking behavior and that the basal level of activity of the VTA dopamine cells is a critical determinant of seeking behavior.

The fact that drug-seeking behavior could only be decreased when impulse-regulating dopamine autoreceptors were normo-sensitive indicates that the functional state of these D2-class receptors plays an important role in modulating seeking behavior and that their impaired function decreases the ability to modify seeking behavior. Interestingly, D2 receptor gene variants are associated with abuse disorders (O'Hara et al. 1993; Comings et al. 1994; Compton et al. 1996; Noble 2000) and lower D2 receptor availability has been reported in psychostimulant abusers (Volkow et al. 1993, 2001); however, it is still unknown whether this can apply to D2-class impulse-regulating autoreceptors. On the basis of our results, it is tempting to speculate that decreased functional activity of D2-class autoreceptors could be responsible, at least in part, for the inability to decrease drug-seeking behavior in

human addicts and that these autoreceptors are necessary to provide a negative feedback signal that can prevent or decrease relapse.

VTA dopamine cells project to various forebrain regions, including the NAc core and shell, and prefrontal cortex (PFC), where motivational and goal-directed information is integrated. Therefore, decreased inhibition of seeking behavior induced by dopamine neuronal inhibition is probably associated with disruption of dopamine signaling in these structures. This is in line with findings showing that administration of dopamine antagonists in the PFC (McFarland and Kalivas 2001) and the NAc shell (Anderson et al. 2003), but not the core (McFarland and Kalivas 2001), decrease reinstatement of drug-seeking behavior.

Although our results show that seeking behavior requires basal levels of dopamine cell activity and can be disrupted by decreasing cell activity, it is unclear whether an increase in neuronal activity can be associated with increased seeking. In control animals, seeking behavior was similar on WD1 (when impulse activity of VTA DA cells was high) and on WD10 (when impulse activity was lower) suggesting that this is not the case. However, reinstatement can be triggered by a phasic increase in dopamine transmission, via local administration of dopamine in the NAc (Cornish and Kalivas 2000) or infusion of excitatory amino acids in the VTA (Vorel et al. 2001). Therefore, it is likely that enhancement of seeking can only be produced by phasic increases in dopamine cell activity. It is also possible that other neuroadaptations that might occur at early withdrawal times could override this increased firing. For example, increased impulse activity at early withdrawal is often accompanied by an upregulation of dopamine transporter levels and might not translate in a functional increase in dopamine transmission (Pierce and Kalivas 1997).

What is the function of the cocaine/withdrawal associated increase in basal excitability of the dopamine cells? As excitatory inputs increase in response to the cocaine-paired context and conditioned stimuli, the increased basal activity of the dopamine cells may provide a coincident postsynaptic signal that facilitates enduring synaptic potentiation. Support for a psychostimulant-induced increase in excitatory synaptic signaling during short but not long-term withdrawal from psychostimulants has recently been reported (Ungless et al. 2001; Saal et al. 2003). Therefore, it is possible that a transient period of strengthening of synaptic input occurring when impulse activity is elevated and spikes are clustered in bursts could facilitate the induction of long lasting drug-associated memories.

In conclusion, these results show that cocaine self-administration induces neuroadaptations of VTA dopamine cells, which play a significant role in modulating drug-seeking behavior. In particular, drug-seeking can be reduced by activating impulse-regulating autoreceptors and inhibiting the activity of midbrain dopamine cells. These findings could have important implications in understanding the neurobiological mechanisms underlying

ing seeking behavior and could help us design better tools to prevent relapse in human addicts.

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