

Restraint Increases Dopaminergic Burst Firing in Awake Rats

Kristin K Anstrom^{*,1} and Donald J Woodward¹

¹Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Winston-Salem, NC, USA

In anesthetized animals, dopamine neurons fire in tonic and phasic firing modes hypothesized to be regulated by dissociable circuit mechanisms. Salient events critical to learning, reward processing, and attentional selection elicit transient phasic bursts. It is unclear, however, how burst activity contributes to sustained firing patterns in awake animals and if behavioral conditions known to affect dopaminergic neurotransmission change impulse activity levels. Acute stress is known to increase extracellular dopamine in the striatum and the prefrontal cortex. In this study, we have used multiunit recording to define and follow activity patterns in single dopaminergic neurons across days and to determine how restraint, a model of acute stress, changes tonic and phasic firing patterns. Long-term recording shows that a population of 23 putative dopamine neurons has heterogeneous firing profiles under baseline conditions. In all, 62% showed significant burst activity under resting conditions, while others showed predominantly regular (17%) or random (21%) activity patterns. Restraint increased mean firing rate in all dopamine neurons, but preferentially increased burst firing in neurons with higher burst rates under resting conditions. Finally, we show that increased burst firing can persist 24 h after a single exposure to stress. These data indicate that subsets of dopamine neurons may be sensitive to circuit mechanisms activated by stress and that persistent changes in burst firing may be evidence of synaptic plasticity. Furthermore, increased burst firing may be a mechanism through which stress augments extracellular dopamine in selected terminal regions.

Neuropsychopharmacology (2005) **30**, 1832–1840. doi:10.1038/sj.npp.1300730; published online 11 May 2005

Keywords: dopamine; stress; burst firing; rat; restraint; VTA

INTRODUCTION

The activity within the mesolimbic system, particularly within ventral tegmental area (VTA) dopamine neurons, has been hypothesized to be critical to motivation, reinforcement, and learning (Koob and Bloom, 1988; Wise, 1996). Although electrophysiological activity patterns of dopamine neurons have been extensively characterized in anesthetized animals (Grace, 1988), there are relatively few studies doing so in awake, freely moving animals. This study is the first to use chronic recording techniques to identify sustained dopaminergic firing patterns in freely moving animals in single neurons across days. With this approach, the goal of this study was to determine how restraint, a model of stress known to increase dopaminergic neurotransmission, affects firing profiles in single dopamine neurons.

In anesthetized rats, dopamine neurons typically have slow, irregular firing patterns punctuated by periodic bursts (Grace, 1991; Bunney *et al*, 1991). Burst activity, defined as transient rapid firing of dopaminergic cell bodies, is

thought to be dependent on afferent input (Kitai *et al*, 1999; Floresco *et al*, 2003) and produces an enhanced release of intrasynaptic dopamine as compared to regular firing at the same rate (Gonon, 1988; Wightman and Zimmerman, 1990). Multiple studies have shown that transient burst activity is correlated with presentation of salient auditory or visual stimuli (Schultz *et al*, 1993; Horvitz *et al*, 1997; Freeman *et al*, 1985) or cues critical to goal-motivated behavior and reward (Phillips *et al*, 2003; Cooper, 2002; Schultz, 1998). Whether or not burst firing contributes to extracellular dopamine levels in freely moving animals has been debated (Phillips and Wightman, 2004; Floresco *et al*, 2003). Furthermore, it is unclear if increased impulse activity is correlated with behavioral conditions such as stress that are known to elevate tonic dopamine levels.

There is evidence that one of the primary physiological responses to acute stress paradigms is to activate the dopaminergic corticomesolimbic system (Roth *et al*, 1988; Imperato *et al*, 1992). Stress is reported to produce a slow, long-term increase in extracellular and DA turnover in the striatum (Keller *et al*, 1983; Serrano *et al*, 1989) and an enhanced dopaminergic response in the prefrontal cortex (Abercrombie *et al*, 1989; Morrow *et al*, 1999). It is unclear whether or not changes in dopaminergic firing rates and/or patterns contribute to stress-induced increases in extracellular dopamine. Stress is reported to selectively activate c-fos staining in VTA dopamine neurons (Redmond *et al*, 2002; Morrow *et al*, 2000). Others have found that stress

*Correspondence: Dr KK Anstrom, Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Medical Center Blvd, Winston-Salem, NC 27157, USA. Tel: +1 336 716 8568, Fax: +1 336 716 8501, E-mail: kanstrom@wfuhs.edu
Received 31 December 2004; revised 16 February 2005; accepted 24 February 2005

Online publication: 2 March 2005 at <http://www.acnp.org/citations/Npp030205040615/default.pdf>

does not increase tonic firing rates in awake cats (Strecker and Jacobs, 1985), leading to the hypothesis that stress may increase extracellular dopamine through other mechanisms such as local glutamatergic signaling (Grace, 1991; Zigmond *et al*, 1998; West *et al*, 2003) or by increasing the number of active dopamine neurons (Floresco *et al*, 2003). Finally, aversive or nonrewarding stimuli have been reported to inhibit dopaminergic firing (Ungless *et al*, 2003; Tobler *et al*, 2003).

The aim of this study was to compare real-time changes in electrophysiological responses patterns of single dopamine neurons across control and restraint periods. By identifying how restraint modulates these response patterns, we will better understand how neural activity contributes to circuit mechanisms underlying regionally specific increases in dopaminergic tone due to stress.

MATERIALS AND METHODS

Animals

In all, 15 experimentally naïve, male Sprague–Dawley rats (Harlan) initially weighing 350–400 g were used for these experiments. Animals were housed under a normal dark–light cycle (lights off 1800). Behavioral testing was performed in the light phase of their cycle. Animals had free access to food and water at all times and were maintained within the standards set forth for the care and use of laboratory animals by the National Institutes of Health. All protocols used to complete this study were reviewed and approved by the Animal Care and Use Committee at Wake Forest University Health Sciences.

Surgery

After the 3-day acclimation period, animals were handled daily for at least 1 week to habituate them to experimental manipulation and then implanted with microwire arrays (Biographics Inc., Winston-Salem, NC). Animals were anesthetized with isoflurane, and a prophylactic dose of antibiotics was administered prior to surgery. Body temperature was maintained between 35 and 37°C and aseptic technique observed. After placing the rat in a stereotaxic apparatus, the scalp was shaved, swabbed with iodine, and a central incision made to expose the skull. Small holes were drilled in the skull and two arrays of eight, stainless-steel, Teflon-coated microwires (45–62 µm in diameter) were lowered bilaterally into the midbrain using the following coordinates: AP, +3.5 mm from Lambda; ML \pm 1.8; DV, –8.3 from skull surface. Uncoated stainless-steel ground wires were positioned 2–3 mm ventral to the cortical surface and wrapped around skull screws. The recording headstage was then secured to the cranium with dental cement using skull screws as anchors.

Recording Protocol

Distributed neural activity was recorded in the midbrain under control and experimental conditions using well-defined protocols (Chang *et al*, 1994). Neuroelectric signals were amplified and filtered (0.5 and 5 kHz 3 dB cutoffs) via software control. Signals were digitized (50 kHz per

channel), and spikes from single units were sorted from background and other units via movable Windows-based parameters using software from Spectrum Scientific (Dallas, TX). Temporal records of extracellular spike activity were superimposed on behavioral data using Magsort software (Biographics Inc., Winston-Salem, NC) with a time resolution of 13 ms.

Before undergoing any experimental manipulation, each animal was subjected to two 30-min baseline sessions where the animal was tethered to the recording system and allowed to move freely in the recording chamber, which consisted of a Plexiglas box placed within a sound attenuating chamber. The effect of restraint on dopamine firing rate and burst firing was determined by recording baseline neural activity for a third 30-min baseline session where the animal was allowed to move freely in the recording chamber. The animal was removed from the chamber and restrained by placing it into a vented Plexiglas hemicylinder in which it could not move, turn, or escape. Once the animal was in the restraint device, the entire ensemble was placed back into the same recording chamber and neural activity was recorded for 30 min in the same population of neurons while the animal was restrained. A subset of animals (4) in which dopamine neurons could be held was subjected to a second exposure to baseline conditions and then restraint the following day. Another subset of animals (5) was subjected to haloperidol injections.

Pharmacology

In a subset of animals with putative dopamine neurons, a 0.5 mg injection of haloperidol (Ortho-McNeil, Raritan, NJ) was administered intramuscularly after a 45-min baseline period. Neural activity 5 min prior to and following injection was excluded from analysis in order to eliminate changes due to injection stress, handling, or arousal.

Neuron Classification

Neuron populations were defined by the mean activity and duration of extracellular spike waveform. The mean activity was calculated across a 30-min time period in a baseline session where the animal was allowed to rest quietly. Extracellular spike waveform duration was determined by measuring the length of the point at which the depolarization phase of the curve left baseline to when the hyperpolarization phase returned to baseline.

Dopamine neurons were categorized according to burst properties. To determine the percentage of spikes that were found in bursts, a burst was defined by a minimum of three successive spikes having an initial interspike interval (ISI) less than or equal to 80 ms and ending with an ISI greater than 160 ms (Grace and Bunney, 1984).

ISI histograms were conducted with a maximum interval of 1 s and 5 ms bins. The number of ISIs/bin was normalized to the percentage of total intervals and the percentage of ISIs that occurred within six time periods calculated. Intervals in the first period (25–125 ms) fall within standard intervals that define dopamine bursts and thus were considered indicative of burst activity. The second period analyzed (225–325 ms) was considered indicative of regular

pacemaker activity and four final periods (400–500, 600–700, 800–900, and 900–1000 ms) random activity.

Dopamine neurons with a clear ISI modal peak between 200 and 300 ms and fewer than 20% of ISIs falling in both the first and last four time periods were classified as pacemaker neurons. Neurons with no modal peak and a minimum of 20% of ISIs falling in the last four periods were classified as randomly firing neurons. Neurons with a modal peak that fell between 20 and 200 ms and at least 20% of ISIs occurring in the first period were classified as bursting neurons.

Statistical Analysis

A total of 142 neurons were recorded across 15 animals with an average of 9.5 neurons recorded per animal, although not all neurons could be held across each experimental condition. Analyses of mean firing rates, burst activity, ISI histograms, and autocorrelograms were performed using STRANGER (Biographics Inc.) and NEX (Plexon Inc.). Before including neural signals in a data set, autocorrelograms were performed on each channel record to assure that single neurons were separated from background noise. The criteria used to identify a single neuron across days included consistency of firing rate, shape of extracellular waveform, and autocorrelogram pattern across recording sessions.

The effects a single exposure to restraint and haloperidol on mean firing rates, the percentage of spikes found in bursts, the number of spikes found in bursts, and bursts per minute across single neurons were determined by a paired *t*-test. A repeated measures one-way ANOVA with a *post hoc* Tukey contrast was conducted to determine if changes in rate and burst firing across 2 days of exposure to restraint as compared to initial baseline levels were significant.

Localization of Recording Electrodes and Immunohistochemistry

To visualize electrode placement, animals were deeply anesthetized with pentobarbital (100 mg/kg) administered intraperitoneally. A 12-s current of 10–15 μ A was passed through at least four electrodes of an eight-electrode array. The animals were then perfused transcardially with a 0.9% saline wash followed by a 2% potassium ferrocyanide in 10% buffered formalin fixative to create a Prussian Blue spot marking electrode placement. The brains were removed and cryoprotected overnight in a 20% sucrose in 10% formalin solution at 4°C. Coronal sections (50 μ m) were cut on a sliding microtome. Free-floating sections with Prussian Blue marks were collected and stored serially in 0.1 M phosphate buffer, pH 7.2. Three out of four sections were stained with neutral red, serially mounted on gelatin-subbed slides, passed through an alcohol/xylene series, and coverslipped with Permount.

One out of four sections containing Prussian Blue staining were stained with an antibody against tyrosine hydroxylase to localize electrodes within the midbrain using the ABC method. Free-floating sections were washed with phosphate-buffered saline, pH 7.2. To reduce nonspecific binding, sections were blocked for 1 h in 10% normal horse serum (NHS) in phosphate-buffered saline containing 1%

Triton X-100, 1% bovine serum albumin, and 0.1 M L-lysine at room temperature. Sections were incubated in the primary antibody (Chemicon; 1:4000 dilution) containing 1% NHS and 0.1% Triton X-100 in phosphate-buffered saline for 12–14 h at room temperature with constant agitation. Sections were rinsed three times with 1% NHS and then immersed in biotinylated anti-mouse IgG diluted 1:500 in phosphate-buffered saline for 60 min at room temperature. Following exposure to the secondary antibody, sections were washed in three 10-min phosphate-buffered saline rinses. The sections were incubated in ABC solution (Vector Laboratories) for 60 min, washed for 30 min in phosphate-buffered saline, followed by two 10-min washes in phosphate buffer. Sections were reacted with 0.05% 3,3'-diaminobenzidine tetrahydrochloride and nickel cobalt in phosphate buffer and 0.01% hydrogen peroxide in phosphate buffer for 1–3 min to produce a black reaction product. The reaction was terminated by rinsing the sections in 0.1 M phosphate buffer. After rinsing in 0.1 M phosphate buffer, sections were mounted on gelatin-coated glass slides, dehydrated with a graduated alcohol/xylene series, and coverslipped with Permount.

Control sections for nonspecific binding underwent the same experimental procedure, except for the omission of primary antibody in the first overnight exposure. Analysis and photomicroscopy were conducted using an Olympus BX60 microscope and a digital camera.

RESULTS

Identification of Neuron Type and Anatomical Confirmation

Putative dopamine neurons recorded with microwires were identified on the basis of electrophysiological and pharmacological parameters used in previous studies, which include long-duration waveforms, firing rates between 1 and 8 Hz, and with excitatory responses to haloperidol (Grace, 1988; Marinelli *et al*, 2003). Two populations of neurons recorded with microwires were separated on the basis of the electrophysiological criteria. The average duration of extracellular spike waveforms for putative dopamine neurons was 2.38 ± 0.14 ms, although these durations may be underestimated due to filtering conditions. Extracellular spike waveforms were triphasic (+/-/+) with variable positive domains, which were at times notched, and had prolonged negative phases (Figure 1a). In comparison, the average duration for 30 representative GABA neurons was 0.40 ± 0.02 ms. The waveforms were, in general, biphasic with consistent, well-defined negative phases.

The average firing rate recorded under resting conditions for putative dopamine neurons had a relatively tight distribution clustered at 3.1 ± 0.27 Hz. A representative population of putative GABAergic neurons ($n=30$) had firing rates that averaged at 19.5 ± 3.2 Hz (Figure 1b). A subset of animals ($n=5$) with putative dopamine neurons was injected with 0.5 mg/kg haloperidol to pharmacologically identify dopamine neurons. Average firing of putative dopamine neurons ($n=6$) was significantly increased after haloperidol injections ($p<0.01$, *df* 1,5) across the entire population (Figure 1c).

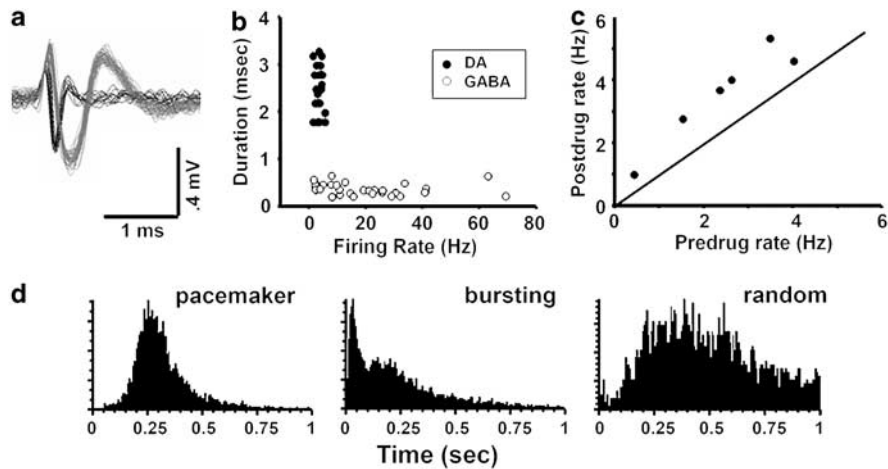


Figure 1 Identification of putative dopamine neurons. (a) A record of a 2.8 ms recording window with an example of a putative dopamine neuron (gray) separated from putative GABA neuron (black). (b) Dopamine neurons (filled circles) could be separated from GABAergic neurons (empty circles) on the basis of firing rate and extracellular spike waveform duration. (c) Haloperidol significantly increased firing rate over a population of putative DA neurons ($p < 0.01$) as determined by a paired t -test. (d) ISI histograms show that dopamine neurons have three firing modes in the resting state in the awake animal: pacemaker, bursting, and random.

Dopamine neurons in this study could be subcategorized by sustained firing patterns (Figure 1d) using ISI histograms and standard burst criteria (Table 1) across resting conditions. Three out of 22 putative dopamine neurons were classified as pacemaker neurons, as indicated by regular firing around a modal interval. ISI histograms had clear modal peaks that ranged from 170 to 250 ms. Across this population, $11.3 \pm 4.3\%$ of ISIs fell between 25 and 125 ms in duration, $32.0 \pm 7.8\%$ between 250 and 350 ms, and $14.1 \pm 3.6\%$ from 400 to 1000 ms. Average firing rate for these neurons was 2.5 ± 0.07 Hz and $4.4 \pm 0.70\%$ of spikes were found in bursts. Out of 22 neurons, 12 showed more of a classic dopaminergic burst profile (Grace, 1988). They had a higher percentage of 25–125 ms intervals ($28.0 \pm 2.1\%$) and varying levels of longer intervals ($18.4 \pm 0.7\%$ —250–350 ms) and ($14.8 \pm 1.5\%$ —400–1000 ms). There were at times two modal peaks showing that bursting neurons shift between burst and regular firing modes under resting conditions. Average firing rate was 3.5 ± 0.06 Hz and $17.6 \pm 0.75\%$ of spikes were found in bursts. Seven neurons were distinguished by lower firing rates (1.8 ± 0.12 Hz), no distinct ISI modal peak, and a large percentage of 400–1000 ms ISIs ($29.9 \pm 3.3\%$). As these neurons had no particular patterned firing, they were classified as ‘random’ neurons.

Finally, immunohistochemical methods showed that electrodes recording putative dopamine signals were localized to tyrosine hydroxylase-positive regions of the VTA (Figure 2). Other neurons with long-duration extracellular spike waveforms that were not localized to tyrosine hydroxylase-positive regions were noted; however, they could be distinguished by consistent, nonvariable positive phases. These neurons were, in general, localized in the red nucleus. Putative dopamine neurons ($n = 23$) met three or more of the above-mentioned criteria.

Effect of Restraint on Dopamine Firing Patterns

Mean firing rates. During the 30-minute period of restraint, animals showed behavioral indices of stress including

urination and defecation. Exposure to restraint significantly increased baseline firing rate across 15 putative DA neurons. Figure 3a compares a dopaminergic rate histogram of the last 1000 s of a baseline session to the first 1000 s of the restraint session and shows an increase in dopaminergic rate. The average rate across a population of 15 putative DA neurons increased from 3.1 ± 0.07 to 4.0 ± 0.09 Hz ($p < 0.01$, $df = 1, 14$) (Figure 3b). This increase in mean firing rate is consistent across the entire population of putative dopamine neurons (Figure 3c), although it does not seem that the degree of firing rate increase is a function of initial firing rate. No ‘new’ dopamine neurons appeared during restraint as compared to baseline periods immediately preceding the restraint period.

Burst firing. Restraint significantly increased burst firing in neurons, with higher initial burst activity, but less so in random or pacemaker neurons. The percentage of spikes in bursts was significantly increased with restraint ($p < 0.01$, $df = 1, 14$) (Figure 3d), although not all neurons showed an increase in burst firing (Figure 3e). Neurons that showed an initial pacemaker or random firing profile showed smaller increases or even decreases in burst firing (Figure 3e and Table 1). Exposure to restraint increased dopaminergic burst rate from 6.7 ± 0.52 to 13.8 ± 0.9 bursts/min, but decreased the number of spikes found within the burst from 4.2 ± 0.01 to 4.1 ± 0.4 spikes/min, indicating that the increase in percentage of spikes found in bursts were achieved by increasing burst frequency.

Effect of repeated restraint. The effect of repeated restraint on burst firing was explored in five neurons across four animals where dopamine neurons could be held across all conditions. Figure 4a shows ISI histograms of a single putative DA neuron across 2 days where the animal was subjected to 30-min baseline and restraint recording sessions each day. Across five neurons, firing rate was significantly increased as compared to initial baseline session across all conditions ($p < 0.01$, $df = 1, 3$, $F = 22.86$),

Table 1 The Resting Profile of Each Category of Recorded Neurons is Documented in the Upper Half of the Table. In the Lower Half, the Percentage of Spikes Found in Bursts was Calculated for 30-Min Periods of Rest, Restraint, and after Haloperidol Injections

Neuron type	Subtype	n	Resting firing rate		
Summary of recorded neurons					
DA	Bursting	14	3.5+0.06		
DA	Random	5	1.4+0.15		
DA	Pacemaker	4	3.3+0.25		
GABA	—	119	16.0+0.13		
Rat/neuron	Type	Base	Restraint	Haloperidol	
Percentage of spikes within bursts					
Ac17n22	h	39.7	58.9	33.5	
Ac11n27	h	34.7	—	—	
LAM1n6	h	27.1	—	—	
PD8n23	h	26.3	43.9	—	
PD8n31	h	21.5	—	—	
Ac5n22	h	21.1	34.1	—	
LAM1n17	h	18.5	35.9	42.5	
Ac6n21	h	17.1	—	—	
Ac11n9	h	13.4	18.1	—	
PD8n29	h	13.2	—	—	
Ac13n25	h	11.6	23.1	18.3	
PD8n2	h	11	19.1	—	
Jenn9	r	9.1	6.6	9.1	
Ac17n27	p	7.5	6.5	—	
LAM1n30	r	7.5	—	18	
PD8n17	h	6	22.3	—	
LAM1n18	h	5.6	12.7	7.8	
AC11n25	r	5.1	3.9	—	
Jann12	p	4.9	0.2	17.3	
AC11n12	p	4.8	19.8	—	
PD8n24	r	3.4	10.1	—	
CPI n5	r	3.3	—	—	
Ac5n17	p	0.47	—	—	

A burst was defined as a minimum of three successive spikes not having an initial ISI greater than 80 ms and any successive ISI greater than 160 ms. Each dopamine neuron was categorized as p (pacemaker), r (random), and h (hybrid) according to its sustained burst properties across resting conditions. Haloperidol increased burst firing uniformly, but burst response to restraint was heterogeneous.

including the second baseline session where the animal was allowed to rest quietly in the recording chamber without any experimental manipulation (Figure 4b). The percentage of spikes found in bursts was also significantly increased across all conditions ($p < 0.01$, $df = 1, 3$, $F = 8.83$) as compared to the initial baseline session.

DISCUSSION

Here, we have presented the initial study following electrophysiological activity of single dopamine neurons

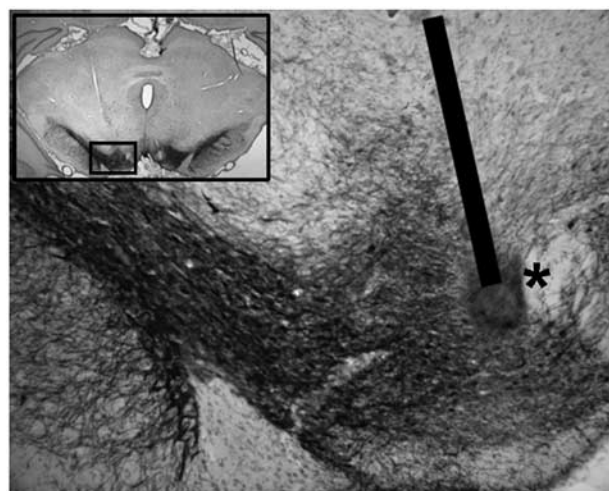


Figure 2 Anatomical localization of dopaminergic recording site. This representative section contains a Prussian Blue stain (marked by asterisk) that marks the site of the electrode tip recording putative dopamine signals. All recording sites for putative dopamine signals were localized to tyrosine hydroxylase-positive regions. Inset shows lower power view of the section.

across baseline and experimental conditions over days in awake rats. Long-term recording showed that putative VTA dopamine neurons have heterogeneous firing profiles as has been suggested in other studies (Hyland *et al*, 2002). Over 50% of these neurons showed a classic dopaminergic firing profile and regularly alternated between burst firing and pacemaker modes in the absence of salient or unpredicted stimuli, while others showed regular pacemaker-like activity or unpatterned firing. Restraint, a behavioral condition known to increase extracellular dopamine (Morrow *et al*, 1997), increased mean firing rate of all putative DA neurons. In neurons that showed higher burst indices under resting conditions, restraint increased burst firing, although by increasing burst rate and not the number of spikes per burst. In contrast, in neurons that showed under baseline conditions, burst firing was minimally affected or even reduced. Finally, we have shown evidence that this shift persists 24 h after initial exposure to restraint.

To identify putative dopamine neurons, we used a combination of anatomical, physiological (Grace and Bunney, 1983; White, 1996), as well as pharmacological (Bunney *et al*, 1973) criteria. The mean firing rate, spike waveform shape, and duration and responses to haloperidol for presumed dopamine neurons recorded with microwire arrays all fell within the range of those previously reported in other studies using other recording techniques (Bunney *et al*, 1980; Freeman *et al*, 1985; Marinelli *et al*, 2003). In addition, all recording sites were localized to the paranigral region of the VTA as determined immunohistochemical staining against tyrosine hydroxylase. These results suggest that dopamine neurons are readily identifiable and the use of microwire electrode arrays does not distort electrophysiological parameters used to identify dopaminergic neurons.

In anesthetized animals, dopamine neurons have relatively slow, irregular firing rates punctuated by periodic bursts of phasic activity (Grace, 1988) hypothesized to encode salient information in awake animals (Schultz,

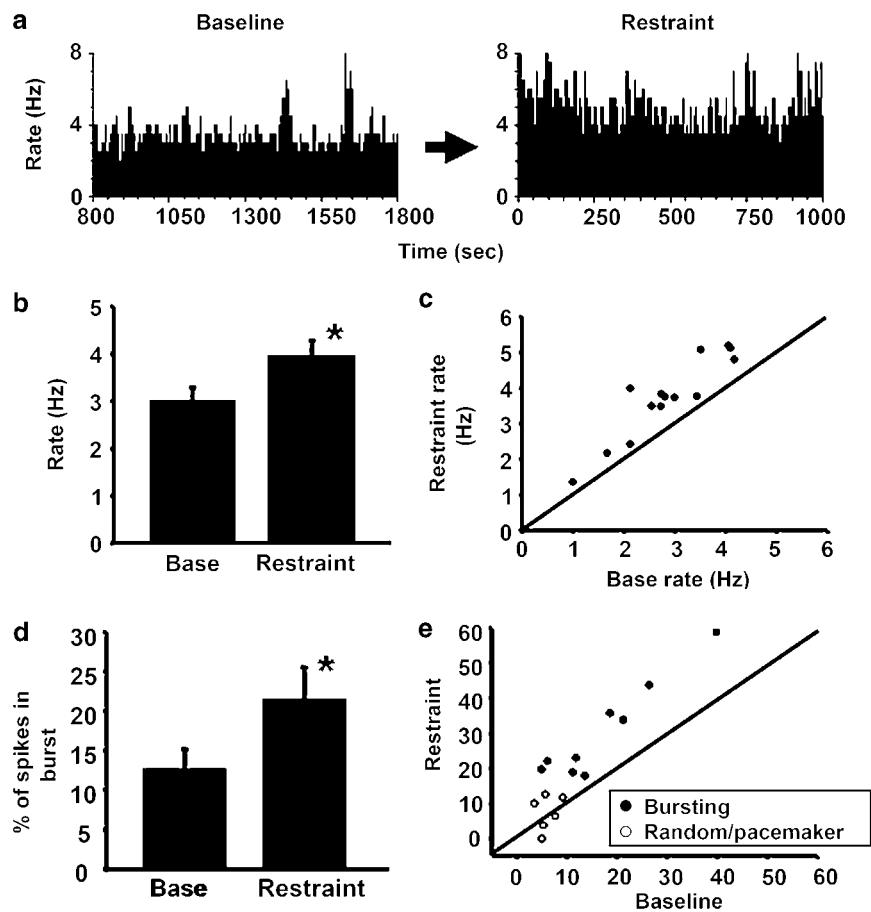


Figure 3 Restraint increases firing rate and burst firing of dopamine neurons. (a) Rate histogram of a single dopaminergic neuron across baseline and restraint conditions (b) \pm SEM of firing rate was significantly increased with restraint-induced stress ($p < 0.01$) across 15 neurons as determined by a paired t -test. (c) Restraint increases average firing rate across the entire population of dopamine neurons. (d) The percentage of spikes found in bursts is significantly increased with restraint as determined by a paired t -test. (e) Restraint does not increase burst firing in neurons in all dopamine neurons. Neurons with lower burst indices (empty circles) have smaller increases or even decreases in burst firing as compared to bursting neurons (filled circles). Increases in burst firing are thus predicted by initial firing profile.

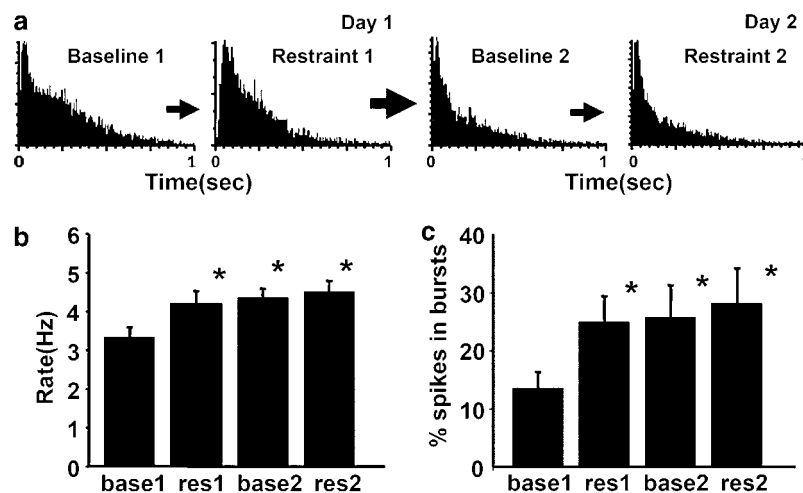


Figure 4 Increased rate and burst firing persists 24 h after exposure to restraint in the same neurons. (a) ISI histogram from a single bursting DA neuron recorded across two baseline and two restraint sessions. Restraint decreases the percentage of longer intervals. This effect persists through the following day. Restraint significantly increases (b) rate and (c) percentage of spikes found in bursts 24 h after initial exposure as determined by one-way ANOVA analysis with a Tukey comparison.

1998). Phasic burst activity is thought to be dependent on afferent input (Kitai *et al*, 1999). In awake animals, over 50% of putative dopamine neurons show this same pattern. Although a subpopulation of putative dopamine neurons fired only in a regular manner without burst activity, variation in ISI length suggests that these neurons are not truly deafferented (Mereu *et al*, 1997). Interestingly, stress did not induce burst firing in these neurons, whereas it increased the percentage of short intervals and spikes found in bursts in neurons showing burst activity under resting conditions, suggesting that longer interval spikes between bursts are eliminated. These data suggest that restraint does not elicit a synchronous response across the entire dopaminergic population and may activate multiple circuits. Finally, slow increases in extracellular dopamine, as measured by dialysis, may not be sufficient to predict the full range of circuit mechanisms activated by stress or other behavioral states.

Multiple studies have shown that various forms of stress increase dopaminergic tone (Roth *et al*, 1988; Imperato *et al*, 1992) in afferent target areas, but there is no previous evidence that increases in extracellular dopamine levels are correlated with elevated firing rates of dopamine neurons. Strecker and Jacobs (1985) report that various forms of stress do not change baseline firing rates of A9 dopamine neurons in cats. Several studies have shown that stress induces c-fos staining in A10 dopamine neurons (Redmond *et al*, 2002; Morrow *et al*, 1999); however, these data do not directly predict changes in dopamine firing rate and rather reflect afferent activity onto these neurons. Inconsistencies in dopaminergic neural responses across these studies may reflect differences in species, recording sites, and experimental protocol.

Acute models of stress, including restraint (Morrow *et al*, 1997), produce an enhanced dopaminergic response in the prefrontal cortex as compared to the striatum (Abercrombie *et al*, 1989; Murphy *et al*, 1996; Morrow *et al*, 2000). The results from this study show that restraint increases mean activity and burst firing in approximately 80% of A10 neurons. Retrograde labeling shows that dopamine neurons projecting to the medial prefrontal cortex comprise a small percentage of VTA dopamine neurons (Fallon and Laughlin, 1995). As stress increased firing rate of all putative dopamine neurons and increased burst firing in approximately 80%, it is unlikely that the enhanced prefrontal response is due to a selective activation of dopamine neurons projecting to the medial prefrontal cortex.

Recent work has suggested that dopamine release dynamically adapts in response to spike activity (Garris *et al*, 1999; Yavich and MacDonald, 2000), and that regionally specific increases in DA signaling may be due to multiple factors such as innervation and autoreceptor density, efficiency of dopamine reuptake, as well as afferent neuronal activity (Montague *et al*, 2004). For example, the kinetics of dopamine release and uptake suggest that the medial prefrontal cortex and the amygdala show a greater synaptic overflow per spike and are termed 'release dominated' due to sparse uptake as compared to the striatum, which is 'uptake dominated' due to denser perisynaptic uptake systems (Garris *et al*, 1993; Garris and Wightman, 1994). As dopamine bursts result in an enhanced release at synaptic terminals (Gonon, 1988), increases in

sustained burst firing could potentially allow for extra-synaptic diffusion of dopamine within the prefrontal cortex (Garris and Wightman, 1994; Wightman and Robinson, 2002), while only mildly affecting tonic dopamine levels in the striatum. Thus, a condition such as stress that increases burst firing may bias dopaminergic neurotransmission by selectively augmenting dopamine levels in these limbic and cognitive areas.

In operant reward paradigms, Pavlovian conditioning has been shown to differentially modulate phasic dopaminergic responses to stimuli according to their predictive value (Schultz, 1998; Tobler *et al*, 2003). In these and other studies, 'aversive' events (Ungless *et al*, 2003) or stimuli predicting aversive outcomes are associated with phasic dopaminergic inhibition. Here we show that exposure to restraint increases burst firing at the time of restraint, and that it remains increased when the animal is reintroduced to the recording chamber 24 h later. Our hypothesis is that a sustained increase in dopaminergic burst firing following restraint as conducted in this study results from a combination of neuroplastic changes at dopaminergic synapses (see Figure 3) and contextual Pavlovian conditioning. Apparent differences in dopaminergic responses to aversive stimuli may be due to differences in behavioral paradigm, appropriate behavioral responses, and type of dopaminergic response being measured. Whereas the above-mentioned studies document how operant cues elicit transient phasic activity within operant tasks (Tobler *et al*, 2003; Schultz, 1998), this study provides evidence that a behaviorally relevant condition such as stress alters frequency of burst firing, a well-defined dopaminergic firing mode that occurs in the absence of predictive cues (Grace and Bunney, 1984).

Burst activity is thought to be dependent on excitatory input from areas such as the prefrontal cortex (Kitai *et al*, 1999). In particular, several studies have shown that activation of NMDA receptors on dopamine neurons promotes burst firing (Overton and Clark, 1992; Kalivas, 1993). Modulation of these synapses is thought to be involved in both acute and long-term changes in dopaminergic activity (Bonci and Malenka, 1999). *In vitro* models suggest that CRF, a peptide released under stressful conditions, increases NMDA receptor activity in a subset of dopamine neurons (Ungless *et al*, 2003). It is therefore possible that stress-induced CRF release increases burst firing that persists over days in a subset of dopamine neurons via activation of VTA CRF receptors. Finally, neurons that are not responsive to CRF may coincide with those neurons that do not have augmented burst activity in response to stress.

There is converging evidence that both stress (Robinson and Berridge, 1993; Le *et al*, 2000; Shaham *et al*, 2000; Saal *et al*, 2003) and increased impulse activity in the dopaminergic system (Marinelli and White, 2000; Marinelli *et al*, 2003) are associated with maladaptive behaviors (Grace, 1991). Although higher cognitive processes have been shown to be dependent on cortical dopamine neurotransmission (Williams and Goldman-Rakic, 1995; Murphy *et al*, 1996), performance is not linearly related to DA receptor activation. It is instead optimized when dopaminergic tone in the prefrontal cortex falls within a critical range of an inverted U-shaped function (Zahrt *et al*,

1997; Phillips *et al*, 2004). Stress and other conditions that increase sustained burst firing may drive release-dominated systems such as the medial prefrontal cortex out of optimal ranges.

Transient phasic bursts are known to occur in response to behaviorally relevant stimuli (Schultz *et al*, 1993; Horvitz *et al*, 1997; Phillips *et al*, 2003; Robinson *et al*, 2001), leading to the hypothesis that burst firing leads to 'attentional switching' (Freeman *et al*, 1985; Redgrave *et al*, 1999). An emerging hypothesis, however, is that multiple circuit mechanisms, including those that elicit burst firing, may lead to increased dopaminergic tone in selected terminal regions. Furthermore, persistent increases in burst firing may disrupt finely tuned extracellular dopamine levels in selected areas such as the prefrontal cortex, while leaving others relatively unaffected, thus promoting maladaptive behaviors such as compulsive drug-seeking or schizophrenic psychoses. Concomitant recording of dopamine neurons and their afferent systems across more complex behavioral paradigms will be required in the future to identify dysfunctions in large-scale circuit mechanisms associated with these behaviors.

ACKNOWLEDGEMENTS

We would like to thank Robert Hampson and Michael Zigmond for helpful comments. We would also like to recognize the excellent technical assistance of Ms Courtney Reed, Mr Darrell Agee, and Mr Donald Collins. This work was supported by an ABMRF grant to KKA, AA13199, EB2092 to DJW, and NS19608 to Michael Zigmond.

REFERENCES

- Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ (1989). Differential effect of stress on *in vivo* dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem* 52: 1655–1658.
- Bonci A, Malenka RC (1999). Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci* 19: 3723–3730.
- Bunney BS, Chiodo LA, Grace AA (1991). Midbrain dopamine system electrophysiological functioning: a review and new hypothesis. *Synapse* 9: 79–94.
- Bunney BS, Skirboll LR, Grace AA (1980). Acute and chronic haloperidol treatment: effects on nigrostriatal dopaminergic system activity. *Adv Biochem Psychopharmacol* 24: 267–273.
- Bunney BS, Walters JR, Roth RH, Aghajanian GK (1973). Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. *J Pharmacol Exp Ther* 185: 560–571.
- Chang JY, Sawyer SF, Lee RS, Woodward DJ (1994). Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats. *J Neurosci* 14: 1224–1244.
- Cooper DC (2002). The significance of action potential bursting in the brain reward circuit. *Neurochem Int* 41: 333–340.
- Fallon JH, Loughlin SE (1995). *Substantia Nigra*. In: Paxinos G (ed). *The Rat Nervous System*, 2nd edn. Academic Press: San Diego, CA. pp 215–237.
- Floresco SB, West AR, Ash B, Moore H, Grace AA (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat Neurosci* 6: 968–973.
- Freeman AS, Meltzer LT, Bunney BS (1985). Firing properties of substantia nigra dopaminergic neurons in freely moving rats. *Life Sci* 36: 1983–1994.
- Garris PA, Collins LB, Jones SR, Wightman RM (1993). Evoked extracellular dopamine *in vivo* in the medial prefrontal cortex. *J Neurochem* 61: 637–647.
- Garris PA, Kilpatrick M, Bunin MA, Michael D, Walker QD, Wightman RM (1999). Dissociation of dopamine release in the nucleus accumbens from intracranial self-stimulation. *Nature* 398: 67–69.
- Garris PA, Wightman RM (1994). Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an *in vivo* voltammetric study. *J Neurosci* 14: 442–450.
- Gonon FG (1988). Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by *in vivo* electrochemistry. *Neuroscience* 24: 19–28.
- Grace AA (1988). *In vivo* and *in vitro* intracellular recordings from rat midbrain dopamine neurons. *Ann NY Acad Sci* 537: 51–76.
- Grace AA (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41: 1–24.
- Grace AA, Bunney BS (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons—1. Identification and characterization. *Neuroscience* 10: 301–315.
- Grace AA, Bunney BS (1984). The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci* 4: 2877–2890.
- Horvitz JC, Stewart T, Jacobs BL (1997). Burst activity of ventral tegmental dopamine neurons is elicited by sensory stimuli in the awake cat. *Brain Res* 759: 251–258.
- Hyland BI, Reynolds JN, Hay J, Perk CG, Miller R (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114: 475–492.
- Imperato A, Angelucci L, Casolini P, Zocchi A, Puglisi-Allegra S (1992). Repeated stressful experiences differently affect limbic dopamine release during and following stress. *Brain Res* 577: 194–199.
- Kalivas PW (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Brain Res Rev* 18: 75–113.
- Keller RW, Stricker EM, Zigmond MJ (1983). Environmental stimuli but not homeostatic challenges produce apparent increases in dopaminergic activity in the striatum: an analysis by *in vivo* voltammetry. *Brain Res* 279: 159–170.
- Kitai ST, Shepard PD, Callaway JC, Scroggs R (1999). Afferent modulation of dopamine neuron firing patterns. *Curr Opin Neurobiol* 6: 690–697.
- Koob GF, Bloom FE (1988). Cellular and molecular mechanisms of drug dependence. *Science* 242: 715–723.
- Le AD, Harding S, Juzysch W, Watchus J, Shalev U, Shaham Y (2000). The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behaviour in rats. *Psychopharmacology* 150: 317–324.
- Marinelli M, Cooper DC, Baker LK, White FJ (2003). Impulse activity of midbrain dopamine neurons modulates drug-seeking behavior. *Psychopharmacology* 168: 84–98.
- Marinelli M, White FJ (2000). Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. *J Neurosci* 20: 8876–8885.
- Mereu G, Lilliu V, Casula A, Vargiu PF, Diana M, Musa A *et al* (1997). Spontaneous bursting activity of dopaminergic neurons in midbrain slices from immature rats: role of N-methyl-D-aspartate receptors. *Neuroscience* 77: 1029–1036.
- Montague PR, McClure SM, Baldwin PR, Phillips PE, Budygin EA, Stuber GD *et al* (2004). Dynamic gain control of dopamine delivery in freely moving animals. *J Neurosci* 24: 1754–1759.
- Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999). The role of mesoprefrontal dopamine neurons in the acquisition

- and expression of conditioned fear in the rat. *Neuroscience* **92**: 553–564.
- Morrow BA, Lee EJ, Taylor JR, Elsworth JD, Nye HE, Roth RH (1997). (S)-(-)-HA-966, a gamma-hydroxybutyrate-like agent, prevents enhanced mesocorticolimbic dopamine metabolism and behavioral correlates of restraint stress, conditioned fear and cocaine sensitization. *J Pharmacol Exp Ther* **283**: 712–721.
- Morrow BA, Roth RH, Elsworth JD (2000). TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. *Brain Res Bull* **52**: 519–523.
- Murphy BL, Arnsten AF, Goldman-Rakic PS, Roth RH (1996). Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc Natl Acad Sci USA* **93**: 1325–1329.
- Overton P, Clark D (1992). Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptors modulate burst firing in A9 dopamine neurons in rats. *Synapse* **24**: 815–818.
- Phillips AG, Ahn S, Floresco SB (2004). Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task. *J Neurosci* **24**: 547–553.
- Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003). Subsecond dopamine release promotes cocaine seeking. *Nature* **422**: 614–618.
- Phillips PE, Wightman RM (2004). Extrasynaptic dopamine and phasic neuronal activity. *Nat Neurosci* **7**: 199.
- Redgrave P, Prescott TJ, Gurney K (1999). Is the short-latency dopamine response too short to signal reward error? *Trends Neurosci* **22**: 146–151.
- Redmond AJ, Morrow BA, Elsworth JD, Roth RH (2002). Selective activation of the A10, but not A9, dopamine neurons in the rat by the predator odor, 2,5-dihydro-2,4,5-trimethylthiazoline. *Neurosci Lett* **328**: 209–212.
- Robinson DL, Phillips PE, Budygin EA, Trafton BJ, Garriss PA, Wightman RM (2001). Sub-second changes in accumbal dopamine during sexual behavior in male rats. *Neuroreport* **12**: 2549–2552.
- Robinson TE, Berridge KC (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev* **18**: 247–291.
- Roth RH, Tam SY, Ida Y, Yang JX, Deutch AY (1988). Stress and the mesocorticolimbic dopamine systems. *Ann NY Acad Sci* **537**: 138–417.
- Saal D, Dong Y, Bonci A, Malenka RC (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* **37**: 577–582.
- Schultz W (1998). Predictive reward signal of dopamine neurons. *J Neurophysiol* **80**: 1–27.
- Schultz W, Apicella P, Ljungberg T (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J Neurosci* **13**: 900–913.
- Serrano A, D'Angio M, Scatton B (1989). NMDA antagonists block restraint-induced increase in extracellular DOPAC in rat nucleus accumbens. *Eur J Pharmacology* **162**: 157–166.
- Shaham Y, Erb S, Stewart J (2000). Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res Rev* **33**: 13–33.
- Strecker RE, Jacobs BL (1985). Substantia nigra dopaminergic unit activity in behaving cats: effect of arousal on spontaneous discharge and sensory evoked activity. *Brain Res* **361**: 339–350.
- Tobler PN, Dickinson A, Schultz W (2003). Coding of predicted reward omission by dopamine neurons in a conditioned inhibition paradigm. *J Neurosci* **23**: 10402–10410.
- Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A (2003). Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. *Neuron* **39**: 401–407.
- West AR, Floresco SB, Charara A, Rosenkranz JA, Grace AA (2003). Electrophysiological interactions between striatal glutamatergic and dopaminergic systems. *Ann NY Acad Sci* **1003**: 53–74.
- White FJ (1996). Synaptic regulation of mesocorticolimbic dopamine neurons. *Annu Rev Neurosci* **9**: 405–436.
- Wightman RM, Robinson DL (2002). Transient changes in mesolimbic dopamine and their association with 'reward'. *J Neurochem* **82**: 721–735.
- Wightman RM, Zimmerman JB (1990). Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Res Rev* **15**: 135–144.
- Williams GV, Goldman-Rakic PS (1995). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* **376**: 572–575.
- Wise RA (1996). Neurobiology of addiction. *Curr Opin Neurobiol* **6**: 243–251.
- Yavich L, MacDonald E (2000). Dopamine release from pharmacologically distinct storage pools in rat striatum following stimulation at frequency of neuronal bursting. *Brain Res* **870**: 73–79.
- Zahrt J, Taylor JR, Mathew R (1997). Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci* **17**: 8528–8535.
- Zigmond MJ, Castro SL, Keefe KA, Abercrombie ED, Sved AF (1998). Role of excitatory amino acids in the regulation of dopamine synthesis and release in the neostriatum. *Amino Acids* **14**: 57–62.