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Stimulus-evoked changes in neostriatal dopamine levels in awake and anesthetized rats as measured by microdialysis

J.M. Tepper, I. Creese and D.H. Schwartz

Center for Molecular and Behavioral Neuroscience, Aidekman Research Center, Rutgers, The State University of New Jersey, Newark, NJ (U.S.A.)

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The effect of medial forebrain bundle (MFB) stimulation on neostriatal dopamine levels was examined using in vivo microdialysis in urethane-anesthetized and awake, freely-moving rats in conjunction with single unit extracellular recordings from antidromically identified nigral dopaminergic neurons. Dialysis samples were collected during baseline periods or while stimulating the MFB with trains of 5 or 10 pulses at different frequencies within a physiologically relevant range. When the perfusion solution contained 1.2 mM Ca^{2+}, even intense, high frequency stimulation was ineffective at producing significant elevations in neostriatal dopamine levels whereas cocaine or amphetamine reliably caused several-fold elevations in dopamine levels. When the perfusate contained 2.4 mM Ca^{2+}, modest MFB stimulation within the range of spontaneous nigral cell firing produced large and reliable increases in dopamine levels. There was a significant correlation between the proportion of dopaminergic neurons that could be antidromically activated from the MFB and the increase in neostriatal dopamine. There was no effect of stimulus pattern on the increase in dopamine levels, and results obtained in awake, freely-moving animals did not differ from those obtained in anesthetized animals. These data provide good evidence that in vivo microdialysis is sensitive to neostriatal dopamine overflow evoked by stimulation within the normal rate of firing of nigrostriatal neurons and that Ringer's Ca^{2+} concentration is a critical variable in the detection of stimulus-induced release of dopamine.

INTRODUCTION

The release of neurotransmitter from nerve terminals as a consequence of impulse activity is generally considered to be both Ca^{2+} and frequency dependent. Depolarization of the nerve terminal due to orthodromic impulse flow is thought to cause Ca^{2+} entry which subsequently results in exocytotic release of the transmitter. It follows that transmitter efflux from the terminal should be greater in the presence of impulse traffic and lower in its absence or under conditions of low extracellular Ca^{2+} concentrations. Furthermore, impulse traffic at a higher mean frequency or that occurring in bursts might lead to a greater release of transmitter per action potential than the same number of impulses at a lower frequency due to a general phenomenon known as facilitation which has been demonstrated in many different systems[35,36,54].

On the other hand, dopaminergic neurons (as well as many other types of neurons) possess an autoreceptor-mediated local regulatory mechanism at their terminals that appears to modify synaptic input–output relations by altering dopamine synthesis, release, and the electrophysiological properties of the terminals themselves[43,44,51]. The operation of these autoreceptors both in vivo and in vitro has been repeatedly shown to depend on the frequency and/or pattern of stimulation or impulse flow[8,21,45]. It is possible, therefore, that the terminal autoreceptors serve to normalize the amount of dopamine released per impulse, compensating for changes in the frequency or pattern of firing. These issues have been examined both in vivo and in vitro; however the findings have been strongly influenced by the particular technique employed. Briefly, in vitro procedures have shown that dopamine release per pulse from neostriatal slices exhibits either a modest inverse relationship or no relationship at all to stimulation frequency[7,21,22], and terminal excitability studies show that the excitability of dopamine nerve terminals is inversely proportional to the frequency of firing or the occurrence of spontaneous bursts in vivo[45]. On the other hand, when monitored by electrochemistry, dopamine overflow or release in vivo appears to be directly related to stimulation frequency with higher stimulation frequencies producing increased dopamine efflux[15,16,33].

Another in vivo technique, intracerebral microdialy-

Correspondence: J.M. Tepper, Center for Molecular and Behavioral Neuroscience, Aidekman Research Center, Rutgers, The State University of New Jersey, 197 University Avenue, Newark, NJ 07102, U.S.A.

sis, has been widely used to evaluate pharmacological changes in monoaminergic systems[1,23,26,38,39,41]. It has not, however, been applied as extensively to the examination of dopamine release caused by changes in impulse traffic within the physiological range.

In the present experiments, we have used in vivo microdialysis to examine changes in extracellular neostriatal dopamine levels evoked by stimulation of the medial forebrain bundle (MFB) at physiologically relevant frequencies. Since dopaminergic axons exhibit relatively high thresholds and are thus difficult to activate[52,53] single-unit extracellular recordings were obtained from a number of electrophysiologically identified nigrostriatal dopaminergic neurons in each animal prior to, and in some cases, during the dialysis measurements. These recordings were used to verify the microdialysis probe location with respect to the MFB electrode location, to test the efficacy of the MFB stimulus for activation of dopaminergic axons, and to measure the ability of these axons to respond to each stimulus within a train with an action potential. The stimulus parameters were chosen to approximate the duration and pattern of the frequent bursts that characterize the spontaneous activity of nigrostriatal dopaminergic neurons in vivo[5]. Portions of these results have previously appeared in abstract form[40].

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats weighing between 250 and 400 g were anesthetized with urethane (1.3 g/kg; i.p.) and installed in a stereotaxic frame. All wound margins and points of contact between the animal and the experimental apparatus were infiltrated with xylocaine ointment (5%) or solution (2%). Body temperature was maintained at 37 ± 1 °C by a solid-state heating pad and the electrocardiogram was continuously monitored on an auxiliary oscilloscope and audio monitor.

Electrical stimulation

Stimulating electrodes consisted of enamel-coated stainless steel wires approximately 200 μm in diameter, insulated with formvar except at the sharply cut tips. Four of these electrodes were cemented together in a square array such that each tip was separated from its adjacent neighbor by 1 mm and the array was then lowered into the MFB (stereotaxic coordinates relative to lambda and dura: anterior 4.5 mm; lateral 1.8; ventral 7.8 mm). Each of the 4 electrodes served as a single pole. A dialysis probe (see below) was coupled to a single bipolar electrode (tip separation < 150 μm, in vitro resistance 10–30 kΩ, insulated except at the sharply cut tips) whose tip resided mid-way along the exposed fiber. The pair was then lowered into the anterior-lateral neostriatum (stereotaxic coordinates relative to bregma and dura: anterior 1.0 mm; lateral 3.5 mm; ventral 4.2 mm), so that the active region of the probe extended from 2.2 to 6.2 mm from the dural surface. Stimulating electrodes and the dialysis probe were affixed to the skull with dental cement and cyanoacrylate glue.

Electrical stimuli were generated by a Winston Electronics A-65 timer coupled to a constant current stimulus isolation unit (Winston Electronics, model SC-100). The stimuli consisted of trains of 5 or 10 single monophasic square-wave pulses of durations ranging from 250–500 μs at current intensities between 0.1 and 4.0 mA. The pattern of these stimuli was varied by altering the interpulse interval within the train. Thus, although the mean frequency of stimulation was held constant (5 pulses/1500 ms = 3.33 Hz or 10 pulses/1500 ms = 6.67 Hz) at values surrounding the mean firing rate of dopaminergic neurons in vivo[5,19] these pulses were delivered within a window of 100–1500 ms, thus mimicking bursts of 5 or 10 spikes at intraburst frequencies ranging from 3.33 to 100 Hz. For the remainder of this paper, these different stimuli are described by the number of pulses and the frequency corresponding to the interpulse interval within the train, e.g. 10 pulses at 12.5 Hz means 10 pulses delivered within an 800 ms train, once every 1.5 s.

Electrodes and electrophysiological identification of dopaminergic neurons

Recording electrodes were fabricated from 2.0 mm o.d. capillary tubing (WPI kwik-fill) on a Narishige vertical pipette puller, and possessed in vitro impedances of approximately 20 MΩ when filled with 2 M NaCl. The electrode impedance was then lowered by passing a 500 ms 150 V DC pulse (Grass stimulator, model S-48) through the electrode which caused the resistance to drop to between 4 and 10 MΩ. Electrodes were then lowered into the region of the substantia nigra pars compacta and single unit extracellular recordings were obtained from neurons identified as dopaminergic using the following previously published criteria.

Dopaminergic neurons displayed a characteristic electrophysiological profile of spontaneous activity consisting of an unusually wide action potential, greater than 2 ms in duration, typically possessing a notch or inflection on the initial positive component and fired in a slow, irregular pattern at rates ranging up to 8 Hz with occasional bursts consisting of 2–10 spikes during which the instantaneous firing rate can reach 15 Hz[5,9,19,20]. In order to make the identification unambiguous, the ultimate criterion for an electrophysiologically identified dopaminergic neuron was antidromic activation from neostriatum at an appropriate latency[20,46].

For each recorded neuron, after antidromic identification was established from the neostriatal site, current was applied between different pairs of MFB electrodes in an attempt to drive the cell antidromically from the MFB. The electrode pair which elicited antidromic responses at the lowest current was then used for the remainder of the experiment. From 3 to 10 electrophysiologically identified nigrostriatal neurons were tested for their responsivity to MFB stimulation in each animal. Measures were taken of the neostriatal and MFB threshold currents for each neuron, the maximum stimulus frequency (up to 100 Hz) at which MFB-evoked antidromic responses were elicited to each stimulus in the train, and the proportion of neurons antidromically activated from neostriatum that were also antidromically activated from the MFB. Following the electrophysiological testing, dialysis sampling commenced.

Microdialysis

Probes were constructed from 26-gauge stainless steel tubing, fused silica glass (150 μm o.d.), cellulose dialysis membranes (250 μm o.d.) and PE-20 tubing[37,39]. The active region of the probe was 4.0 mm long. A modified rat Ringer's solution consisting of 140 mM NaCl, 4.0 mM KCl and 1.2 or 2.4 mM $CaCl_2$ was constantly perfused through the probe at a rate of 1 or 2 μl/min.

Samples were separated on a reverse phase C-18 (3.2 mm bore with 3 μm packing) column (ESA, Inc. HR-80). The mobile phase consisted of 40 mM NaH_2PO_4, 1.3 mM octane sulphonic acid, 238 μM EDTA, and 8% v/v methanol at pH 3.6. A Coulochem electrochemical detector (ESA, Inc. model 5100A) was used to quantify levels of DOPAC, dopamine, 5-HIAA and HVA as they eluted off the column as well-separated peaks at 4, 5, 7 and 10.5 min respectively. The output of the electrochemical detector was digitized with a Nicolet model 4094 digital oscilloscope and fed into a Macintosh II computer. Peak heights and latencies were measured using custom-designed software. All dopamine values are expressed as a percentage of from three to five 12 min baseline samples taken at the beginning of the experiment, no less than 2 h after probe implantation.

In order to examine the relationship between impulse pattern and dopamine release, trains of 5 or 10 pulses (250–500 μs; 0.1–4 mA) were delivered to the MFB every 1.5 s for an entire 12 min sampling period. From one to four (usually two) 12 min non-stimulated baseline samples were interspersed between stimulation sampling periods.

Microdialysis in freely-moving animals

Animals were anesthetized with a mixture of ketamine (50 mg/kg) and chloral hydrate (150 mg/kg) i.p., and installed in a stereotaxic apparatus. A 21-gauge guide cannula was implanted into the neostriatum (stereotaxic coordinates relative to bregma and dura: anterior 1.0 mm; lateral 3.5 mm; ventral 3.0 mm) and an array of 4 stimulating electrodes was placed into the MFB, as described above. After a recovery period of at least 4 days, a dialysis probe was inserted in the guide cannula. The tubing was prevented from tangling by a fluid swivel and was attached to the animals' headpiece via a spring and counterbalance arm. Samples were collected directly above the animals' head (approximately 20 cm) in a 400 μl collection vial clipped to the spring. At least 16 h elapsed between probe implantation and the collection of the first dialysis sample.

RESULTS

Effect of MFB stimulation and Ca^{2+} concentration on dopamine overflow

Our initial experiments were performed with a perfusion solution containing 1.2 mM Ca^{2+}. At this Ca^{2+} concentration, MFB stimulation, even at high frequencies and intensities (e.g. 10 pulses at 100 Hz; 2.0–4.0 mA, 250–500 μs), was ineffective at producing significant increases in extracellular neostriatal dopamine levels, despite the fact that the same stimulation consistently produced antidromic responses in identified dopaminergic neurons, as shown in Fig. 1 for a representative experiment. This was not due to a failure of the dialysis or HPLC system, since a marked increase in dopamine levels was produced by intravenous administration of cocaine (5 mg/kg) in this animal or amphetamine in other experiments (amphetamine 0.5 mg/kg i.v.; mean ± S.E.M. = 327 ± 40% increase; $n = 3$; data not shown).

However, quite different results were obtained when the Ca^{2+} concentration in the perfusion medium was increased to 2.4 mM. Under these conditions, MFB stimulation was effective at producing increases in neostriatal dopamine levels as shown for one representative experiment in Fig. 2. Reliable increases in dopamine levels could be obtained under conditions of modest MFB stimulation (e.g. 5 pulses at 3.33 Hz; 1.0 mA, 500 μs) which produced antidromic activation of dopaminergic neurons at currents within the same range as those that failed to elicit significant increases in neostriatal dopamine levels at a perfusate Ca^{2+} concentration of 1.2 mM.

That the ability of MFB stimulation to produce increases in neostriatal dopamine levels displayed a critical dependence on the Ca^{2+} concentration in the perfusion medium could also be demonstrated by altering the Ca^{2+} concentration within a single microdialysis session, as shown for one representative example in Fig. 3. When the initial perfusate Ca^{2+} concentration was 2.4 mM, reliable increases in extracellular dopamine levels were obtained in response to modest MFB stimulation (10 pulses, 6.67–12.5 Hz at 1.0 mA, 500 μs). When the Ca^{2+} concentration was reduced to 1.2 mM Ca^{2+}, the stimulation-induced increase in dopamine was reduced after 36 min and was completely abolished after approximately 70 min. When it was returned to 2.4 mM, the stimulation-induced increase was reinstated in a similar manner.

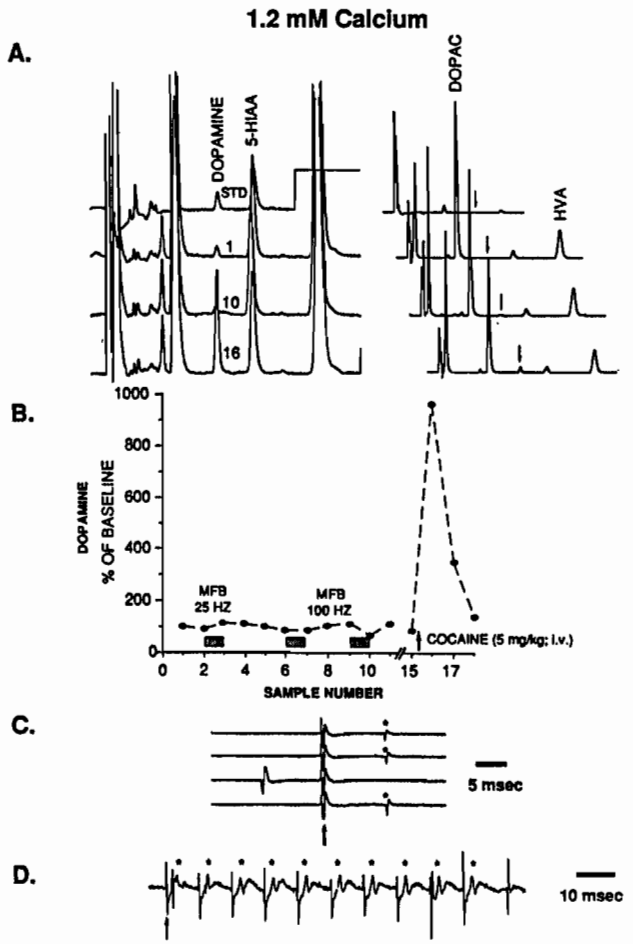

Fig. 1. Neostriatal dopamine levels as a function of MFB stimulation and cocaine administration when the perfusion solution contained 1.2 mM Ca^{2+}. A: raw chromatographs (*left panel*: high gain trace; *right panel*: low gain trace) from a representative case showing standards (20 pg dopamine; 200 pg DOPAC, 5-HIAA and HVA) and samples before (sample 1) and during MFB stimulation (sample 10) and after cocaine administration (sample 16). B: extracellular dopamine levels did not change even at high stimulation frequencies and currents but cocaine (5 mg/kg i.v. at arrow) produced a dramatic increase in dopamine level. C: neostriatal-evoked antidromic responses (*). Note collision with spontaneous spike in the third trace. Arrow denotes stimulus artifact. D: train of MFB stimulation (100 Hz) elicits antidromic responses (*) to each stimulus demonstrating effective stimulation of nigrostriatal axons. MFB stimuli consist of 10 pulses at 1 mA, 500 μs, delivered once every 1.5 s for 12 min. Arrow denotes first stimulus artifact in train.

Overall, stimulation-induced increases in dopamine overflow were significantly greater using a perfusion medium Ca^{2+} concentration of 2.4 mM compared to 1.2 mM ($F_{1,8} = 9.76$; $P < 0.02$), as shown in the bottom panel of Fig. 3.

Basal (unstimulated) dopamine levels were also dependent on the perfusion calcium concentration, being approximately 40% greater at 2.4 mM Ca^{2+} (19.12 ± 1.84 pg/ 12 min = 10.35 ± 1.2 fmol/min; mean ± S.E.M.) than at 1.2 mM Ca^{2+} (13.75 ± 2.55 pg/12 min = 7.48 ± 1.66 fmol/min). As measures of basal levels of dopamine release tended to be somewhat variable across animals, this difference was only of borderline statistical significance with a one tailed *t*-test ($t = 1.74$, $df = 13$, $P = 0.0524$).

An estimate of the effects of the perfusion Ca^{2+} concentration on the excitability of dopaminergic nigrostriatal terminals in the region of the dialysis probe was obtained by measuring the threshold currents for antidromic activation from the probe site[44–46]. Although there was clearly a difference in MFB stimulation-induced dopamine overflow with 1.2 vs 2.4 mM Ca^{2+} in the perfusion medium, there was no significant difference in the antidromic thresholds of nigrostriatal dopaminergic nerve terminals at 1.2 (1.72 ± 0.25 mA) vs 2.4 mM (2.20 ± 0.20 mA) Ringer's Ca^{2+} as shown in Fig. 4.

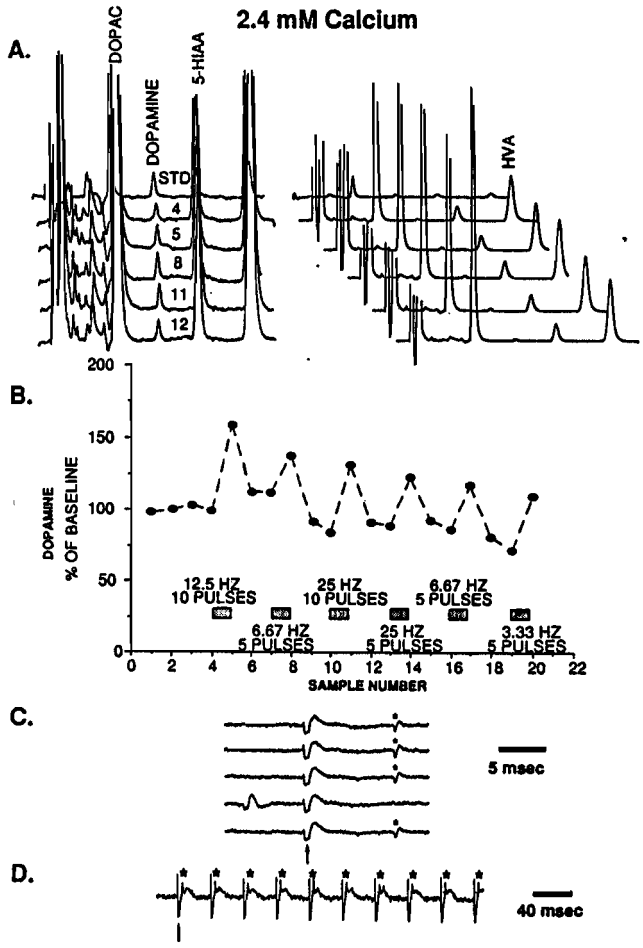

Fig. 2. Neostriatal dopamine levels as a function of MFB stimulation when the perfusion solution contained 2.4 mM Ca^{2+}. A: raw chromatographs (*left panel*: high gain trace; *right panel*: low gain trace) from a representative experiment showing standards (20 pg dopamine; 200 pg DOPAC, 5-HIAA and HVA) and samples before (sample number 4), during (samples number 5, 8 and 11 respectively) and after (sample number 12) various stimulation periods. Note lack of effect of stimulus frequency. B: extracellular dopamine showed consistent increases during each MFB stimulation period. C: neostriatal-evoked antidromic responses (*). Note the collision in the fourth trace. Arrow denotes stimulus artifact. D: MFB-elicited antidromic responses faithfully follow 25 Hz stimulation. MFB stimuli consist of 10 pulses at 1 mA, 500 μs, delivered once every 1.5 s. Arrow denotes first stimulus artifact in train.

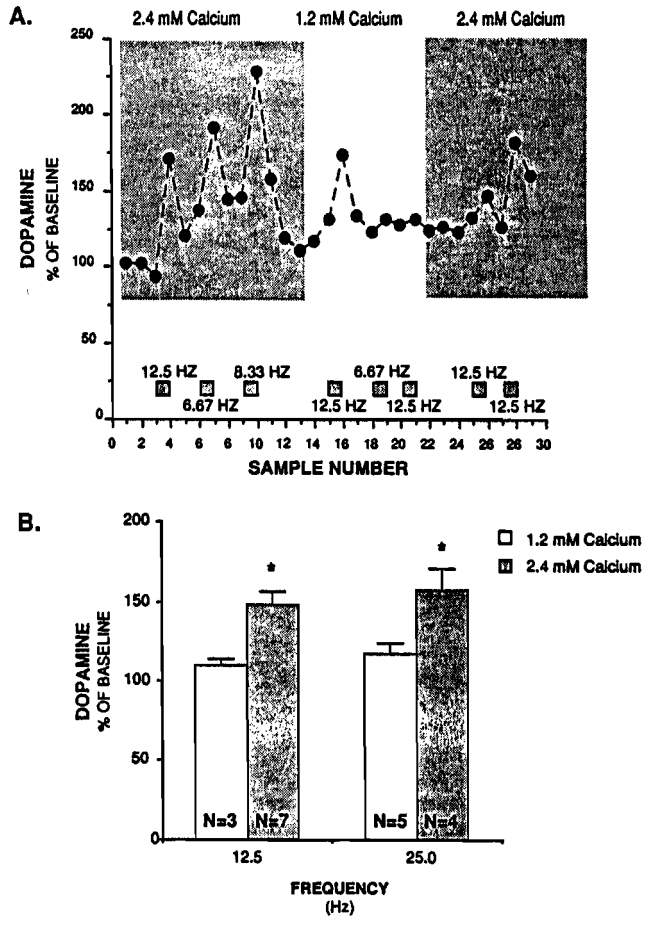

Fig. 3. A: stimulation-induced increases in neostriatal dopamine overflow with 2.4 mM Ca^{2+} in perfusion medium (gray areas) were abolished when the Ca^{2+} concentration was reduced to 1.2 mM (white area). These stimulation-induced increases were reinstated when the Ca^{2+} concentration was returned to 2.4 mM. B: summary chart showing significantly greater stimulation-induced dopamine overflow at 2.4 mM as compared to 1.2 mM (*$P < 0.05$). Error bars represent S.E.M. Numbers within bars represent number of cases in each condition. MFB stimuli consist of 10 pulses at 1 mA, 500 μs, delivered once every 1.5 s.

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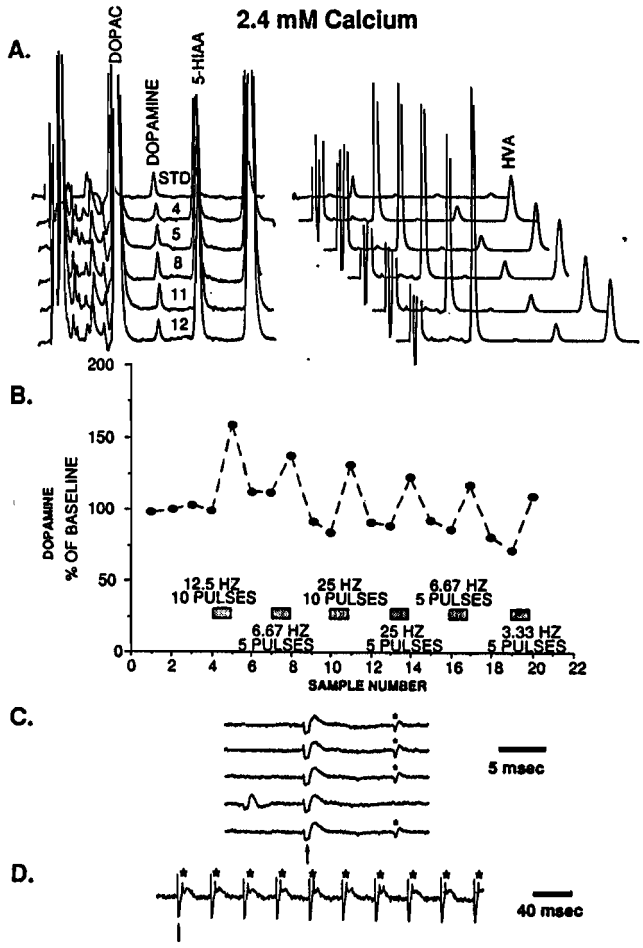
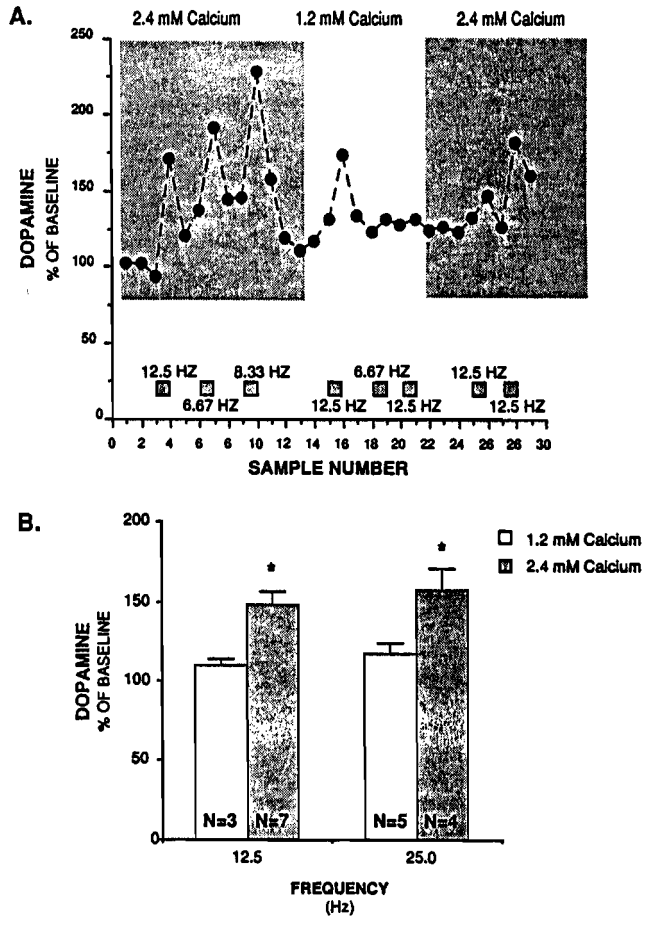


Fig. 2. Neostriatal dopamine levels as a function of MFB stimulation when the perfusion solution contained 2.4 mM Ca^{2+}. A: raw chromatographs (*left panel*: high gain trace; *right panel*: low gain trace) from a representative experiment showing standards (20 pg dopamine; 200 pg DOPAC, 5-HIAA and HVA) and samples before (sample number 4), during (samples number 5, 8 and 11 respectively) and after (sample number 12) various stimulation periods. Note lack of effect of stimulus frequency. B: extracellular dopamine showed consistent increases during each MFB stimulation period. C: neostriatal-evoked antidromic responses (*). Note the collision in the fourth trace. Arrow denotes stimulus artifact. D: MFB-elicited antidromic responses faithfully follow 25 Hz stimulation. MFB stimuli consist of 10 pulses at 1 mA, 500 μs, delivered once every 1.5 s. Arrow denotes first stimulus artifact in train.



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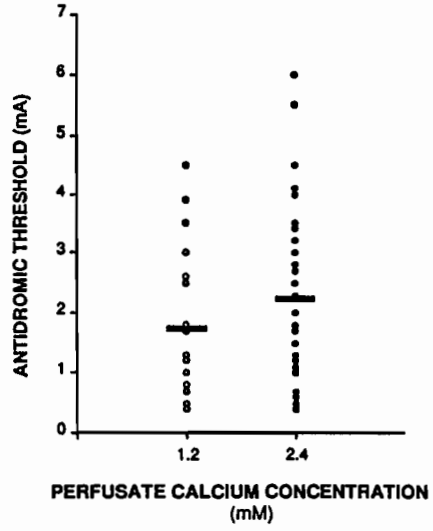

Fig. 4. Threshold currents required to antidromically activate single dopaminergic neurons were not significantly different at 1.2 mM (open circles) as compared to 2.4 mM (closed circles) Ringer's Ca^{2+} concentration. Each circle represents the current necessary to elicit antidromic response on 100% of non-collision trials from individual dopaminergic neurons. Horizontal bars represent means.

Correlation between stimulation-induced dopamine overflow and antidromic responding from MFB

There was a significant positive correlation between the proportion of dopaminergic neurons that could be antidromically driven from the MFB and the increase in dopamine overflow induced by identical stimuli (10 pulses at 12.5 Hz; 1 mA, 500 μs) across animals at 2.4 mM Ringer's Ca^{2+} ($r = 0.778$; $F_{1,13} = 19.99$; $P < 0.001$) as shown in Fig. 5. Animals in which a large proportion of the nigrostriatal neurons were antidromically activated from the MFB site showed larger increases in dopamine levels in response to the MFB stimulation than animals in which only a small fraction of the nigrostriatal neurons recorded could be antidromically driven from the MFB. Figure 6 shows a representative example of an animal in which 9 neurons could be antidromically driven from the neostriatum, but only one could be antidromically activated by MFB stimulation. Stimulation at that MFB site was also ineffective in producing significant increases in neostriatal dopamine levels. An i.v. injection of cocaine (1 mg/kg), however, produced a large (>5-fold), transient elevation in neostriatal dopamine levels.

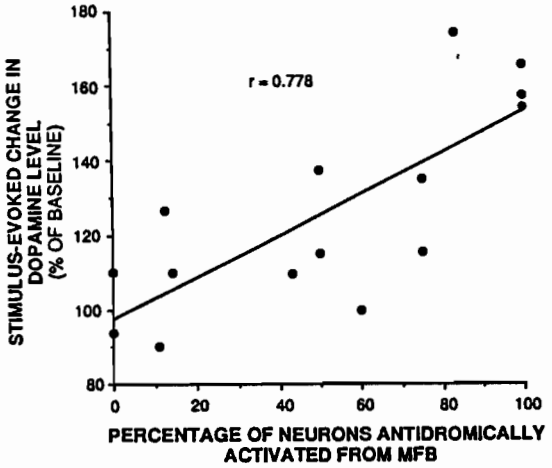

Fig. 5. Significant correlation of stimulation-induced dopamine overflow vs percentage of dopaminergic neurons antidromically activated by identical MFB stimuli in different animals (train of 10 pulses, 12.5 Hz, 1 mA, 500 μs, once every 1.5 s; $r = 0.778$, $F_{1,13} = 19.99$, $P < 0.001$).

Effect of MFB stimulation pattern

When the number of pulses per train was held constant at 5 or 10, stimulation-induced dopamine overflow was not dependent on the pattern of stimulation, despite the fact that robust increases were obtained at each frequency examined as shown in Fig. 7. No frequency dependence was observed at stimulation frequencies within (6.67 Hz, 8.33 Hz, 12.5 Hz) and beyond (25 Hz) the normal physiological firing rates of dopaminergic neu-

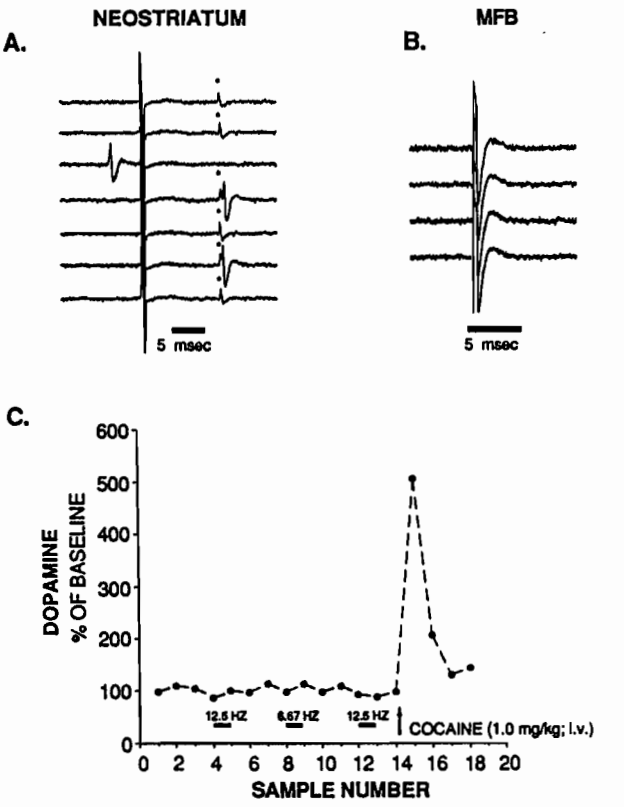

Fig. 6. MFB sites that were ineffective in antidromically activating dopaminergic neurons were also ineffective in producing increases in neostriatal dopamine overflow. A: neostriatal-evoked antidromic responses (*). Note collision in the third trace. B: single pulse MFB stimulation fails to elicit antidromic responses in the same dopamine cell. C: MFB stimulation fails to increase striatal dopamine overflow; however cocaine administration (1 mg/kg i.v. at arrow) caused over a 500% increase.

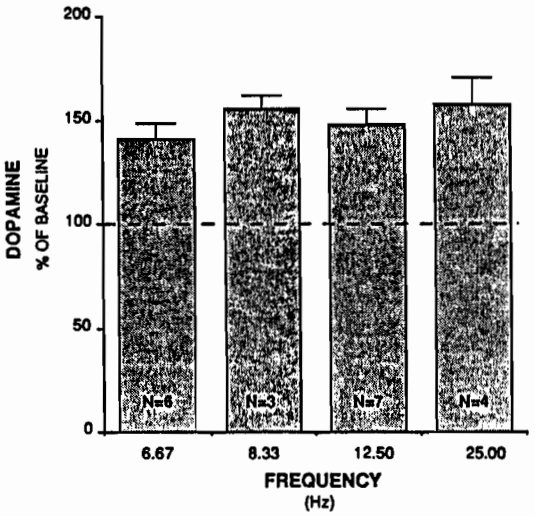

Fig. 7. Stimulation-induced neostriatal dopamine overflow was not differentially affected by various stimulation frequencies (10 pulses; 1.0–1.5 mA; 500 μs; 6.67–25 Hz). Error bars represent S.E.M. Numbers within bars represent number of cases at each frequency.

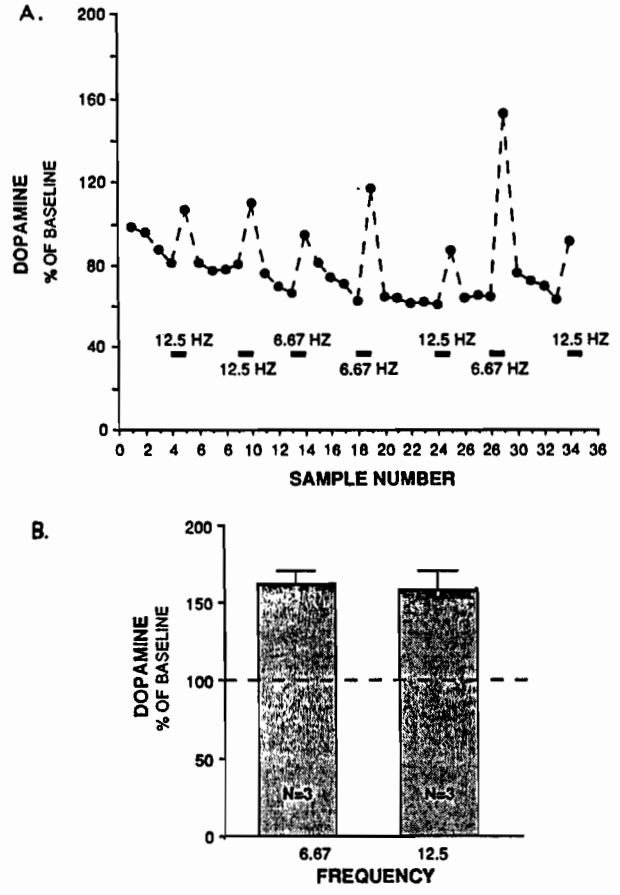

Fig. 8. MFB stimulation-induced increases in neostriatal dopamine overflow in awake, freely-moving animals. A: representative animal shows reproducible increases at different stimulation frequencies. B: summary chart showing no difference in striatal dopamine overflow at 6.67 Hz compared to 12.5 Hz in freely-moving animals (n = 3). Error bars represent S.E.M. Numbers within bars represent number of cases at each frequency.

rons, despite the fact that in all cases, recordings from nigrostriatal neurons demonstrated that the dopaminergic axons were able to faithfully follow the MFB stimuli.

To test the hypothesis that the lack of dependence on stimulus pattern was an artifact of general anesthesia or immobilization in the stereotaxic apparatus, similar experiments were conducted in unanesthetized, freely moving animals with a perfusion Ca^{2+} concentration of 2.4 mM, as illustrated for one representative case in the upper panel of Fig. 8, and averaged across experiments (n = 3) in the lower panel. The results obtained in freely moving animals were similar in all respects to those observed in anesthetized animals; the percent increase in dopamine levels as a function of MFB stimulation relative to baseline was similar in magnitude to those observed in urethane-anesthetized animals and no significant effect of stimulus pattern was observed.

DISCUSSION

The present experiments demonstrate that increases in neostriatal dopamine levels elicited by stimulating dopaminergic axons at rates within the range of their normal physiological activity can be detected in vivo by microdialysis. Dopamine levels rose rapidly upon stimulation (within a single 12 min sample period), and decayed with a similar rapid time course. DOPAC levels showed a much poorer temporal correlation to MFB stimulation, and did not decay consistently during the interstimulus baseline periods, indicating that the extracellular level of DOPAC is not very useful as an index of dopamine release in microdialysis studies of this kind where stimuli are repeated and varied many times in a single animal.

There have been a number of reports attempting to demonstrate that brain compounds recovered from dialysates are neuronal in origin, by showing that levels of the compound can be reduced under exposure to zero Ca^{2+} or calcium antagonists, sodium channel blockade by tetrodotoxin or by decreased neuronal activity following administration of autoreceptor agonists e.g.[2,6,23,24,40,47,49]. Although detailed pharmacological manipulations were not performed in the present study, we did observe that basal levels of neostriatal dopamine release were some 40% higher when the perfusion medium contained 2.4 mM Ca^{2+} than when it contained 1.2 mM Ca^{2+}, consistent with the recent findings of others[31,48]. These data suggest that the basal dopamine levels reflect, at least in part, dopamine released by the spontaneous activity of nigrostriatal neurons.

The fact that use of a perfusion medium containing 1.2 mM Ca^{2+} was insufficient to reveal stimulation-induced dopamine increases is interesting and unexpected,

since 1.2 mM has been reported to be the extracellular Ca^{2+} concentration in rat neostriatum[31]. In contrast to the failure of MFB stimulation to increase neostriatal dopamine overflow at 1.2 mM Ca^{2+}, administration of cocaine or amphetamine produced extremely large increases in neostriatal dopamine levels, indicating that the dialysis system was sensitive to changes in dopamine levels at this lower Ca^{2+} concentration.

One possible explanation for the requirement for a high perfusion Ca^{2+} concentration is that the excitability of the nigrostriatal terminals varied with the Ca^{2+} concentration and thereby affected the impulse-dependent release of dopamine. Decreased terminal excitability has been shown to be associated with decreased dopamine release per pulse and increased terminal excitability with increased release[44–46]. However, this was not the case in the present experiments; the current threshold necessary to antidromically activate dopaminergic neurons from neostriatum was not significantly different at 1.2 mM vs 2.4 mM Ca^{2+}. The slight difference that was observed indicated that the excitability of the nigrostriatal terminals was slightly lower at the higher Ca^{2+} concentration, rather than higher. Thus, changes in evoked release due to a change in the electrical excitability of the terminals as a function of Ca^{2+} concentration can be ruled out.

Another possibility is that the requirement for high Ca^{2+} is related to the lack of Mg^{2+} in our perfusion solution. This does not seem likely, however, since basal levels of dopamine are not affected by the presence or absence of Mg^{2+} in the perfusion medium[31]. Furthermore, early electrophysiological studies of neurotransmitter release clearly show an antagonistic effect of Mg^{2+} on Ca^{2+}-dependent neurotransmitter release[10], and local infusion of Mg^{2+} has been shown to antagonize calcium-dependent dopamine release in previous microdialysis experiments[49]. Based on these results, one would predict that the addition of Mg^{2+} ions to the perfusion medium would, if anything, raise the Ca^{2+}-requirement for stimulated release, not lower it.

A more likely explanation may be that some aspect of the dialysis or MFB stimulation procedure itself somehow reduces the extracellular Ca^{2+} concentration in the region surrounding the probe in such a way as to require perfusion with a higher Ca^{2+} concentration in order to support and maintain the Ca^{2+}- and impulse-dependent exocytotic release of dopamine throughout the 12 min stimulation and sampling period. It has been shown, for example, that repetitive neuronal activity leads to rather large decreases in extracellular Ca^{2+} in cerebellar cortex, as measured with ion-sensitive microelectrodes[34]. When the MFB is stimulated under the conditions of the present experiment, it is likely that hundreds and perhaps thousands of dopaminergic axons are driven to fire synchronously. It is conceivable that the resulting synchronous firing of millions of tightly packed dopaminergic terminals in neostriatum removes enough Ca^{2+} from the extracellular pool due to influx into presynaptic terminals to depresses the extracellular Ca^{2+} concentration enough to interfere with depolarization–secretion coupling, particularly when the stimulation is maintained for 12 min. It is interesting to note that in prefrontal cortex where the density of dopamine terminals and the basal levels of dopamine are far less than in neostriatum, stimulus-evoked release can be demonstrated when the Ca^{2+} concentration of the perfusion solution is 1.2 mM[3].

Stimulation-induced increases in neostriatal dopamine overflow were not dependent on the pattern of MFB stimulation. When using anesthetized preparations, the possibility always exists that the anesthesia is somehow exerting confounding factors on the results. Indeed, various electrophysiological investigations have reported differential effects of pharmacological manipulations on anesthetized vs awake animals[12,13,14,29]. However, in the present experiments, the lack of frequency or pattern dependence was not an artifact of general anesthesia, since it was observed both in the urethane-anesthetized rats as well as in awake, freely-moving animals. Not only was the lack of a pattern effect consistent, but the magnitude of the effect (approximately a 50% increase above non-stimulated baseline samples) was also similar in the two conditions.

The lack of a pattern effect similarly cannot be attributed to an inappropriate choice of stimulus frequencies since the range examined (3.33–100 Hz) included and extended beyond the firing frequency of dopaminergic neurons in both the single-spike mode and within spontaneous bursts[5,9,20]. Likewise, the lack of frequency dependence is not due to the failure of dopaminergic axons to support impulse conduction within this range, since our electrophysiological data clearly demonstrate that dopaminergic axons reliably followed stimuli at these rates.

A similar lack of frequency dependence is also found when endogenous or pre-labeled [^{3}H]dopamine release per pulse is measured from neostriatal slices in vitro using a low number of total pulses, and with a sufficiently long stimulus period, dopamine release actually shows a slight inverse relationship to stimulus frequency[7,21,22]. The present data therefore are consistent with and extend the in vitro striatal slice data to the in vivo condition. Electrophysiological data suggest that this lack of dependence on stimulus pattern may be due to an autoreceptor-mediated, terminal regulatory mechanism that is more strongly activated at shorter interpulse intervals corresponding to faster spontaneous firing rates and/or occurrence of burst firing[44–46]. When MFB con-

ditioning stimuli very similar to those employed in the present study were applied, the excitability of dopamine terminals in neostriatum in vivo was transiently and dramatically reduced via an autoreceptor-mediated mechanism[45]. The overall role of this mechanism may be to maintain dopamine release per pulse at a relatively constant level, regardless of changes in firing rate or pattern. Further experiments will address this issue by examining the frequency dependence of stimulation-induced dopamine overflow in the presence and absence of autoreceptor blockade.

In contrast to the present results, when the electrochemical signal corresponding to dopamine is assessed, there appears to be a direct relationship between this signal and stimulation frequency or patterning both in vitro[4] and in vivo[15,16,33,42]. Differential pulse voltammetry[18] has been most commonly used to address these issues; however there has been some controversy as to whether this technique can separate DOPAC and ascorbate from dopamine[17,27,32]. Furthermore, in many such studies, long continuous trains of high frequency stimuli (25–100 Hz) well in excess of the highest firing rates ever achieved by dopaminergic neurons are used, and frequency effects are sometimes confounded with the number of stimuli delivered[11,25,30,33,42]. Thus, methodological differences may account for some of the discrepancies between results from voltammetric studies and those reported in the present study which are in good agreement with results from previous in vitro experiments[7,8,21,43].

The MFB sites that were most effective at eliciting increases in neostriatal dopamine overflow were those that also elicited antidromic action potentials in the highest proportion of single dopaminergic neurons recorded in a given animal. Combining single-cell recording with in vivo microdialysis provides an independent criterion with which to judge the effectiveness of MFB stimulation sites. In fact, quite a few stimulation sites that did not activate dopaminergic axons electrophysiologically or increase neostriatal dopamine overflow appeared to be quite close to or directly in lateral hypothalamic sites through which the MFB passes, as verified histologically. This is consistent with other reports suggesting that dopaminergic axons are relatively difficult to activate as compared to other myelinated fibers in the region[52,53].

Thus far, there have been relatively few reports demonstrating stimulation-evoked dopamine increases by microdialysis[3,23,28] and the effect of perfusate Ca^{2+} concentration on evoked release has not previously been reported. The present data provide good evidence that in vivo microdialysis is sensitive to stimulation-induced increases in neostriatal dopamine overflow, and that there is no significant difference between stimulated release in urethane-anesthetized and awake animals. The concentration of Ca^{2+} in the perfusion medium is a critical factor in the ability to demonstrate stimulus-evoked increases in neostriatal dopamine levels, and under the conditions of the present experiments, stimulated release is not dependent on the frequency or patterning of stimuli so long as the number of pulses remains constant.

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REFERENCES

1 Abercrombie, E.D., Bonatz, A.E. and Zigmond, M.J., Effects of L-DOPA on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats, *Brain Research*, 525 (1990) 36–44.
2 Auerbach, S.B., Minzenberg, M.J. and Wilkinson, L.O., Extracellular serotonin and 5-HIAA in hypothalamus of the unanesthetized rat measured by in vivo dialysis coupled to high performance liquid chromatography with electrochemical detection-dialysate serotonin reflects neuronal release, *Brain Research*, 499 (1989) 281–290.
3 Bean, A.J., During, M.J. and Roth, R.H., Stimulation-induced release of coexistent transmitters in the prefrontal cortex: an in vivo microdialysis study of dopamine and neurotensin release, *J. Neurochem.*, 53 (1989) 655–657.
4 Bull, D.R., Palij, P., Sheehan, M.J., Millar, J., Stamford, J.A., Kruk, Z.L. and Humphrey, P.P.A., Application of fast cyclic voltammetry to measurement of electrically evoked dopamine overflow from brain slices in vitro, *J. Neurosci. Methods*, 32 (1990) 37–44.
5 Bunney, B.S., Walters, J.R., Roth, R.H. and Aghajanian, G.K. Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity, *J. Pharmacol. Exp. Ther.*, 185 (1973) 560–571.
6 Carboni, E. and Di Chiara, G., Serotonin release estimated by transcortical dialysis in freely moving rats, *Neuroscience*, 32 (1989) 617–645.
7 Cubeddu, L.X. and Hoffmann, I.S., Operational characteristics of the inhibitory feedback mechanism for regulation of dopamine release via presynaptic receptors, *J. Pharmacol. Exp. Ther.*, 223 (1982) 497–501.
8 Cubeddu, L.X. and Hoffmann, I.S. Frequency-dependent release of acetylcholine and dopamine from rabbit striatum: its modulation by dopaminergic receptors, *J. Neurochem.*, 41 (1983) 94–101.
9 Deniau, J.M., Hammond, C., Riszk, A. and Feger, J., Electrophysiological properties of identified output neurons of the rat substantia nigra (pars compacta and pars reticulata): evidence for the existence of branched pathways, *Exp. Brain Res.*, 32 (1978) 409–422.
10 Dodge, F.A. and Rahamimoff, R., Cooperative action of calcium ions in transmitter release at the neuromuscular junction, *J. Physiol.*, 193 (1967) 419–432.
11 Ewing, A.G., Bigelow, J.C. and Wightman, R.M., Direct in vivo monitoring of dopamine released from two striatal compartments in the rat, *Science*, 221 (1983) 169–171.
12 Fornal, C.A. and Jacobs, B.L., Physiological and behavioral

correlates of serotonergic single-unit activity. In N.N. Osborne and M. Osborne (Eds.), *Neuronal Serotonin*, John Wiley, New York, 1988, pp. 305–345.

13 Freeman, A.S. and Bunney, B.S., Activity of A9 and A10 dopaminergic neurons in unrestrained rats: further characterization and effects of apomorphine and cholecystokinin, *Brain Research*, 405 (1987) 46–55.

14 Freeman, A.S., Meltzer, L.T. and Bunney, B.S., Firing properties of substantia nigra dopaminergic neurons in freely moving rats, *Life Sci.*, 36 (1985) 1983–1994.

15 Gonon, F.G., Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry, *Neuroscience*, 24 (1988) 19–28.

16 Gonon, F.G. and Buda, M.J., Regulation of dopamine release by impulse flow and by autoreceptors as studied by in vivo voltammetry in the rat striatum, *Neuroscience*, 14 (1985) 765–774.

17 Gonon, F., Buda, M., Cespuglio, R., Jouvet, M. and Pujol, J., In vivo electrochemical detection of catechols in the neostriatum of anesthetized rats: dopamine or DOPAC?, *Nature*, 286 (1980) 902–904.

18 Gonon, F.G., Navarre, F. and Buda, M., In vivo monitoring of dopamine release in the rat brain with differential normal pulse voltammetry, *Anal Chem.*, 56 (1984) 573–575.

19 Grace, A.A. and Bunney, B.S., Intracellular and extracellular electrophysiology of nigral dopaminergic neurons-1. Identification and characterization, *Neuroscience*, 10 (1983) 301–315.

20 Guyenet, P.G. and Aghajanian, G.K., Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra, *Brain Research*, 150 (1978) 69–84.

21 Hoffmann, I.S. and Cubeddu, L.X., Rate and duration of stimulation determine presynaptic effects of haloperidol on dopaminergic neurons, *J. Neurochem.*, 39 (1982) 585–588.

22 Hoffmann, I.S., Talmaciu, R.K., Ferro, C.P. and Cubeddu, L.X., Sustained high release at rapid stimulation rates and reduced functional autoreceptors characterize prefrontal cortex dopamine terminals, *J. Pharm. Exp. Ther.*, 245 (1988) 761–772.

23 Imperato, A. and Di Chiara, G., Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the in vivo release of endogenous dopamine and metabolites, *J. Neurosci.*, 4 (1984) 966–977.

24 Kalen, P., Strecker, R., Rosengren, E. and Björklund, A., Endogenous release of neuronal serotonin and 5-HIAA in the caudate-putamen of the rat as revealed by intracerebral dialysis coupled to high performance liquid chromatography with fluorimetric detection, *J. Neurochem.*, 51 (1988) 1422–1435.

25 Kuhr, W.G. and Wightman, R.M., Real-time measurement of dopamine release in rat brain, *Brain Research*, 381 (1986) 168–171.

26 Kuczenski, R. and Segal, D., Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis, *J. Neurosci.*, 9 (1989) 2051–2065.

27 Lyne, P.D. and O'Neill, R.D., Selectivity of stearate-modified carbon paste electrodes for dopamine and ascorbic acid, *Anal. Chem.*, 61 (1989) 2323–2324.

28 Manley, L.D., Kuczenski, R., Young, S.J., Segal, D.S. and Groves, P.M., The effect of medial forebrain bundle (MFB) stimulation on striatal dopamine and serotonin release as measured by microdialysis, *Soc. Neurosci. Abstr.*, 16 (1990) p. 234.

29 Mereu, G., Casu, M. and Gessa, G.L., Sulpiride activates the firing rate and tyrosine hydroxylase activity of dopaminergic neurons in unanesthetized rats, *Brain Research*, 264 (1983) 105–110.

30 Michael, A.C., Ikeda, M. and Justice, J., Mechanisms contributing to the recovery of striatal releasable dopamine following MFB stimulation, *Brain Research*, 421 (1987) 325–335.

31 Moghaddam, B. and Bunney, B.S., Ionic composition of microdialysis perfusing solution alters the pharmacological responsiveness and basal outflow of striatal dopamine, *J. Neurochem.*, 53 (1989) 652–654.

32 Mueller, K., In vivo voltammetric recording with nafion-coated carbon paste electrodes: additional evidence that ascorbic acid release is monitored, *Pharmacol. Biochem. Behav.*, 25 (1986) 325–328.

33 Nicolaysen, L.C., Ikeda, M., Justice, J.B. and Neill, D.B., Dopamine release at behaviorally relevant parameters of nigrostriatal stimulation: effects of current and frequency, *Brain Research*, 460 (1988) 50–59.

34 Nicholson, C., ten Bruggencate, G., Steinberg, R. and Stockle, H., Calcium modulation in brain extracellular environment demonstrated with ion-sensitive micropipette, *Proc. Natl. Acad. Sci. U.S.A.*, 74 (1977) 1287–1290.

35 Parnas, H. and Segal, L.A., A theoretical explanation for some effects of calcium on the facilitation of neurotransmitter release, *J. Theor. Biol.*, 84 (1980) 3–29.

36 Rahamimoff, R., A dual effect of calcium ions on neuromuscular facilitation., *J. Physiol.*, 195 (1968) 471–480.

37 Robinson, T.E. and Wishaw, I.Q., Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats, *Brain Research*, 450 (1988) 209–224.

38 Robinson, T.E., Jurson, P.A., Bennett, J.A. and Bentgen, K.M., Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats, *Brain Research*, 462 (1988) 211–222.

39 Schwartz, D.H., Hernandez, L. and Hoebel, B.G., Fenfluramine increases extracellular serotonin in the lateral hypothalamus of the rat as measured by microdialysis, *Brain Research*, 482 (1989) 261–270.

40 Schwartz, D.H., Creese, I. and Tepper, J.M., Factors controlling the stimulated overflow of striatal dopamine measured by in vivo microdialysis, *Soc. Neurosci. Abstr.*, 16 (1990) p. 1049.

41 Sharp, T., Zetterström, T., Ljungberg, T. and Ungerstedt, U., A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis, *Brain Research*, 401 (1987) 322–330.

42 Stamford, J.A., Kruk, Z.L. and Millar, J., The influence of stimulus parameters on the potentiation of striatal dopamine release by metoclopramide: in vivo voltammetric data, *Eur. J. Pharmacol.*, 127 (1986) 57–65.

43 Starke, K., Gothert, M. and Kilbinger, H., Modulation of neurotransmitter release by presynaptic autoreceptors, *Physiol. Rev.*, 69 (1989) 864–989.

44 Tepper, J.M. and Groves, P.M., In vivo electrophysiology of central nervous system terminal autoreceptors. In S. Kalsner and T.C. Westfall (Eds.), *Presynaptic Autoreceptors and the Question of the Autoregulation of Neurotransmitter Release.*, *Ann. New York Acad. Sci. Vol. 604*, The New York Academy of Sciences, New York, 1990, pp. 470–487.

45 Tepper, J.M., Young, S.J. and Groves, P.M., Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of increases in impulse flow, *Brain Research*, 309 (1984) 309–316.

46 Tepper, J.M., Nakamura, S., Young, S.J. and Groves, P.M., Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of striatal drug infusions, *Brain Research*, 309 (1984) 317–333.

47 Westerink, B.H.C., Damsma, G., Rollema, H., De Vries, J.B. and Horn, A.S., Scope and limitations of in vivo brain dialysis: a comparison of its application to various neurotransmitter systems, *Life Sci.*, 41 (1987) 1763–1776.

48 Westerink, B.H.C., Damsma, G. and De Vries, J.B., The significance of extracellular calcium for the release of dopamine acetylcholine and amino acids in conscious rats, evaluated by brain microdialysis, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 337 (1988) 373–378.

49 Westerink, B.H.C., Hofsteede, R.M., Tuntler, J. and De Vries, J.B., Use of calcium antagonism for the characterization of drug-evoked dopamine release from the brain of conscious rats determined by microdialysis, *J. Neurochem.*, 52 (1989) 722–729.
50 Wightman, R. and Wipf, D.O., High-speed cyclic voltammetry, *Accounts Chem. Res.*, 23 (1990) 65–70.
51 Wolf, M.E. and Roth, R.H., Dopamine autoreceptors. In I. Creese and C.M. Fraser (Eds.), *Dopamine Receptors*, Alan R. Liss, New York, 1987, pp. 45–96.
52 Yeomans, J.S., Two substrates for medial forebrain bundle self-stimulation: myelinated axons and dopamine axons, *Neurosci. Biobehav. Rev.*, 13 (1989) 91–98.
53 Yeomans, J.S., Maidment, N.T. and Bunney, B.S., Excitability properties of medial forebrain bundle axons of A9 and A10 dopamine cells, *Brain Research*, 450 (1988) 86–93.
54 Zucker, R.S., Characteristics of crayfish neuromuscular facilitation and their calcium dependence, *J. Physiol.*, 241 (1974) 91–110.