Published in final edited form as:

Neuroscience. 2008 February 6; 151(3): 824–835.

Electrophysiological evaluation of the time-course of dopamine uptake inhibition induced by intravenous cocaine at a reinforcing dose

Yoshihiko Wakazono and Eugene A. Kiyatkin *

Behavioral Neuroscience Branch, National Institute on Drug Abuse - Intramural Research Program, National Institutes of Health, DHHS, 5500 Nathan Shock Drive, Baltimore, Maryland 21224

Abstract

Cocaine is an effective dopamine (DA) uptake inhibitor and this action appears to be the primary cause for increased DA transmission following systemic cocaine administration. Although this action had been reliably demonstrated *in vivo* with cocaine at high doses, data on the extent and the time-course of DA uptake inhibition induced by intravenous (iv) cocaine at low, reinforcing doses remain controversial. To clarify this issue, we examined how cocaine affects striatal neuronal responses to repeated iontophoretic DA applications in urethane-anesthetized rats. Because most striatal neurons during anesthesia have low, sporadic activity, DA tests were performed on cells tonically activated by continuous glutamate application.

DA phasically decreased the activity of most dorsal and ventral striatal neurons; these responses in control conditions (iv saline) were current (dose)-dependent and remained highly stable following repeated DA applications at the same currents. DA also consistently decreased the activity of striatal neurons after iv cocaine (1 mg/kg); the magnitude of DA-induced inhibition slowly increased from ~5 min, became significantly larger from ~9 min, and peaked at 13–15 min after a single iv injection. Then, the difference in the DA response slowly decreased toward the pre-cocaine baseline. A similar enhancement of DA induced-inhibition was also seen after ip cocaine administration at a high dose (15 mg/kg). In this case, the DA response becomes significantly stronger at 7–9 min and remained enhanced vs. a pre-drug control up to 24–26 min after the injection. Both regimens of cocaine treatment did not result in evident changes in either onset or offset of the DA-induced inhibitions.

Our data confirm that cocaine at low, reinforcing doses inhibits DA uptake, resulting in potentiation of DA-induced neuronal inhibitions, but they suggest that this effect is relatively weak and delayed from the time of iv injection. These slow and prolonged effects of iv cocaine on DA-induced neuronal responses are consistent with previous binding and our electrochemical evaluations of DA uptake, presumably reflecting the total time necessary for iv delivered cocaine to reach brain microvessels, cross the blood-brain barrier, passively diffuse within brain tissue, interact with the DA transporters, and finally inhibit DA uptake.

Keywords

striatum; dopamine transporter; iontophoresis; rats

*Correspondence should be addressed to Eugene A. Kiyatkin at the above address. Fax: (410) 550-5553; tel.: (410) 550-5551; e-mail: ekiyatki@intra.nida.nih.gov

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Cocaine interaction with the dopamine (DA) transporter inhibits DA uptake, which is believed to cause increased extracellular DA levels following both passive cocaine administration (Di Chiara and Imperato, 1988) and cocaine self-administration (Pettit and Justice, 1989; Wise et al., 1995). This interaction is viewed as the primary mechanism for cocaine's reinforcing properties (Wise and Bozarth, 1987).

While *in vitro* data suggest that cocaine is an effective DA uptake inhibitor ($ED_{50}=0.3-0.8\text{ }\mu\text{M}$; Hennings et al., 1999; Rothman et al., 2001) with no effect on DA release (Heikkila et al., 1975), *in vivo* data are more controversial. Electrochemical evaluations confirm that cocaine decreases the clearance of locally applied DA and potentiates the increases in DA induced by K^+ and electrical stimulation of DA cell bodies or terminals (Cass et al., 1992; Zahniser et al., 1999; Reith et al., 2001). Although these data suggest that cocaine at relatively large systemic doses (10-20 mg/kg) inhibits DA uptake, the extent to which DA uptake is inhibited by intravenous (iv) cocaine at much lower, reinforcing doses (0.5-2.0 mg/kg) remains unknown.

Another point of controversy is how quickly cocaine influences DA uptake after iv administration. Although electrochemical data suggest that iv cocaine at low doses (0.3-1.0 mg/kg) rapidly (20-30 s) increases extracellular DA levels (Heien et al., 2005), assuming rapid DA uptake inhibition, previous attempts to measure its time-course produced conflicting results. Using the technique of [^3H]WIN displacement, a 3 min delay was shown between iv cocaine injection and reliable inhibition of striatal DA uptake even with large drug doses (Pogun et al., 1991; Stathis et al., 1995). These values coincide with ~ 2.5 min onset latencies in appearance of [^{11}C]-cocaine specific binding in human striatum after iv injection (Fowler et al., 1989). Using another *ex vivo* approach, reliable inhibition of DA uptake in the ventral striatum was found 5 min post-injection only at high cocaine doses (10 mg/kg, iv), while no changes were seen in dorsal striatum at this time point (Wang et al., 2007). Weak inhibition of DA uptake in the dorsal striatum, however, was found in this study at 15th post-injection min.

To evaluate the time-course of DA uptake inhibition, we combined fast-scan cyclic voltammetry with iontophoretic DA delivery (Kiyatkin et al., 2000). By constructing a combined microelectrode, which allowed repeated iontophoretic DA ejections and selective detection of iontophoretically released DA, it was possible to quantify the changes in DA clearance induced by a systemically administered drug in a fully awake, unrestrained rat. Using this approach, we showed that iv cocaine (1 mg/kg) reliably inhibits DA uptake, but with some latency ($\sim 1.5-2.0$ min), peak at 7-9 min and disappearance at ~ 20 min after a single injection. Later on, this finding was questioned by another study, in which cocaine-induced uptake was evaluated based on changes in amplitude and decay time of electrically stimulated DA release (Mateo et al., 2004). This study concluded that iv cocaine might inhibit DA uptake almost immediately (from 4 s, peaking at 20 s) after a single 1.5 mg/kg injection. While this short latency appears to be inconsistent with the total time necessary for iv injected cocaine to reach brain microvessels, cross the blood-brain barrier, passively diffuse within brain tissue, and finally interact with the DA transporters, these data also contrast to other electrochemical findings, suggesting a later appearance of DA after iv injection in drug-naïve rats (Heien et al., 2005; Stuber et al., 2005).

To further clarify this issue we decided to evaluate DA uptake induced by cocaine using a different, fully independent approach. This approach is based on evaluation of alterations in neuronal impulse activity to repeated iontophoretic DA applications before and after systemic cocaine administration. Due to cocaine's inhibition of DA uptake, the clearance of iontophoretic DA should be delayed and the resulting neuronal response amplified or prolonged. If cocaine reaches brain DA transporters with some latency, the amplification of neuronal response should increase with repeated DA applications, peaking some time after iv injection. For evaluating DA responses, we choose the striatum, which receives abundant DA

input and where DA consistently inhibits most spontaneously active and GLU-stimulated neurons (Kiyatkin and Rebec, 1996,1999a). These effects, moreover, are current (dose)-dependent, stable during repeated applications, and similar in both dorsal (caudate putamen) and ventral (nucleus accumbens) striatal compartments. In addition, the striatal neuronal population is highly homogenous, with ~95% cells representing medium spiny projection neurons (Parent and Hazrati, 1995), which *in vivo* have slow, sporadic impulse activity (Rebec, 2006;Wilson, 1993). In contrast to the awake, freely moving conditions of our previous electrochemical study, the present evaluations were performed in rats during urethane anesthesia. General anesthesia blocks cocaine-induced locomotor activation, thus eliminating both the recording artifacts and movement-related activations that occur in most striatal neurons in awake, freely moving conditions (Kiyatkin and Rebec, 1996;Rebec, 2006). Since anesthesia has a clear inhibiting effect on striatal neuronal activity, cells were tested during tonic stimulation by continuous, low-current GLU iontophoresis.

Hence, three tasks were formulated in the present study. First, to evaluate striatal neuronal responses to DA and determine their current (dose) dependence and stability following repeated applications before and after iv saline (control). Second, to evaluate changes in these responses after iv cocaine at 1 mg/kg. Third, to examine alterations in DA responses after intraperitoneal (ip) cocaine at 15 mg/kg. At this dose, cocaine presumably should have a stronger effect on DA uptake.

Materials and Methods

Animals and Surgery

Data were obtained from 16 male Long-Evans rats (400±50 g) supplied by Charles River Laboratories (Greensboro, NC). All animals were housed individually under standard laboratory conditions (12-hr light cycle beginning at 07:00) with free access to food and water. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publication 865-23) and were approved by the NIDA-IRP Animal Care and Use Committee. Maximal care was taken to minimize the number of animals used and their possible suffering.

Single-unit recording combined with iontophoresis was performed with the use of a microdriver (Rebec et al., 1993) that allowed manual fine movements of four-barrel glass microelectrodes within the recording track. The surgical procedures used have been described previously (Kiyatkin and Rebec, 1996). Briefly, under general anesthesia (Equithesin 3.3 ml/kg ip; dose of sodium pentobarbital 32.5 mg/kg and chloral hydrate 145 mg/kg), rats were implanted with a plastic, cylindrical hub, designed to mate with a microelectrode during recording. This hub was centered over a hole drilled above the striatum (1.2 mm anterior and 1.2 mm lateral to bregma). During the same session, most rats (n=12) were also implanted with a chronic jugular catheter, which was run subcutaneously to the head mount and secured with dental cement. After a 5-day period of recovery and one-day habituation to the experimental chamber, each rat underwent one recording session (6-8 hrs) during urethane anesthesia (1.25 mg/kg, ip).

Single-unit recording and iontophoresis

Four-barrel, microfilament-filled, glass pipettes (Omega Dot 50744, Stoelting, Wood Dale, IL), pulled and broken to a diameter of 4±1 µm, were used for single-unit recording and iontophoresis. The recording barrel contained 2% Pontamine Sky Blue (BDH Chemicals Ltd, Poole, England) in 3 M NaCl; the balance barrel contained 0.25 M NaCl. The remaining barrels were filled with 0.25 M solutions of l-GLU monosodium salt (GLU) and DA hydrochloride (DA) each dissolved in distilled water (pH 7.5 and 4.5, respectively). All drugs were obtained

from Sigma (St. Louis, MO). The resistance, measured at 100 Hz, was 3-5 M Ω for the recording barrel and 10-35 M Ω for the iontophoretic barrels. GLU was ejected by anionic (5-40 nA) and DA by cationic currents (5-60 nA), with a continuous retaining current of opposite polarity ($\pm$ 8 to 10 nA), using constant current generators (Ion 100 and Ion 100T, Dagan, Minneapolis, MN). Each multibarrel pipette was filled with fresh solutions less than one hour before use and fixed in a microdrive assembly that later was inserted into the skull-mounted hub. The electrode was then advanced 4.0 mm below the skull surface to the starting point of unit recording.

Neuronal discharge signals were sent to a head-mounted preamplifier (OPA 404KP, Burr Brown, Tucson, AZ) and then additionally amplified and filtered (band pass: 300-3,000 Hz) with a Neurolog System (Digitimer, Hertfordshire, UK). The filtered signal was then recorded using a Micro 1401 MK2 interface (Cambridge Electronic Design, Cambridge, UK). Spike activity was monitored with a digital oscilloscope and audio amplifier and analyzed using a Spike2 interface (Cambridge Electronic Design).

Experimental protocol

Search for units was typically performed during continuous low-current GLU applications (10-20 nA). After isolating a single-unit discharge (signal to noise ratio at least 3:1), GLU currents were adjusted to provide a relatively stable and moderate discharge rate. Units were then tested with repeated DA applications, which were always performed for 20 s with 120-s inter-application intervals. Our previous data and preliminary experiments of this study suggest that 2 min intervals between individual DA ejections are sufficient to eliminate a possible influence of previous ejections on subsequent ones. Individual units were exposed to several testing programs. First, to establish dose-response relationships, cells were tested with DA at increasing ejection currents (5-40 nA or more). Second, cells were tested with repeated DA applications at the same moderate currents (20-30 nA) before and after a single iv saline administration. These data provided an essential control for our cocaine data. Third, cells were tested with repeated DA applications at the same moderate currents before and after a single iv cocaine administration (1 mg/kg dissolved in 0.3 ml saline and injected over 20 s). This dose is optimal for self-administration (Wise and Bozarth, 1987) and it was used in our previous behavioral (Kiyatkin and Brown, 2004) and electrochemical studies (Kiyatkin et al., 2000). Fourth, cells were tested with repeated DA applications before and after ip injection of cocaine (15 mg/kg). Cocaine at this dose induces robust and prolonged increases in NAcc DA levels (Kalivas and Duffy, 1990) and is usually used in experiments with behavioral sensitization, a phenomenon dependent upon DA uptake inhibition (Kalivas and Stewart, 1991). In experiments with iv cocaine and saline, each cell was exposed to at least 14 DA applications with three before (-5, -3, and -1 min) and 11 after (+1, +3, and so on) cocaine or saline injection. In experiments with ip cocaine, units were exposed to at least 16 DA applications (3 before and 13 after the ip injection; to ~30 min post-injection). Rats typically received only one drug injection in a session. All high-dose cocaine injections were performed in different animals and only in two rats was cocaine injected at low dose twice, with inter-injection intervals exceeding 1 hour.

Histology

After the last recording session, animals were anesthetized and Pontamine sky blue was deposited by current ejection (-20 μ A for 20 min) at the last recording site. Rats were perfused with 10% formalin and brains were removed and stored in a formalin solution. Coronal 30 μ m tissue sections were prepared at -20°C using a microtome cryostat. The Paxinos and Watson atlas (Paxinos and Watson, 1998) served as the basis for histological analyses. Slices were analyzed to verify the location of dye spot and the recording track, which were used to reconstruct the location of recorded units within the striatum.

Data analysis

Impulse activity of individual striatal neurons was characterized by mean rate (X), standard deviation (SD) and coefficient of variation ($CV=SD/X \times 100$) calculated based on twenty 1-s values of discharge rate preceding DA applications. These values were grouped together and further analyzed by using standard statistical procedures (i.e., mean and modal group values, variability, distributions).

Individual responses to DA were analyzed based on statistical comparisons of equal durations of discharge rates (20 s) before and during DA applications and the time-course of activity changes (i.e., onset latency, peak effect, after-effect). For quantitative comparisons of DA responses, we used relative change in discharge rate (% of baseline activity that was calculated for 20 s and set as 100%). Standard statistical techniques (one-way ANOVA with repeated measures, correlation and regression analyses) were used for group analyses and verification of the effect of time (i.e., the effects of saline and cocaine in both doses) following repeated DA applications. Additional data on specifics of statistical analyses will be presented in Results.

Results

In contrast to what is seen in awake, unrestrained conditions, few spontaneously active units were encountered in the striatum during urethane anesthesia and, when found, their basal activity was typically low and sporadic. Cells recorded during continuous GLU ejection at low currents (5-15 nA) typically maintained relatively stable activity (range: 2-27 imp/s, mode: 6-8 imp/s) and showed decreases in discharge rate during DA applications. There were several units that appeared to be insensitive to DA; these cells were excluded from further testing. A total of 32 units recorded from 16 rats were accepted for analyses. All these units were histologically verified to be located within the striatum (Fig. 1); 23 were located in dorsal striatum (caudo-cutamen) and 9 units were located in ventral striatum (nucleus accumbens). Among these cells, 8 were exposed to DA with increasing currents (5-40 nA) to establish dose-response relationships. 15 and 14 units were exposed to repeated DA applications following iv saline and cocaine, respectively. Finally, 8 neurons were exposed to repeated DA applications before and after ip cocaine (15 mg/kg).

Responses of striatal neurons to iontophoretic DA

Iontophoretic DA decreased GLU-stimulated activity of most striatal neurons. As shown in Fig. 2A (15 units, mean for 210 DA applications), discharge rate slowly decreased after the DA ejection current was on, stabilized at about a half of the initial rate, and then slowly returned to baseline within 7-10 s. Along with a decrease in mean rate (X), DA also decreased SD of impulse activity (see B). Because of a weaker change in this latter parameter, CV , an index of relative irregularity of impulse activity, significantly increased during DA iontophoresis (see C).

As shown in Fig. 2D, individual responses to DA were highly variable, and a DA-induced decrease in activity was stronger in units with higher discharge rate and weaker in slow-firing units. In contrast to the tight correlation between DA-induced and basal activities ($r=0.92$, $p<0.01$), relative change in activity was independent of basal discharge rate, varying in most cases within 40-60% (Fig. 2E). Although there were much stronger and weaker responses, DA-induced activity was consistently lower than baseline (=100%) during virtually all DA tests (E).

As shown in Fig. 3, DA-induced decrease in activity was directly dependent upon ejection current (8 units, 32 DA tests). The decrease was about 62% at the smallest ejection current (5

nA) and became stronger to 53, 44 and 34% of baseline with 10, 20 and 40 nA currents, respectively. Importantly, impulse activity of striatal neurons, which was highly variable before DA application, remained highly variable at decreased levels during DA iontophoresis. As with awake conditions (Kiyatkin and Rebec, 1996), inhibition increased only slightly with large increases in ejection currents. Although onset latencies became slightly shorter as currents increased, after-effects were altered to a greater extent. After high-current DA application, return to the pre-injection baseline took about 10 s.

Since DA-induced decreases in discharge rate were dependent upon ejection currents, when testing with iv cocaine and saline ejections, currents were adjusted to induce a ~50% decrease in discharge rate. Following repeated, constant current DA applications (15 cells, n=210 tests; 3 before and 11 after iv saline injection), DA responses were surprisingly similar. All three analyzed parameters (basal discharge rate, discharge rate during DA iontophoresis and percent change) remained stable over ~30 min of repeated testing. Fig. 4 shows rate histogram of striatal activity during repeated DA applications before and after iv saline. Fig. 5 shows averaged DA responses for the first four and the last four tests of this series (A) as well as the mean values of DA-induced inhibition (B) and discharge rate (C) during this testing. As can be seen, in each case DA induced a significant decrease in discharge rate (~3 s latency), which restored within 3-6 s after the ejection current was switched off. Although percent change was highly stable and not significantly different, there was a tendency for a slightly weaker response to DA with repeated applications (see mean response values above the graphs and mean values in Fig. 5B). Importantly, repeated DA applications did not affect discharge rate of striatal neurons, which remained highly stable (~8 imp/s) within the 30 min of repeated testing (see Fig. 5C).

Responses of striatal neurons to iontophoretic DA after iv cocaine administration

As can be seen in Fig. 6, iv cocaine induced changes in DA responses, which were seen only with statistical analyses and with respect to relative magnitude of DA-induced inhibition (14 cells, 196 DA tests). This parameter slowly increased (i.e., the response became stronger) from the third post-cocaine test (5 min) and became significantly larger during the fifth test (9 min). At 13 and 15 post-cocaine minute, the difference was maximal but then slowly decreased toward the pre-cocaine baseline. In contrast to expectations, there were no evident changes in onset latencies and after-effects; both remained similar before and after cocaine injection. As an average, the DA response after cocaine injection was not more prolonged at any time point and in some cases discharge rate restored even quicker than it did before drug injection (see after-effects at 1, 3, 7, 15 and 19 mins). Contrary to expectations, basal discharge rate did not decrease after iv cocaine injection (Fig. 6C) and tended to be higher; all post-cocaine values were larger than those in baseline. This effect was not seen after saline injection (see Fig. 5C).

Responses of striatal neurons to iontophoretic DA after ip cocaine administration

As can be seen in Fig. 7, responses to DA also remained highly stable after ip cocaine administration (15 mg/kg, 8 cells, 120 DA applications). Like iv administration, relative amplitude of the DA-induced inhibition decreased across repeated DA applications (see Fig. 7A). This parameter decreased significantly (i.e., inhibition became stronger) at 7-9 min and remained lower than in control for subsequent tests (B). In this case, there were minimal changes in DA response after-effects, which were longer at 18 and 22 min after cocaine administration. Similar to iv administration, discharge rate also slightly increased after ip injection, but this change did not reach statistical significance (C).

4. Discussion

We found that iv cocaine at a low, reinforcing dose increases the magnitude of the DA-induced inhibition of striatal neurons. However, this effect is relatively weak and delayed from the time of injection. A similar effect was also seen with ip cocaine at a higher dose, but it appeared at later times and was more delayed. If these changes occur because of cocaine-induced DA uptake inhibition, our present results generally agree with previous data (Pogun et al., 1991; Stathis et al., 1995), suggesting a significant time delay between iv cocaine administration and inhibition of DA uptake. Our present data also fit a time-course of cocaine-induced DA uptake inhibition suggested by previous electrochemical evaluations in awake rats (Kiyatkin et al., 2000). However, both our previous and present results are inconsistent with electrochemical data based on cocaine-induced alterations in electrically evoked DA release (Mateo et al., 2004), which suggest that iv cocaine might inhibit DA uptake virtually immediately after iv injection.

DA responses of striatal neurons and their mechanisms

Although an awake, freely moving animal best mimics natural conditions, iv cocaine induces powerful motor activation, thus making single-unit recording with fine glass electrodes virtually impossible. In addition, most striatal neurons show movement-related phasic excitations (Kiyatkin and Rebec, 1996; Rebec, 2006), which presumably result from phasic GLU release from cortico-striatal and thalamo-striatal afferents (Parent et al., 1995; Rebec, 2006; Wilson, 1993). Since the iontophoretic response is determined as the change in activity with respect to baseline, these naturally occurring excitations make it impossible accurate evaluation of the iontophoretic response. Therefore, general anesthesia was essential for accurate evaluation of neuronal responses to repeated DA applications—an approach used in this study to evaluate the effects of cocaine on DA uptake. In contrast to the evaluation of DA clearance after electrical stimulation-induced endogenous DA release, this approach allows DA uptake to be studied independently of possible effects of cocaine on DA release. Since DA is applied by iontophoresis in very small amounts and neuronal responses to DA remain highly stable following repeated tests in drug-free conditions, this approach evades the problem of uptake site and receptor saturation. This is an important consideration in experiments employing large-dose DA applications or robust, non-physiological DA release induced by electrical stimulation of DA cell bodies or their axons. Furthermore, this procedure results in dramatic alterations in DA cell activity and responsiveness, thus affecting evoked DA release - the parameter which is used to evaluate the effect of cocaine on DA uptake.

Consistent with our previous studies in awake rats (Kiyatkin and Rebec, 1996; Kiyatkin, 2002), iontophoretic DA decreased GLU-stimulated activity of most striatal neurons in anesthetized conditions. This decrease is often viewed as neuronal inhibition, but it has several important differences from “true” inhibition that is induced by GABA. While the DA response becomes stronger with increasing currents (dose dependence), it rarely progresses to full inactivity despite large increases in currents, with discharge rate highly irregular during DA application (see Fig. 2 and 3). In contrast, the GABA-induced inhibition is associated with regularization of impulse flow and rapidly progresses into inactivity with slight increases in ejection currents (Kiyatkin and Rebec, 1999b). Although the DA-induced decrease occurs with some definite latency (3–6 s), the effect is transient and discharge rate rapidly returns to the pre-application levels after the ejection current is switched off. While relatively short onset latencies might result from rapid interaction of iontophoretically released DA with DA receptors, rapid diffusion of DA from these receptors appears to be the primary factor determining relatively short offset latencies. Therefore, although re-uptake is the primary way to remove endogenously released DA from its receptor sites, diffusion appears to be the main factor for clearance of iontophoretic DA.

Neuronal responses induced by iontophoretic DA were highly stable following repeated applications (see Fig. 5), arguing against some kind of response saturation or receptor desensitization. Although this stability contrasts with the rapid DA response desensitization found *in vitro* with local high-concentration DA exposure (Arbilla et al., 1985; Hanbauer and Sanna, 1986), DA responses had a slight tendency to become weaker with repeated applications, possibly suggesting a decreased DA receptor responsiveness. This change, however, was not accompanied by evident alterations in either the onset or offset latencies of DA responses, nor in impulse activity.

Slow and delayed changes in DA uptake induced by iv cocaine

In contrast to control conditions, the relative magnitude of DA responses increased following iv cocaine. This effect appeared at 3-5 min, became significant at 9 min, peaked at 13-15 min, and slowly returned toward baseline at the later times. These changes in magnitude of DA-induced inhibition were not associated with evident changes in either onset or offset latencies, though basal activity of striatal neurons tended to increase. Despite a higher dose (15 mg/kg), a similar pattern also occurred after ip cocaine. DA response magnitude increased in these conditions from ~8 min after drug administration, with a maximal effect at final testing points (20-24 min). Similar, there was a clear trend to higher basal activity with no significant changes in offset and offset latencies of DA responses. However, in this case, a more prolonged return to baseline was seen at 18-22 post-cocaine min.

Although it may seem surprising that the inhibiting effect of DA on striatal neurons was not prolonged after iv cocaine, a larger response magnitude suggests a stronger action of DA on relevant receptors because of DA uptake inhibition. The lack of response prolongation may be related to the principal differences between natural DA release and its iontophoretic application. Following DA cell activation, DA is rapidly released at high concentrations within the synaptic cleft (acting on low-affinity postsynaptic DA receptors), later diffusing in the extrasynaptic space and affecting (at lower concentrations) the high-affinity presynaptic receptors located on striatal afferents. In this case, DA uptake effectively regulates local DA concentrations, determining the strength and duration of its action on relevant receptor pools. In contrast, iontophoretic DA is released from some point outside of the cell, affecting the receptors within the sphere of its passive diffusion. While cocaine's interaction with the DA transporter effectively inhibits uptake of naturally released DA, because of different release sites it might affect only a minor portion of exogenously applied DA near the DA receptors. Therefore, the effect of cocaine in our model might be weak because most applied DA is spontaneously diffusing from the electrode tip, resulting in a rapid fall of its local concentrations after the ejection current is off. Our previous electrochemical measurements of DA following its iontophoretic ejection (Kiyatkin et al., 2000) support this view, suggesting quite a rapid diffusion of DA from the release site and the importance of a continuous ejection to maintain stable DA concentration.

However, weak and delayed effects of cocaine on DA responses might be related to its relatively late appearance in the brain and low concentrations at the DA transporter sites. Previous attempts to measure brain cocaine levels after iv administration produced quite contrasting results. For example, [^3H] cocaine has been detected in striatal tissue one min after iv injection (10 mg/kg), peaking at ~1.2 μM levels at 5th min (Wang et al., 2007). If these estimates are accurate, brain cocaine levels after 1 mg/kg iv injection should be lower than ED_{50} (0.3-0.8 μM ; Hennings et al., 1999; Rothman et al., 2001), only bordering the range for minimal affecting DA uptake. Similarly low values of extracellular cocaine (peak of ~0.5 μM at 10th min) were found in striatal tissue by microdialysis after 1.5 mg/kg iv injection (Hurd et al., 1988). By measuring the displacement of [^3H] WIN 35,428 from striatal transporter sites *in vivo*, the first time point of significant DA uptake was 3rd min after 7 mg/kg iv injection in

mice (Stathis et al., 1994). Maximal effect on DA uptake in this case occurred on 15th min post-injection. Additional support for this slow dynamics comes from human studies with [¹¹C] cocaine, which revealed specific binding (i.e. striatal radioactivity greater than cerebellar radioactivity) only at about 2.5 min after iv injection (Fowler et al., 1989). However, later studies by the same group showed more rapid appearance (30 s) and much higher brain cocaine levels (up to 32 μ M) using the same PET technology with [¹¹C] cocaine (Fowler et al., 1998). Although these values were interpreted as striatal cocaine levels, it is unclear whether they reflect cocaine levels in head microvessels (i.e. outside of brain tissue) or in extracellular space (i.e. inside of brain tissue). Using *ex vivo* binding of [³H] DA, no changes in DA uptake in the dorsal striatum were found 5 min after iv cocaine injection with 1-10 mg/kg doses, though a weak effect was found in the ventral striatum (Wang et al, 2007). The ED₅₀ value of DA uptake (22.3 μ M) in this *ex vivo* study was much higher than those reported *in vitro* (<1 μ M; Hennings et al., 1999; Rothman et al., 2001). Finally, relatively slow DA uptake inhibition is suggested by electrophysiological studies (Peterson et al., 1990), which evaluated the effects of iv cocaine on inhibitions of prefrontal cortical neurons induced by electrical stimulation of the VTA in anesthetized rats. Although this approach allowed evaluation of neuronal responses to endogenously released DA, response potentiation was absent at 2 min but was evident at 10 min after an iv cocaine injection at 2 mg/kg dose.

Although it is quite difficult to measure how quickly cocaine reached its brain substrates and what its effective brain concentrations are, from a pharmacodynamic point of view a measurable delay should exist between cocaine infusion into a peripheral vein and its effects mediated via interactions with the DA transporters. Although cocaine is quickly transported to the brain after iv administration (in humans it takes about 18 s after injection via an ulnar vein), much more time is necessary to cross cellular membranes of the brain-blood barrier, passively diffuse through brain tissue, interact with monoamine transporters, and affect uptake.

In addition to the slow and weak increases in brain cocaine levels, which may determine a relatively small and delayed effect on DA responses, two other procedural factors could affect their quantitative parameters. The first factor is general anesthesia, which is known to affect circulation, decrease brain temperature and slow transmitter uptake (see Kiyatkin, 2005 for review). While in awake, freely moving conditions the decrease in DA clearance was greatest at 7-9 min after cocaine injection (Kiyatkin et al., 2000), maximal changes in DA responses were seen at 13-15 min after the same dose injection in urethane-anesthetized conditions. The second factor could be a continuous low-current GLU stimulation, which was used to increase basal discharge rate and make possible accurate evaluation of DA responses. Although this procedure affects the activity state and neuronal responsiveness, possibly triggering some kind of compensatory plastic changes, it is unlikely that it can seriously affect the time-course of DA responses and their changes induced by cocaine. Our evaluation of DA responses on striatal neurons made in awake, unrestrained rats revealed that they are virtually identical in spontaneously active and GLU-stimulated conditions. With small GLU currents used in our study, striatal neurons, moreover, maintained a relatively low and stable discharge rate and showed similar responses to repeated DA applications within a relatively long time intervals (20-30 min). Finally, our cocaine data were compared with similar control data, which both were obtained during identical regimens of continuous GLU stimulation.

How could rapid, transient physiological, behavioral and psychoemotional effects of iv cocaine be explained in the light of possibility of slow and prolonged DA uptake inhibition?

Although there is a tendency to explain physiological, behavioral and psychoemotional effects of cocaine via the central DA mechanism, many important effects of this drug are resistant to DA receptor blockade and they occur within seconds after iv administration. Such rapid and brief dynamics is typical of cocaine-induced EEG desynchronization (Lukas et al., 1990;

Matsuzaki et al., 1978), arterial blood pressure increase (Poon and van den Buuse, 1998) and acute skin hypothermia that reflects peripheral vasoconstriction (Kiyatkin and Brown, 2005); all these effects remain generally intact during DA receptor blockade. Acute euphoria induced by iv cocaine in humans also has second-scale latencies (Zernig et al., 2003), remaining generally intact during DA receptor blockade (Gawin, 1986). The rapid appearance and relatively short duration of all these effects are difficult to reconcile with the much slower appearance and longer duration of brain monoamine uptake inhibition suggested by literature and this study.

Although discussion of the possible mechanisms underlying rapid, transient effects of iv cocaine exceeds the limits of this study, it is known that, in addition to brain monoamine transporters, cocaine interacts with different neural substrates both in the brain and periphery. Our recent thermorecording (Brown and Kiyatkin, 2006) and single-cell data (Kiyatkin and Brown, 2007) suggest that iv cocaine may induce central excitatory effects via its direct action on peripheral neural elements. While the nature of these substrates needs to be clarified, cocaine has a high affinity to K⁺ and Na⁺ channels abundantly expressed on terminals of sensory nerves (Lee et al., 2003) that densely innervate peripheral vessels (Goder et al., 1993; Michaelis et al., 2005). Via this direct interaction and involvement of visceral somato-sensory pathways, iv cocaine might trigger an excitatory drive to the CNS and neural activation, thus playing a role in its rapid, transient central effects. For example, iv cocaine induced rapid but brief (5-20 s) excitation of most striatal neurons in awake rats during full DA receptor blockade; this effect was virtually fully blocked during urethane anesthesia. Despite having longer onset latencies, these effects were similar to those induced by somato-sensory stimuli such as tail-touch and tail-pinch. Short response latencies of these neuronal effects point to its peripheral trigger and rapid neural transmission, a mechanism supported by data with cocaine methiodide, cocaine's derivative that cannot cross the blood-brain barrier (Shriver and Long, 1971). This drug mimics such important, centrally mediated effects of cocaine as acute increase in arterial blood pressure (Dickerson et al., 1999), brain hyperthermia and vasoconstriction (Brown and Kiyatkin, 2006) as well as phasic excitations of striatal neurons (Kiyatkin and Brown, 2007). Similar to usual cocaine, neuronal effects of cocaine methiodide were fully blocked during general anesthesia. This peripheral trigger may also be activated by procaine, a local anesthetic structurally similar to cocaine but with minimal effects on DA uptake (Ritz et al., 1987). Procaine with rapid iv administration is able to mimic cardio-vascular effects of cocaine (Pitts et al., 1987) and induce powerful limbic activation as well as sensory and emotional effects in humans (Servan-Shreiber et al., 1998). Despite the lack of primary reinforcing properties, procaine also mimics cocaine-induced euphoria in human cocaine abusers (Adinoff et al., 1998; Fischman and Shuster, 1983) and maintains drug-taking behavior in cocaine-trained animals (Johanson, 1980; Kiyatkin and Stein, 1995).

Via this peripherally triggered, DA transporter-independent mechanism, iv cocaine, like other salient somato-sensory stimuli (Horvitz et al., 1997; Kiyatkin, 1988) may rapidly affect DA neurons, inducing phasic DA release. Although cocaine has no effect on DA release *in vitro* (Heikkila et al., 1975), *in vivo* studies are consistent with the idea that cocaine, in addition to uptake inhibition, also increases DA release (Stamford et al., 1989; Venton et al., 2006). This rapid, peripherally triggered action of cocaine on DA release will be combined with its slower and more prolonged action on uptake, resulting in DA accumulation. This rapid action on DA release may explain unusually rapid detection of extracellular DA (20-30 s) revealed by fast-scan voltammetry in awake, drug-naïve rats following iv cocaine (Heien et al., 2005; Stuber et al., 2005). While this hypothesis could be directly verified by recording DA cell activity following iv cocaine administration in awake, freely moving animals, this task is enormously difficult and experimental data are still lacking.

Hence, in addition to a direct interaction with centrally located monoamine transporters, which appears to be relatively slow and prolonged, there are other rapid mechanisms, which can be activated by cocaine. Because of differing time-course, these different pharmacological effects interact with each other and behavioral variables, jointly contributing to the development of drug-taking behavior. Therefore, although cocaine's interaction with the DA transporters appears to be essential in mediating its psychomotor stimulant and reinforcing properties, many important behavioral, physiological and psychoemotional effects of this drug cannot be fully explained via DA uptake inhibition.

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, NIDA. We wish to thank Drs. Roy A. Wise, Barry Hoffer and Carlos Mejias-Aponte for their valuable comments on the matter of this manuscript.

References

- Adinoff B, Brady K, Sonne S, Mirabella RF, Kellner CH. Cocaine-like effects of intravenous procaine in cocaine addicts. *Addiction Biol* 1998;3:189–196.
- Arbilla S, Nowak JZ, Langer SZ. Rapid desensitization of presynaptic dopamine autoreceptors during exposure to exogenous dopamine. *Brain Res* 1985;337:11–17. [PubMed: 4005600]
- Brown PL, Kiyatkin EA. The role of peripheral Na⁺ channels in triggering the central excitatory effects of intravenous cocaine. *Eur J Neurosci* 2006;24:1182–1192. [PubMed: 16930444]
- Cass WA, Gerhardt GA, Mayfield RD, Curella P, Zahniser NR. Differences in dopamine clearance and diffusion in rat striatum and nucleus accumbens following systemic cocaine administration. *J Neurochem* 1992;59:259–266. [PubMed: 1613502]
- Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 1988;85:5274–5278. [PubMed: 2899326]
- Dickerson LW, Rodak DJ, Kuhn FE, Wahlstrom SK, Tessel RE, Visner MS, Schaer GL, Gillis RA. Cocaine-induced cardiovascular effects: Lack of evidence for a central nervous system site of action based on hemodynamic studies with cocaine methiodide. *J Cardiovasc Pharmacol* 1999;33:36–42. [PubMed: 9890394]
- Fischman MW, Schuster CR. A comparison of the subjective and cardiovascular effects of cocaine and procaine in humans. *Pharmacol Biochem Behav* 1983;18:711–716. [PubMed: 6856646]
- Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schyler DJ, MacGregor RR, Hitzemann R, Logan J, Bindriem B, Gattley SJ, Christman D. Mapping cocaine binding sites in human and baboon brain in vivo. *Synapse* 1989;4:371–377. [PubMed: 2557686]
- Fowler JS, Volkow ND, Logan J, Gattley SJ, Pappas N, King P, Ding Y-S, Wang G-J. Measuring dopamine transporter occupancy by cocaine in vitro: radiotracer considerations. *Synapse* 1998;28:111–116. [PubMed: 9450511]
- Gawin F. Neuroleptic reduction of cocaine-induced paranoia but not euphoria? *Psychopharmacology* 1986;90:142–143. [PubMed: 2876452]
- Goder R, Habler HJ, Janig W, Michaelis M. Receptor properties of afferent nerve fibers associated with the rat saphenous vein. *Neurosci Lett* 1993;164:175–178. [PubMed: 8152597]
- Hanbauer I, Sanna E. Molecular mechanisms involved in the desensitization of dopamine receptors in slices of corpus striatum. *Prog Brain Res* 1986;69:161–168. [PubMed: 2965820]
- Heien MLAV, Khan AS, Ariansen JL, Cheer JF, Phillips PEM, Wassum KM, Wightman RM. Real-time measurements of dopamine fluctuations after cocaine in the brain of behaving rats. *PNAS* 2005;102:10023–10028. [PubMed: 16006505]
- Heikkila RE, Orlanski H, Cohen G. Studies on the distinction between uptake inhibition and release of [³H]-dopamine in rat brain tissue slices. *Biochem Pharmacol* 1975;24:847–852. [PubMed: 1125084]
- Hennings EC, Kiss JP, De Oliveira K, Toth PT, Vizi ES. Nicotinic acetylcholine receptor antagonistic activity of monoamine uptake blockers in rat hippocampal slices. *J Neurochem* 1999;73:1043–50. [PubMed: 10461893]

- Horvitz JC, Stewart T, Jacobs BL. Burst activity of ventral tegmental dopamine neurons is elicited by sensory stimuli in the awake cat. *Brain Res* 1997;759:251–258. [PubMed: 9221945]
- Hurd YL, Kehr J, Ungerstedt U. In vivo microdialysis as a technique to monitor drug transport: correlation of extracellular cocaine levels and dopamine overflow in the rat brain. *J Neurochem* 1988;51:1314–1316. [PubMed: 3418351]
- Johanson CE. The reinforcing properties of procaine, chlorprocaine and proparacaine in rhesus monkeys. *Psychopharmacology* 1980;67:189–194. [PubMed: 6768094]
- Kalivas PW, Duffy P. Effects of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse* 1990;5:48–58. [PubMed: 2300906]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Rev* 1991;16:223–244. [PubMed: 1665095]
- Kiyatkin EA. Functional properties of presumed dopamine-containing and other ventral tegmental area neurons in conscious rats. *Int J Neurosci* 1988;42:21–43. [PubMed: 3209370]
- Kiyatkin EA. Dopamine in the nucleus accumbens: cellular actions, drug- and behavior-associated fluctuations, and a possible role in an organism's adaptive activity. *Behav Brain Res* 2002;137:27–46. [PubMed: 12445714]
- Kiyatkin EA. Brain hyperthermia as physiological and pathological phenomena. *Brain Res Rev* 2005;50:27–56. [PubMed: 15890410]
- Kiyatkin EA, Brown PL. Brain temperature fluctuations during repeated passive vs. active cocaine administration: Clues for understanding the pharmacological determination of drug-taking behavior. *Brain Res* 2004;1005:101–116. [PubMed: 15044070]
- Kiyatkin EA, Brown PL. Dopamine-dependent and dopamine-independent actions of cocaine as revealed by brain thermorecording in freely moving rats. *Eur J Neurosci* 2005;22:9430–938.
- Kiyatkin EA, Brown PL. I.v. cocaine induces rapid, transient excitation of striatal neurons via its action on peripheral neural elements: Single-cell, iontophoretic study in awake and anesthetized rats. *Neuroscience* 2007;148:978–995. [PubMed: 17706878]
- Kiyatkin EA, Rebec GV. Dopaminergic modulation of glutamate-induced excitations of neurons in the neostriatum and nucleus accumbens of awake, unrestrained rats. *J Neurophysiol* 1996;75:142–153. [PubMed: 8822548]
- Kiyatkin EA, Rebec GV. Striatal neuronal activity and responsiveness to dopamine and glutamate after selective blockade of D1 and D2 dopamine receptors in freely moving rats. *J Neurosci* 1999a;19:3594–3609. [PubMed: 10212318]
- Kiyatkin EA, Rebec GV. Modulation of striatal neuronal activity by glutamate and GABA: iontophoresis in awake, unrestrained rats. *Brain Res* 1999b;822:88–106. [PubMed: 10082887]
- Kiyatkin EA, Kiyatkin DE, Rebec GV. Phasic inhibition of dopamine uptake in nucleus accumbens induced by intravenous cocaine in freely behaving rats. *Neuroscience* 2000;98:729–741. [PubMed: 10891616]
- Kiyatkin EA, Stein EA. Fluctuations in nucleus accumbens dopamine during cocaine self-administration behavior: an in vivo electrochemical study. *Neuroscience* 1995;64:599–617. [PubMed: 7715774]
- Lee Y, Lee C-H, Oh U. Painful channels in sensory neurons. *Mol Cells* 2005;20:315–324. [PubMed: 16404144]
- Lukas SE, Mendelson JH, Amass L, Benedikt R. Behavioral and EEG studies of acute cocaine administration: comparisons with morphine, amphetamine, pentobarbital, nicotine, ethanol and marijuana. *NIDA Res Monogr* 1990;95:146–152. [PubMed: 2561820]
- Mateo Y, Budygin EA, Morgan D, Roberts DCS, Jones SR. Fast onset of dopamine uptake inhibition by intravenous cocaine. *Eur J Neurosci* 2004;20:2838–2842. [PubMed: 15548229]
- Matsuzaki M, Spingler PJ, Whitlock EG, Misra AL, Mule SJ. Comparative effects of cocaine and pseudococaine on EEG activities, cardiorespiratory functions, and self-administration behavior in the rhesus monkey. *Psychopharmacology (Berlin)* 1978;57:12–20.
- Michaelis M, Goder R, Habler HJ, Janig W. Properties of afferent nerve fibers supplying the saphenous vein in the cat. *J Physiol (London)* 1994;474:233–243. [PubMed: 8006810]
- Parent A, Hazrati LN. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res Rev* 1995;20:91–127. [PubMed: 7711769]

- Paxinos, J.; Watson, C. The rat brain in stereotaxic coordinates. Academic Press; San Diego: 1998.
- Peterson SL, Olsta SA, Matthews RT. Cocaine enhances medial prefrontal cortex neuron response to ventral tegmental area activation. *Brain Res Bul* 1990;24:267–273.
- Pettit HO, Justice JB. Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. *Pharmacol Biochem Behav* 1989;34:899–904. [PubMed: 2623043]
- Pitts DK, Udom CE, Marwah J. Cardiovascular effects of cocaine in anesthetized and conscious rats. *Life Sci* 1987;40:1099–1111. [PubMed: 3821374]
- Pogun S, Scheffel U, Kuhar M. Cocaine displaces [3H]WIN 35,428 binding to dopamine uptake sites in vivo more rapidly than mazindol or GBR 12909. *Eur J Pharmacol* 1991;198:203–205. [PubMed: 1864307]
- Poon J, van den Buuse M. Autonomic mechanisms in the acute cardiovascular effects of cocaine in conscious rats. *Eur J Pharmacol* 1986;363:147–152. [PubMed: 9881582]
- Rebec GV. Behavioral electrophysiology of psychostimulants. *Neuropsychopharmacology* 2006;31:2341–2348. [PubMed: 16855534]
- Rebec GV, Langley PE, Pierce RC, Wang Z, Heidenreich BA. A simple micromanipulator for multiple uses in freely moving rats: electrophysiology, voltammetry, and simultaneous intracerebral infusions. *J Neurosci Meth* 1993;47:53–59.
- Reith ME, Kuhar MJ, Carroll FI, Garris PA. Preferential increases in nucleus accumbens dopamine after systemic cocaine administration are caused by unique characteristics of dopamine transmission. *J Neurosci* 2001;21:6338–6347. [PubMed: 11487657]
- Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors of dopamine transporters are related to self-administration of cocaine. *Science* 1987;237:1219–1223. [PubMed: 2820058]
- Rothman RB, Bauman MH, Dersch CM, Romero DV, Rice KC, Carrol FI, Partilla HS. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 2001;39:32–41. [PubMed: 11071707]
- Stamford JA, Kruk ZL, Millar J. Dissociation of the actions of uptake blockers upon dopamine overflow and uptake in the rat nucleus accumbens: in vivo voltammetric data. *Neuropharmacology* 1989;28:1383–1388. [PubMed: 2533329]
- Shriver DA, Long IP. A pharmacological comparison of some quaternary derivatives of cocaine. *Arch Int Pharmacodyn* 1971;189:198–208. [PubMed: 5130150]
- Stathis M, Scheffel U, Lever SZ, Boja JW, Carroll FI, Kuhar MJ. Rate of binding of various inhibitors at the dopamine transporter. *Psychopharmacology* 1995;119:376–384. [PubMed: 7480516]
- Stuber GD, Roitman MF, Phillips PEM, Carelli RM, Wightman RM. Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. *Neuropsychopharmacology* 2005;30:853–863. [PubMed: 15549053]
- Venton BJ, Seipel AI, Phillips PE, Wetsel WC, Gitler D, Greengard P, Augustine GJ, Wightman RM. Cocaine increases dopamine release by mobilization of a synapsin-dependent pool. *J Neurosci* 2006;22:3206–3209. [PubMed: 16554471]
- Wang Z, Ordway GA, Woolverton W. Effects of cocaine on monoamine uptake as measured ex vivo. *Neurosci Lett* 2007;413:191–195. [PubMed: 17169488]
- Wilson CJ. The generation of natural firing patterns in neostriatal neurons. *Prog Brain Res* 1993;99:277–297. [PubMed: 8108553]
- Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev* 1987;94:469–492. [PubMed: 3317472]
- Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB. Fluctuations in nucleus accumbens dopamine concentrations during intravenous cocaine self-administration in rats. *Psychopharmacology* 1995;120:10–20. [PubMed: 7480530]
- Zahniser NR, Larson GA, Gerhardt GA. In vivo dopamine clearance in rat striatum: regulation by extracellular dopamine concentrations and dopamine transporter inhibitors. *J Pharmacol Exp Ther* 1999;289:266–277. [PubMed: 10087014]
- Zernig G, Giacomuzzi S, Riemer Y, Wakonigg G, Sturm K, Saria A. Intravenous drug injection habits: Drug users' self-reports versus researchers' perception. *Pharmacology* 2003;68:49–56. [PubMed: 12660479]

Fig. 1. Brain microphotographs showing Pontamine Sky Blue deposit within the recording track in the caudate putamen. Top graph shows a brain slice with a square, which is magnified in bottom graph.

Fig. 2.

Changes in impulse activity of striatal neurons induced by iontophoretic DA in urethane-anesthetized rats. A, B and C show averaged changes in mean rate (X, imp/s), standard deviation (SD, imp/s) and coefficient of variation of impulse activity (CV, %; 15 units, 210 tests). Filled symbols show values significantly different ($p < 0.05$) from the pre-application baseline. D and E show relationships between basal impulse activity and its changes (D absolute and E relative) induced by DA. Vertical hatched lines on A, B and C show duration of DA iontophoresis and horizontal lines show basal values.

Fig. 3. Mean changes in discharge rate of striatal neurons induced by DA ejected at different currents (5, 10, 20 and 40 nA). Each graph shows relative changes in activity rate (in percents with respect to baseline =100%) with F values evaluated with one-way ANOVA with repeated measures and mean values of DA-induced inhibition. Filled symbols represent values significantly lower than baseline. Data were obtained in 8 neurons, repeatedly tested with DA at increasing currents. Vertical hatched lines show timing of DA application and horizontal hatched line shows basal values (=100%).

Fig. 4. Rate histogram of individual striatal neurons repeatedly tested with iontophoretic DA (black squares above the graph) before and after iv saline administration (black arrow). Top graphs show individual responses to DA at higher temporal resolution. Black lines above discharge trains show duration of DA application.

Fig. 5.

Mean changes in discharge rate of striatal neurons induced by DA ejected at the same currents before and after iv saline injection. A. Each graph shows changes in activity rate in percents with respect to baseline (=100%) at different times before (-5, -3 and -1 min) and after (+15, 17, 19, 21 min) iv saline injection. Filled symbols represent values significantly lower than baseline. Mean magnitude of DA response ($\pm$ standard errors) is also shown in each graph. Vertical hatched lines show timing of DA application and horizontal hatched line shows basal values (=100%). Data were obtained in 15 neurons, repeatedly tested with DA ($n=210$ tests). B. Mean values of DA-induced inhibition averaged for all tested neurons. C. Mean values of discharge rate averaged for all tested neurons.

Fig. 6. Mean changes in discharge rate of striatal neurons induced by DA ejected at the same currents before and after iv cocaine injection (1 mg/kg). **A.** Each graph shows changes in activity rate in percents with respect to baseline (100%) for basal pre-cocaine conditions (averaged for -5, -3 and -1 min and shown as small circles) and different times after cocaine injection (shown as large circles). Filled symbols represent values significantly lower than baseline. Data were obtained in 14 neurons, repeatedly tested with DA ($n=196$ tests). Vertical hatched lines show timing of DA application and horizontal hatched line shows basal values ($=100\%$). **B** shows mean values of DA-induced inhibition ($\pm$ sem) at each testing point (asterisks indicate values significantly lower than baseline with $p<0.05$ (*) and $p<0.01$ (**)). **C** shows mean discharge rate ($\pm$ sem) averaged for each time point

Fig. 7.

Mean changes in discharge rate of striatal neurons induced by DA ejected at the same currents before and after ip cocaine injection (15 mg/kg). A. Each graph shows changes in activity rate in percents with respect to baseline (100%) for basal pre-cocaine conditions (averaged for -5, -3 and -1 min and shown as small diamonds) and different times after cocaine injection (shown as large circles). Data were obtained in 8 neurons, repeatedly tested with DA. Each post-cocaine point represents averaged results of two tests (2 min= sum of tests at +1 and +3 min, and so on). Vertical hatched lines show timing of DA application and horizontal hatched line shows basal values (=100%). B shows mean ($\pm$ sem) values of DA-induced inhibition at different time points (asterisks show values significantly lower than baseline with $p < 0.05$). C shows mean values of impulse activity assessed at each time point.