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Dopamine Uptake Changes Associated with Cocaine Self-Administration

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Abstract

The present study was designed to reveal the relationship between cocaine-induced dopamine uptake changes and patterns of cocaine self-administration observed under a fixed ratio schedule. Cocaine was intravenously infused into anesthetized rats, according to inter-infusion intervals obtained from self-administering animals, and dopamine uptake changes (apparent K_m) were assessed in the nucleus accumbens using voltammetry. The data demonstrate that cocaine-induced dopamine transporter (DAT) inhibition accounts for the accumbal dopamine fluctuations, which are associated with the cyclic regularity of cocaine intake observed during self-administration. Specifically, the inter-infusion intervals that are maintained during cocaine self-administration correlate with the maintenance of a rapidly changing level of dopamine uptake inhibition, which appears to be tightly regulated. Furthermore, this maintained level of dopamine uptake inhibition was found to shift upward using intervals from animals that had shown an escalation in the rate of cocaine self-administration. Although no significant change in the apparent K_m was revealed in animals that exhibited an escalation in the rate of cocaine intake, an increased dopamine uptake rate was found suggesting an up-regulation of DAT number in response to a history of high cocaine intake. This is the first demonstration of the tight correlation that exists between the level of dopamine uptake inhibition and rates of cocaine self-administration. Moreover, a new mathematical model was created that quantitatively describes the changes in cocaine-induced dopamine uptake and correctly predicts the level of dopamine uptake inhibition. This model permits a computational interpretation of cocaine-induced dopamine uptake changes during cocaine self-administration.

Keywords

dopamine transporter; addiction; psychostimulants; pharmacokinetics; Michaelis-Menten kinetics; tolerance

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DISCLOSURE/CONFLICT OF INTEREST

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INTRODUCTION

It is commonly accepted that dopamine neurotransmission is essential for the stimulating, reinforcing, and addictive effects of cocaine and other abused drugs (Koob and Bloom, 1988; Volkow, *et al* 2004). Acutely administered cocaine enhances extracellular dopamine concentrations in specific brain regions, including the caudate putamen and nucleus accumbens (Di Chiara and Imperato, 1988). The magnitudes of cocaine-induced psychomotor activation are positively and highly correlated with dopamine responses detected in these areas (Sabeti, *et al* 2002; Budygin, 2007). Moreover, subsecond dopamine fluctuations in the nucleus accumbens are associated with cocaine seeking behavior (Phillips, *et al* 2003; Stuber, *et al* 2005).

Levels of extracellular dopamine in the nucleus accumbens appear to regulate the rate of cocaine intake. Early studies showed that cocaine infusions are self-administered at regular intervals and that the inter-injection interval depends on the unit injection dose (Pickens and Thompson, 1968; Wilson, *et al* 1971). The timing of this behavior was initially suggested to be associated with fluctuating blood or brain levels of cocaine (Yokel and Pickens, 1974; Gerber and Wise, 1989) with responding being initiated when cocaine levels fall below a threshold level. This idea was extended to include brain dopamine levels by Justice and colleagues (1989) who used microdialysis to show that extracellular dopamine rapidly increases following each cocaine injection and drug seeking appears to be initiated when dopamine levels decline to some critical concentration (Pettit and Justice, 1989; Wise, *et al* 1995). This level has been variously called a trigger-point (Wise, *et al* 1995), set-point (Ahmed and Koob, 1998), or satiety threshold (Tsibulsky and Norman, 1999).

While it is commonly believed that the inhibition of the dopamine transporter (DAT) by cocaine is the mechanism responsible for the elevation of extracellular dopamine in the nucleus accumbens (Wu, *et al* 2001; Garris and Rebec, 2002; Budygin, 2007); it remains unclear to what extent cocaine-induced DAT inhibition is involved in the timing of inter-infusion intervals or whether other mechanisms are involved. For example, subsecond dopamine release (i. e., dopamine transients) detected when an animal approaches a cocaine-paired lever (Phillips, *et al* 2003; Stuber, *et al* 2005) could play an essential role in cocaine self-administration. Moreover, the onset and time course of cocaine-induced dopamine uptake inhibition observed in some studies (Kiyatkin, *et al* 2000; Wakazono and Kiyatkin, 2008) is too slow and gradual to account for the rapid fluctuations in dopamine observed during cocaine self-administration. The fact that rats self-administer cocaine during the peak of dopamine uptake inhibition (Kiyatkin, *et al* 2000) is inconsistent with the hypothesis that decreases in DAT inhibition trigger responding. However, dopamine uptake inhibition has not been investigated during an actual time-course of cocaine self-administration.

In the present study we sought to establish the time course of cocaine-induced dopamine uptake inhibition by using fast-scan cyclic voltammetry to assess changes in dopamine with high temporal resolution (milliseconds) following electrical stimulation. Measurements were taken every 1-minute to allow dopamine uptake inhibition to be evaluated on a time-scale relevant to cocaine self-administration. Specifically, dopamine uptake was assessed in the nucleus accumbens of anesthetized rats using infusion rates obtained from two distinct self-

administration procedures, one of which produces stable responding across sessions, and another of which results in an escalation of the rate of cocaine intake over a two week period (Ahmed and Koob, 1998). A new mathematical model is proposed that accurately describes and predicts the changes of cocaine-induced dopamine uptake inhibition as they occur after cocaine administration.

MATERIALS AND METHODS

Inter-Infusion Interval Determinations

These experiments were designed to allow *in vivo* voltammetric recordings to be performed while intravenous cocaine infusions were being administered in accordance with rates and patterns of responding observed during cocaine self-administration. The access conditions under which animals self-administer cocaine can change the rate at which infusions are self-administered. In the present study, two access conditions were of interest. Under short-access conditions (2 hr sessions) the rate of responding remains stable over daily sessions. Under long-access conditions (6 hr sessions) the rate of responding increases, or escalates, over daily sessions (Ahmed and Koob, 1998). For both conditions, rates were analyzed after 14 self-administration sessions had occurred. Rates from a short-access group (n=6) remained stable across sessions; therefore multiple sessions were averaged from the final self-administration sessions (30 total sessions). Rates from a long-access group (n=8) increased across sessions; therefore, only rates from the final day of self-administration were averaged across animals. Inter-infusion intervals were determined by averaging the time in seconds occurring between responses for each animal. Each response was considered separately - so, for example - the inter-infusion interval between response one and two would be different from the inter-infusion interval between response eight and nine. In order to investigate the effects of a history of long-access to cocaine during self-administration on dopamine uptake, an experimental group (n=7) was given long-access to cocaine (see long-access training) before individual animals were infused with predetermined rates during voltammetric recording. Response rates from the experimental group did not differ from the response rates used to calculate the predetermined long-access inter-infusion intervals [$F(13,169) = 0.75$; n.s.].

General Cocaine Self-Administration

Male Sprague-Dawley rats weighing approximately 350g at the start of the experiment were used as subjects. Rats were anesthetized via an injection of ketamine (100 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.) and implanted with a chronically indwelling Silastic® cannula (CamCaths, Cambridgeshire, UK) into the right jugular vein. The cannula exited through the skin on the dorsal surface in the region of the scapulae (Roberts and Goeders, 1989). Animals were then individually housed in 30 × 30 × 30 cm operant chambers. Following surgery, a stainless steel protective tether that enclosed Tygon® tubing was connected to a counterbalanced fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, Pa., USA) mounted above the chamber. The swivel was connected to an infusion pump (Razel Scientific Instruments, Inc., Stamford, Conn., USA). The cannulae were flushed daily with heparinized saline to maintain patency. Following a recovery period (3-5 days), animals were given access to a cocaine-paired lever during an acquisition phase. During the

acquisition phase, cocaine (0.75 mg/kg/infusion) was available on a fixed ratio 1 schedule of reinforcement. Rats were given access to cocaine in daily training sessions, which terminated after a maximum of 20 infusions or a period of six hours had elapsed. An animal was considered to have acquired stable self-administration if 20 injections were self-administered during a single session and the pattern displayed consistent inter-infusion intervals. Upon completion of the acquisition phase animals began the long-access training procedure.

Long-Access Training

One group of animals (n=7) was used for this experiment. Animals were given access to cocaine (0.75 mg/kg/infusion) under a fixed ratio 1 schedule of reinforcement during daily 6-hour session for 14 consecutive days. Under long-access conditions animals increase, or 'escalate,' cocaine intake and the rate of cocaine infusions over 14 days (Ahmed and Koob, 1998; 1999). In the present study, as expected, the long-access training procedure resulted in a significant increase in the rate of responding across sessions [$F(6,78) = 7.32$; $p < 0.01$]. Voltammetry was performed 24 hours following the final self-administration session for each individual animal.

Voltammetric Recordings

All of the voltammetric experiments were performed on anesthetized rats. There were at least three important reasons why this design was chosen versus experiments on freely moving animals which self-administer cocaine. First of all, an electrical stimulation which is a necessary part of the procedure would change patterns of cocaine self-administration (Phillips, *et al* 2003). Secondly, anesthetized animals permit the application of larger electrical current than can be applied to awake rats. These stimulations permit larger dopamine efflux that allows more accurate evaluation of dopamine uptake changes. Thirdly, the pure pharmacological effects of cocaine without any conditioned influence on dopamine neurotransmission that would be observed during cocaine self-administration in awake animals (Phillips, *et al* 2003; Stuber, *et al* 2005), was the main focus of this study. It should also be noted, urethane anesthesia was chosen because it does not alter dopamine uptake dynamics (Garris, *et al* 2003; Sabeti, *et al* 2003).

Rats were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. A carbon fiber electrode was positioned in the nucleus accumbens core (AP + 1.3, L + 1.3, V - 6.6 mm from bregma) and a Ag/AgCl reference electrode was implanted in the contralateral hemisphere. A bipolar stimulating electrode was lowered to the ventral tegmental area ipsilateral to the working electrode at 5.2 mm posterior and 1.0 mm lateral to bregma. The stimulating electrode depth was optimized to evoke dopamine release in the nucleus accumbens which was monitored using a carbon fiber microelectrode. The working electrode was prepared using single carbon fibers (T-650; 6- μ m diameter; ThorneI, Amoco, Greenville, SC), which were pulled and sealed in glass capillaries. For voltammetric recordings, the exposed carbon fiber extended 120 ± 21 μ m from glass seal. The reference and carbon fiber electrodes were connected to a head-mounted voltammetric amplifier (UNC Electronics Design Facility, Chapel Hill, NC) and voltammetric recordings were made at the carbon fiber electrode every 100 ms by applying a triangular waveform (-0.4 to

+1.2 V, 300 V/s). The signals had an oxidation peak at + 0.6 V and a reduction peak at - 0.2 V versus Ag/AgCl reference, identifying the released species as dopamine. Data were digitized (National Instruments, Austin, TX) and stored on a computer. Dopamine release was evoked every 1 min with electrical stimulations (24 rectangular pulses, 60 Hz, 300 μ A, 2 ms/phase, biphasic) and detected by a carbon fiber electrode. Importantly, with this type of stimulation, physiological dynamics of dopamine release were revealed (Montague, et al 2004). At least four stable stimulations of dopamine were collected, and then either a single (0.75, 1.5, 3.0 mg/kg, i.v.) or multiple injections (0.75 mg/kg i.v.) of cocaine (cocaine hydrochloride obtained from the National Institute on Drug Abuse, Rockville, MD, USA; the drug was dissolved in sterile 0.9% saline) was administered. The drug was administered as an experimenter-delivered bolus over 4 s in a volume of 0.10-0.13 ml.

Carbon fiber microelectrodes were calibrated with known concentrations of dopamine (2-5 μ M) in artificial CSF (aCSF) at room temperature. The aCSF consisted of (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), CaCl_2 (2.4), MgCl_2 (1.2), NaHCO_3 (25), glucose (11) and was pH adjusted to 7.4. Calibrations were done in triplicate and the average value for the current at the peak oxidation potential was used to normalize in vivo signals to dopamine concentration. Dopamine uptake was determined from the clearance rate of dopamine following the termination of the stimulus and was assumed to follow Michaelis-Menten kinetics (Wightman and Zimmerman, 1990; Garris and Rebec, 2002). A detailed description regarding a model characterizing changes in extracellular dopamine concentrations evoked by electrical stimulation as a balance between the opposing mechanisms of release and uptake was provided in previous publications (Wightman, *et al* 1988; Wightman and Zimmerman, 1990; Wu, *et al* 2001; Garris and Rebec, 2002). The changes in dopamine during and after electrical stimulation were fit using equation 1.

$$d[DA]/dt = (f)[DA]_p - (V_{max}/\{(K_m/[DA]) + 1\}) \quad (1)$$

where f is the stimulation frequency (Hz), $[DA]_p$ is the concentration of dopamine released per stimulus pulse. V_{max} is a maximal velocity of dopamine uptake, which is proportional to the number of available DAT proteins. K_m is the substrate (dopamine) concentration at one half of V_{max} . This second uptake parameter (K_m) is a complex constant, related to the affinity of dopamine for the DAT and its rate of turnover. The baseline value of K_m was taken to be $\approx 0.2 \mu\text{M}$, a value determined in rat brain synaptosomes (Near, *et al* 1988; Garris and Rebec, 2002). The integral form of the above equation was used to simulate the dopamine response using single curve analysis. This method, which also agrees favorably with other analyses, has proven to be particularly convenient for the evaluation of dopamine uptake changes induced by competitive DAT inhibitors such as cocaine (Wu, *et al* 2001; Garris and Rebec, 2002; Budygin, 2007). Dopamine signals for each rat were fit individually at all time points before and after cocaine injections. The K_m was fixed and the other variables were determined, when pre-drug parameters were calculated. The calculation of K_m requires knowledge of V_{max} and $[DA]_p$, and a steady-state response of electrically evoked dopamine concentration (Wu, *et al* 2001). Consequently, when the cocaine effect on the dopamine efflux was modeled, K_m became the main subject of manipulation, while V_{max} was kept close to the pre-drug value. As a final point of the fitting procedure, $[DA]_p$ was adjusted in order to obtain a best fit.

Statistical Analysis

Data were analyzed in GraphPad Prism (GraphPad Software, San Diego, CA). A t test, one-way repeated measures and two-way ANOVAs with Bonferroni post tests were used to determine statistical significance. The data are presented as mean \pm SEM and the criterion of significance was set at $p < 0.05$. For the mathematical model, which describes cocaine-induced dopamine uptake changes, MATLAB was used to fit the experimental data using a least-squares minimization procedure. The predicted curves were also generated using MATLAB.

RESULTS

Intravenous Cocaine Dose-Dependently Increases Apparent K_m in the Rat Nucleus Accumbens (Experiment 1)

In this experiment, dopamine uptake inhibition was assessed in naïve rats following single intravenous infusions of cocaine. Electrically evoked dopamine concentrations in the nucleus accumbens were stable before drug injections. Saline administration (0.3 ml/inf, i.v.) did not significantly modify dopamine signals over the time course of this experiment (Figure 1a). The administration of single doses of cocaine elicited fast and robust (2-4 fold) increases in extracellular dopamine (Figure 1b). Kinetic analysis of the evoked dopamine signals indicated that the increases were associated with an increase in the apparent K_m ($K_m(app)$) for dopamine uptake. This effect reached a maximum within one minute following all doses of cocaine and then gradually decayed. Figure 2a shows dose-dependent increases in $K_m(app)$ occurring one minute after an intravenous infusion when dopamine uptake inhibition was maximal [$F(3,16) = 52.3$; $p < 0.01$]. Bonferroni post tests indicated significant differences in $K_m(app)$ after 3.0 versus 1.5 mg/kg of cocaine ($p < 0.01$) and after 0 versus 0.75 mg/kg ($p < 0.01$). There was a trend toward significance between 1.5 and 0.75 mg/kg ($p = 0.056$). No significant changes in the maximal rate of dopamine uptake (V_{max}) were revealed following any cocaine dose (Figure 2b). The pre-drug value for V_{max} ($1.79 \pm 0.15 \mu\text{M/s}$) is consistent with previously published V_{max} values obtained in the nucleus accumbens core region (Mateo, *et al* 2004). The amplitude of electrically evoked dopamine release was increased by cocaine [$F(3,16) = 9.1$; $p < 0.05$] in the same fashion as $K_m(app)$ (Figure 2c). Bonferroni post tests revealed significant differences in this parameter after 1.5 and 3.0 mg/kg of cocaine versus pre-drug values ($p < 0.05$). Importantly, electrically evoked dopamine release does not reflect the basal dopamine concentration in rat brain.

Two Distinct Phases of Dopamine Uptake Inhibition are Observed When Cocaine is Infused Using Intervals Observed During Self-Administration (Experiment 2)

In this experiment, cocaine (0.75 mg/kg) was intravenously infused into naïve rats using inter-infusion intervals which were predetermined from rats that self-administered cocaine under a fixed ratio 1 schedule (2-hour session) of drug delivery (short-access training) (Figure 3). These intervals were 1.11, 2.18, 3.82, 4.52, 4.47, 4.67, 4.83, 4.70, 5.33, 5.37 and 5.18 min. Each cocaine infusion significantly increased $K_m(app)$ compared to the drug pre-infusion value [$F(18,72) = 49.21$; $p < 0.01$, $n=5$]. Following the first four cocaine infusions (loading phase), the level of dopamine uptake inhibition reached a steady-state oscillation that persisted for the duration of the experiment (maintenance phase). During the

maintenance phase upper and lower thresholds were discernible. The upper threshold, which is distinguishable as the crests of the fluctuating level of dopamine uptake inhibition, is defined as the average of the maximal values of $K_m(app)$. Likewise, the lower threshold, which is evident as the troughs of dopamine uptake inhibition, is defined as the average of the minimal values of $K_m(app)$. There was a significant difference between the upper and lower thresholds ($t = 4.8$; $p < 0.01$, $n=6$, paired t test).

As expected, the amplitude of electrically evoked dopamine efflux was also significantly increased following cocaine infusions [$F(41,164) = 6.2$; $p < 0.01$, $n=5$]. The time course of the changes in evoked dopamine efflux paralleled the time course of the increase in $K_m(app)$ (Figure 3, inset), suggesting that the effect of cocaine on dopamine peak height is preferentially driven by competitive antagonism of the DAT. However, multiple mechanisms are also involved in the effect of cocaine on electrically evoked dopamine. For example, cocaine can enhance dopamine release by mobilizing a synapsin-dependent reserve pool (Venton, *et al* 2006). Moreover, electrically-evoked dopamine release is also potentially subject to D₂ dopamine receptor-mediated autoinhibition (Schmitz, *et al* 2001; 2002; Wu, *et al* 2002), which takes place during cocaine-induced DAT blockade (Grace, 2000). Since the dopamine peak amplitude is affected by many factors, the interpretation of cocaine effects on dopamine neurotransmission using this parameter can be complicated.

Dynamics of Dopamine Uptake Inhibition Can Be Mathematically Modeled and Predicted (Experiment 3)

A new mathematical model was developed to quantitatively predict the behavior of the apparent Michaelis-Menten constant $K_m(app)$ as a function of time—The model depends on three parameters: the initial cocaine concentration of each injection, the probability of dopamine diffusion and transport in the absence of DAT, and the rate of dopamine removal in the presence of DAT.

This model assumes a very simple form for the apparent Michaelis constant $K_m(app)$ based on the assumption that the DAT acts as though it can be in one of two states or conditions, i.e. that the DAT is either occupied or unoccupied by cocaine. In this case, the probability that a transporter is occupied by a cocaine molecule is represented by p_o . If p_u represents the probability that a transporter is unoccupied by a cocaine molecule, then these two probabilities must sum to one, i.e. $p_o + p_u = 1$. This relation applies since the model is binary, and the DAT is either occupied or unoccupied by a cocaine molecule. Cocaine acts as an inhibitor, by attaching to the DAT that prevents a dopamine molecule from attaching to the same transporter. Thus, a physically reasonable model for these probabilities involves the ratio of the number of available inhibitor (cocaine) molecules $[I]$ to the number of accessible DATs $[T]$. The number of accessible transporters is assumed fixed. The number of available inhibitor molecules is simply proportional to the inhibitor concentration. If this ratio is defined to be

$$s = \frac{[I]}{[T]}, \quad (2)$$

then the probability that cocaine will occupy a transporter is equal to s , i.e. $p_o = s$. This last relation, that $p_o = s$, is only true as long as the number of occupied DATs remains small, i.e. the occupation probability is much less than 1. However, when the number of available inhibitors becomes large, i.e. comparable to or higher than the numbers of DATs, then the probability must be modified to account for this moderate to high ratio of inhibitors to transporters. In the case where the transporters are basically saturated with inhibitors, then the occupation probability will reach its maximum value of 1. An appropriate model for such a system is assumed that the inhibitor occupation probability p_o is given by

$$p_o = \frac{s}{1+s} \quad (3)$$

As an aid to demonstrating that such a model is reasonable, note that this probability has the correct form in the two extreme cases of low inhibitor-to-transporter ratios ($s \ll 1$, $p_o = s$) and large inhibitor concentration-to-transporter ratios ($s \gg 1$, $p_o = 1$). This inhibitor-occupied-transporter probability can be related to the apparent Michaelis constant. In enzyme reaction rate theory (see Voet and Voet, 2004), an apparent $K_m(app)$ in the presence of an inhibitor is given by

$$K_m (app) = \left(1 + \frac{[I]}{K_1}\right) K_m, \quad (4)$$

where K_m is the Michaelis constant in the absence of inhibitor, and the constant K_1 is the equilibrium constant associated with the enzyme-inhibitor complex. To mimic this standard relation from enzyme reaction rate theory, it is assumed that a Michaelis constant depends on the inhibitor-occupied-transporter probability in the following way

$$K_m (app) = \left(\frac{1}{1 - p_o}\right) K_m. \quad (5)$$

The factor multiplying K_m in both of these cases can be related, in the case of small inhibitor concentration, using the geometric series expansion of the prefactor in the last equation. The geometric series $1 + p_o + p_o^2 + \dots$ sums to the prefactor in Eq. (5) above. So if all the small numbers in the geometric series are dropped, such as p_o^2 and higher powers of p_o , then for small p_o or $[I]$ Eq. (5) for $K_m(app)$ can be written:

$$K_m (app) \approx (1 + p_o) K_m = \left(1 + \frac{[I]}{K_1}\right) K_m. \quad (6)$$

Thus, this model, Eq. (5), gives the expected result in the extreme case of low inhibitor numbers (or concentrations), Eq. (4). In the extreme case of high inhibitor concentrations the occupation probability p_o approaches 1, and the effective Michaelis constant approaches infinity - see Eq. (5). This means that inhibitors occupy all the available transporter sites and so the reaction with dopamine shuts down and dopamine is not taken up at all.

This last discussion, about the behavior of the DAT under high inhibitor number conditions, brings up one more issue that requires modification of the model. If the inhibitor completely

binds to all transporter sites, the dopamine concentration will still decrease with time, through reactions with molecules other than DATs, and through diffusion. The apparent Michaelis constant can never, in practice, reach infinity, which Eq.(5) would predict if $p_o = 1$. Thus, a term must be included in the model that keeps $K_m(app)$ finite in the extreme case of very high inhibitor numbers. To implement this last important concept into the model, its final form becomes

$$K_m (app) = \left(\frac{1}{1 - p_o + b} \right) K_m. \quad (7)$$

The constant b is a small number with a value around $b \sim 0.005$. Physiologically, it accounts for dopamine diffusion and uptake in the presence of saturating amounts of cocaine, which means that the cocaine-responsive DATs are effectively absent (actually occupied by cocaine). Note that under cocaine-saturating conditions, the apparent Michaelis constant becomes K_m/b . In the application of the final model, Eq. (7), to the experimental data, there are three parameters whose values are varied to fit the model to the data. Two of the constants are: 1) $s(0)$, the initial cocaine-to-transporter number ratio at the time of an injection, i.e. at time $t = 0$, when cocaine first floods the probed region, and 2) the constant b . Note that the initial inhibitor-occupied-transporter probability $p_o(0)$ is directly related to $s(0)$. The third parameter needed is the decay rate k_{decay} of the inhibitor number (or concentration) $[I]$ after an injection of cocaine. In this case it is assumed that s , which is proportional to $[I]$, decays exponentially in time after an inhibitor (cocaine) injection.

To explicitly describe the detailed time dependence of the model as it is used to fit the experimental data (see Figure 3), consider a set of data that has cocaine injections at times t_0, t_1, t_2, \dots . This means that the inhibitor number $[I]$, and therefore s , during the whole time series has the form

$$s(t) = s(0) \sum_{i=1}^N e^{-k_{decay}(t-t_i)}, \quad (8)$$

where N is the total number of injections.

The model was used on the data from naïve rats repeatedly receiving a dose of cocaine (0.75 mg/kg), as described in the previous section and is shown in Figure 3. The model fits these data extremely well with the best fit values being: $s(0) = 3.83$, $b = 0.0047$, and $k_{decay} = 0.0628/\text{minute}$. The reduced chi-square for this fit is: $\chi_R = 0.177$, with the number of degrees of freedom = 39.

To definitively test the predictive capability and efficacy of the model, it was used to blindly predict the values of $K_m(app)$ in the case where the inter-infusion intervals of the maintenance phase were made very short (2 minutes) and very long (10 minutes). Note these intervals do not correspond to inter-infusion intervals selected by rats during the self-administration of cocaine, but were chosen outside of this range to determine if the model would predict outcomes of future experiments. An experiment was then initiated for the long inter-infusion intervals with naïve rats. The results demonstrated that the model can capture not only the important qualitative features of the dynamics of dopamine concentration, but

the model successfully described the outcome of the experiment (Figures 3 and 4). Note that no parameters were adjusted in generating the model points - these points were generated prior to voltammetric experiments based on the model's fit to rat responses in the case where the inter-infusion intervals were much shorter (5 minutes).

DAT Inhibition Thresholds are Shifted Upward with Escalated Inter-Infusion Intervals (Experiment 4)

Previous studies have demonstrated that providing long-access to cocaine (6-hr session) under a fixed ratio 1 schedule for 14 days results in animals increasing, or 'escalating' the rate of cocaine intake across sessions (Ahmed and Koob, 1998; 1999). As illustrated in figure 5, dopamine uptake inhibition ($K_m(app)$) was assessed while cocaine (0.75 mg/kg, i.v.) was infused into naïve rats using inter-infusion intervals which were predetermined from rats that either escalated (long-access) or did not escalate (short-access) cocaine self-administration rates. The escalated intervals were determined to be 1.00, 1.00, 1.48, 1.65, 3.00, 3.37, 3.88, 4.00, 3.00, 3.00, 3.20, 3.17, 4.00, 3.12 min (see inter-infusion interval determinations in the Material and Methods). Similarly to the previous experiment, cocaine-induced dopamine uptake inhibition (changes in $K_m(app)$) appeared in two distinct phases, which correspond to the loading and maintenance phases observed during self-administration under a fixed-ratio schedule (Ahmed and Koob, 1999; Wee, *et al* 2007; Specio, *et al* 2008). The total level of DAT inhibition was extensively higher with escalated (long-access) inter-infusion intervals, compared with that from unescalated (short-access) intervals (Figure 5). There was a significant main effect of the access condition from which rates were determined (i.e., long-access versus short-access) on $K_m(app)$ [$F(1,336) = 60.65$; $p < 0.01$]. There was also a significant main effect of time [$F(41, 336) = 5.45$; $p < 0.01$] on $K_m(app)$, although the interaction of access condition and time was not statistically significant. Upper and lower thresholds were compared between escalated (long-access) and unescalated (short-access) groups by averaging the peaks and troughs of the oscillatory pattern of $K_m(app)$ changes observed during the maintenance phase. For the escalated (long-access) rats the upper threshold was $2.81 \pm 0.03 \mu\text{M}$ and the lower threshold was $2.42 \pm 0.04 \mu\text{M}$ ($n = 11$). For the unescalated (short-access) rats the upper threshold was $2.28 \pm 0.03 \mu\text{M}$ and the lower threshold was $1.94 \pm 0.03 \mu\text{M}$ ($n = 6$). There was a significant difference between the escalated (long-access) and unescalated (short-access) groups upper [$t = 11.33$; $p < 0.01$] and lower [$t = 8.976$; $p < 0.01$] thresholds.

Escalated Cocaine Self-Administration Modifies the Maximal Rate of Dopamine Uptake (Experiment 5)

In experiment 5, the dopamine uptake parameters before and after four consequent cocaine (0.75 mg/kg, i.v.) infusions were compared between the cocaine naïve group and rats which demonstrated escalated cocaine intake under a fixed ratio 1 schedule (long-access training). The experiment indicated that the basal (pre-drug) value of $K_m(app)$ was not different between cocaine-naïve and cocaine-exposed rats (0.18 ± 0.008 versus $0.18 \pm 0.008 \mu\text{M}$; n.s.; $n=7$). However, there was a significant difference in the basal V_{max} between these two groups [$t = 4.249$; $p < 0.01$] (Figure 6a). As was expected (see results with single cocaine injection), acute cocaine infusions did not significantly alter this parameter of dopamine uptake in both cocaine-naïve and drug exposed animals (data not shown). Cocaine-induced

$K_m(app)$ changes were indistinguishable between these groups (Figure 7). A two-way ANOVA demonstrated that the effect of prolonged cocaine exposure was not significant [$F(1, 40) = 0.043$; n.s.], while the effect of acute cocaine was significant [$F(4, 40) = 0.043$; $p < 0.01$].

DISCUSSION

The present study demonstrates that cocaine-induced DAT inhibition fluctuates over a time course which can account for patterns of cocaine self-administration reinforced under a fixed-ratio 1 schedule. Specifically, the inter-infusion intervals that are maintained during cocaine self-administration correlate with the maintenance of a rapidly changing level of DAT inhibition. These oscillating changes in dopamine uptake inhibition were modeled and can be predicted in a manner relevant to cocaine self-administration. Furthermore, this tightly maintained level of DAT inhibition was found to shift upward using intervals obtained after a history of escalated (long-access training) cocaine intake. Although, daily 6-hr access (long-access) during cocaine self-administration did not significantly alter the efficacy of cocaine for the DAT, it did result in a facilitated uptake of dopamine (V_{max} changes) suggesting an up-regulation of DAT number in response to a history of high cocaine intake.

Single Intravenous Cocaine Administration and Uptake of Endogenous Dopamine

The effect of intravenously injected cocaine on dopamine uptake occurs with a rapid onset and offset. The present data - together with previously published *in vivo* voltammetry data using intraperitoneal cocaine administration (Wu, *et al* 2001), as well as with *in vitro* drug application (Jones, *et al* 1995) - confirm that competitive inhibition of the DAT is the primary mechanism of acute cocaine action on increasing accumbal dopamine transmission. Indeed, intravenous cocaine dose dependently decreased the uptake of dopamine, acting through an alteration in $K_m(app)$, while V_{max} remained unaffected. According to single curve analysis, the maximum effect of cocaine on dopamine uptake inhibition ($K_m(app)$) was reached within 1-2 minutes after a single cocaine infusion and then gradually decreased (Figure 1b). Importantly, the maximal effect of cocaine on $K_m(app)$ is consistent with a peak in cocaine brain concentrations (Fowler, *et al* 1998; Ahmed, *et al* 2003) and the maximal inhibition of dopamine cells in the ventral tegmental area (Einhorn, *et al* 1988). These data should be contrasted to the results of Kiyatkin and colleagues who demonstrated that cocaine-induced changes in clearance of exogenous dopamine began two minutes following a cocaine infusion (Kiyatkin, *et al* 2000), when extracellular dopamine concentrations were clearly elevated (Wise, *et al* 1995; Ahmed, *et al* 2003; Heien, *et al* 2005) and peaked at 6-8 minutes (Kiyatkin, *et al* 2000). Therefore, the dynamics of dopamine uptake inhibition following intravenous cocaine administration (see also Mateo, *et al* 2004; Samaha, *et al* 2004) appears to differ depending on whether stimulated dopamine release or exogenously applied dopamine is assessed.

Levels of Dopamine Uptake Inhibition are Tightly Linked to Cocaine Self-Administration

The present study demonstrates a strong association between the pattern of cocaine self-administration and the level of dopamine uptake inhibition. Two stages of dopamine uptake

inhibition are revealed in accordance with patterns of self-administration observed during cocaine self-administration under a fixed-ratio schedule. Previous reports, in addition to the data from the current study, have demonstrated that responding at the beginning of a self-administration session (approximately 10 minutes) occurs at a faster rate than at any other period of the session (Ahmed and Koob, 1999; Wee, *et al* 2007; Specio, *et al* 2008). This initial high rate of responding, termed the loading phase, is also reflected by a rapid increase in $K_m(app)$ at the onset of a session (Figure 3). Following the loading phase, the rate of responding appears to subside and stabilize, presumably corresponding to the point in time at which an effective brain level of cocaine-induced DAT inhibition is reached. This level, which is reflected by a lower threshold of $K_m(app)$, is then sustained through a maintenance phase during which responses are separated by stable inter-infusion intervals (Ahmed and Koob, 1999; Specio, *et al* 2008). During the maintenance phase, the level of DAT blockade fluctuates around a narrow range within lower and upper thresholds.

The present data suggest that responding maintained by cocaine occurs in association with a lower threshold of dopamine uptake inhibition. This lower threshold has also been referred to as a trigger-point, set-point, or priming threshold (Wise, *et al* 1995; Ahmed and Koob, 1998; Tsibulsky and Norman, 1999). In fact, a lower level of extracellular dopamine within the nucleus accumbens has previously been implicated as a 'trigger' to respond during cocaine self-administration (Wise, *et al* 1995). The data from the current study link the 'trigger' to a lower threshold of dopamine uptake inhibition, thereby demonstrating the mechanism that provides the trigger-dopamine concentration.

The upper threshold has also received speculative attention (Petitt and Justice, 1989; Tsibulsky and Norman, 1999; Lynch and Carroll, 2001). For example, Petitt and Justice (1989) suggested that the upper threshold may be associated with aversive cocaine effects; whereas, Tsibulsky and Norman (1999) suggested this threshold occurs due to satiety mechanisms alone. Although the data from the present study can not reconcile why an upper threshold is observed, the observation that this threshold shifts upward when cocaine infusions are maintained at a high rate suggests that the upper threshold does not result from maximal DAT occupancy (see Figure 5).

Cocaine-induced dopamine uptake inhibition may alter phasic dopamine release through a D_2 dopamine autoreceptor-mediated feedback mechanism (Grace, 2000; Phillips, *et al* 2003). It was discovered that a lever press for a cocaine infusion appears to be associated with subsecond dopamine release in the nucleus accumbens (Phillips, *et al* 2003; Stuber, *et al* 2005). Importantly, these short-lived dopamine changes - which may be influential in an animal's approach behavior toward a cocaine-paired lever or other drug-paired stimulus (Phillips, *et al* 2003; Stuber, *et al* 2005) - do not translate to a significant elevation in tonic dopamine levels. In contrast, DAT inhibition has a pronounced effect on tonic dopamine levels (Di Chiara and Imperato, 1988; Budygin, *et al* 2000; Heien, *et al* 2005). An intriguing speculation is that subsecond dopamine release can be promoted through feedback mechanisms resulting from a decrease in extrasynaptic dopamine as the level of cocaine-induced DAT inhibition approaches the lower threshold. Future studies are necessary to determine whether this connection exists.

Consequences of Escalated Cocaine Self-Administration on Accumbal Dopamine Uptake

Providing long access to cocaine during self-administration produces an increase, or 'escalation,' in the rate of cocaine self-administration (Ahmed and Koob, 1998), which is reflected by the maintenance of an increased level of dopamine uptake inhibition ($K_m(app)$). As illustrated in figure 5 (experiment 4), the inter-infusion intervals obtained from animals with a history of long-access training were applied to determine the effect of an increased rate of self-administration on dopamine uptake inhibition in naïve animals. It was found that cocaine-induced dopamine uptake inhibition reached a proportionally higher level during the loading phase consistent with an escalation of cocaine intake. Likewise, the dopamine uptake inhibition thresholds associated with the maintenance of responding during self-administration were shifted upward with 'escalated' intervals.

The neuroadaptations explaining the resulting changes in behavior during long-access training remain unknown. There is convincing evidence that changes in cocaine pharmacokinetics, and changes in baseline concentrations of dopamine in the nucleus accumbens, do not have a critical role in the escalation of cocaine intake observed during long-access training (Ahmed, *et al* 2003). Furthermore, no apparent sensitization or desensitization to the effect of cocaine on accumbal dopamine concentrations was observed in animals with a history of long-access cocaine self-administration (Ahmed, *et al* 2003). However, it may be argued that prolonged cocaine exposure could modify the DAT inhibiting efficacy of cocaine. Examination of Figure 6b (experiment 5) shows that long-access escalation training did not result in significant changes in the efficacy ($K_m(app)$) of cocaine for the DAT. Therefore, the higher level of DAT blockade and subsequent maintenance of higher accumbal dopamine concentrations, which repeatedly take place during long-access cocaine self-administration, are not capable of significantly modifying the affinity of the DAT for cocaine. Thus, these data suggest that changes in the affinity of the DAT for cocaine are not involved in the escalation of cocaine intake.

Other possible neuroadaptations may occur during long-access training, such as post-synaptic adaptations and an up-regulation of DAT number, which may explain the escalation of cocaine intake. Several studies have reported post-synaptic adaptations following extended cocaine exposure demonstrating a down-regulation of both striatal D₁ (Graziella de Montis, *et al* 1998) and D₂ (Nader, *et al* 2002) dopamine receptors. Assuming post-synaptic changes occur during an escalation of cocaine intake, it can be hypothesized that increased dopamine uptake inhibition thresholds are maintained to produce a level of reinforcement equal to that occurring prior to escalation. One of the possible consequences of prolonged increases in extracellular dopamine concentrations following administration of addictive substances, including cocaine, is an increase in the maximal rate of dopamine uptake (Budygin, *et al* 2003; 2007; Mateo, *et al* 2005). The data presented here demonstrate the existence of enhanced dopamine uptake in the rat nucleus accumbens after a history of long-access during cocaine self-administration (Figure 6a). The increased rate of dopamine uptake is likely the result of an up-regulation in functional DAT number. Previous studies have reported increases in DAT availability following prolonged cocaine self-administration in both rats (Tella, *et al* 1996; but see also Ben Shahar, *et al* 2006) and non-human primates (Letchworth, *et al* 2001). Moreover, human postmortem analyses report an increase in

striatal DAT binding sites in cocaine addicts (Little, *et al* 1998; Little, *et al* 1999). These presynaptic changes, which may occur following any regimen of prolonged cocaine exposure, may be a compensatory response of the dopamine system to persistently elevated dopamine concentration in the extrasynaptic space.

Conclusion

This study has implications for understanding the role of the DAT in regulating responding for cocaine during self-administration. The present study took advantage of fast-scan cyclic voltammetry, which provides high temporal and spatial resolution, and clearly demonstrates that a tight correlation exists between the level of dopamine uptake inhibition and the rate of responding for cocaine under a fixed-ratio schedule.

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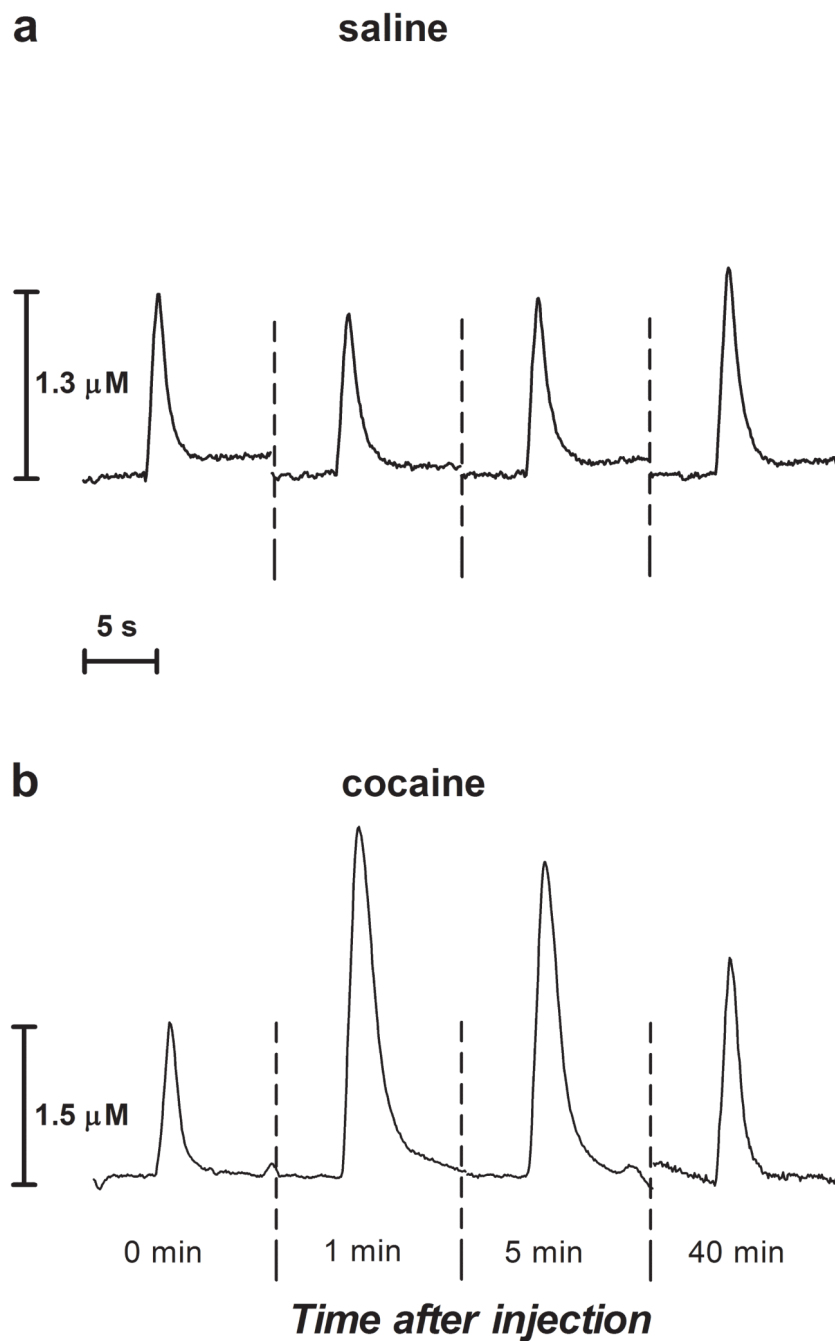


Figure 1. Representative concentration-time plots of dopamine measured in the rat nucleus accumbens before and following a single intravenous infusion of saline (0.3 ml/inf) (a) and cocaine (1.5 mg/kg) (b). An infusion of saline did not induce any changes in dopamine peak height or in $K_m(\text{app})$. In contrast, cocaine significantly increased dopamine peak height and $K_m(\text{app})$. Maximal dopamine peak height occurred within approximately 1 min after cocaine administration and then gradually decayed (1.33 ± 0.20 (pre-drug), 2.82 ± 0.24 (1 min), 1.74

± 0.21 (5 min), 1.42 ± 0.13 (40 min) μM (n=5)). $K_m(\text{app})$ values were 0.18 ± 0.01 (pre-drug), 1.13 ± 0.17 (1 min), 0.94 ± 0.19 (5 min), 0.43 ± 0.07 (40 min) μM (n = 5).

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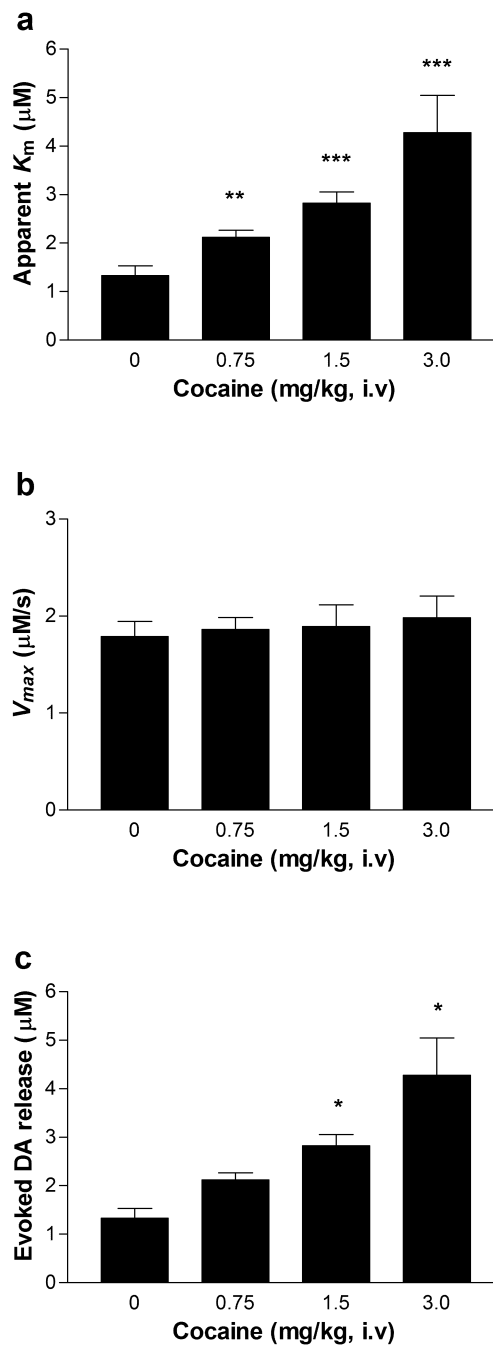


Figure 2.

Effect of single cocaine (i.v.) injection on dopamine release and uptake in rat nucleus accumbens. Electrically evoked dopamine concentrations and uptake parameters, reported as an $K_m(app)$ and V_{max} , were measured 1 min after a 4 s cocaine infusion. (a) $K_m(app)$ was dose dependently increased following a single infusion of intravenous cocaine (0.75, 1.5, or 3 mg/kg). (b) No changes in V_{max} were detected. (c) Similarly to the effect on $K_m(app)$, cocaine significantly enhanced electrically evoked dopamine release. Data are means \pm SEM of 5 rats per group. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

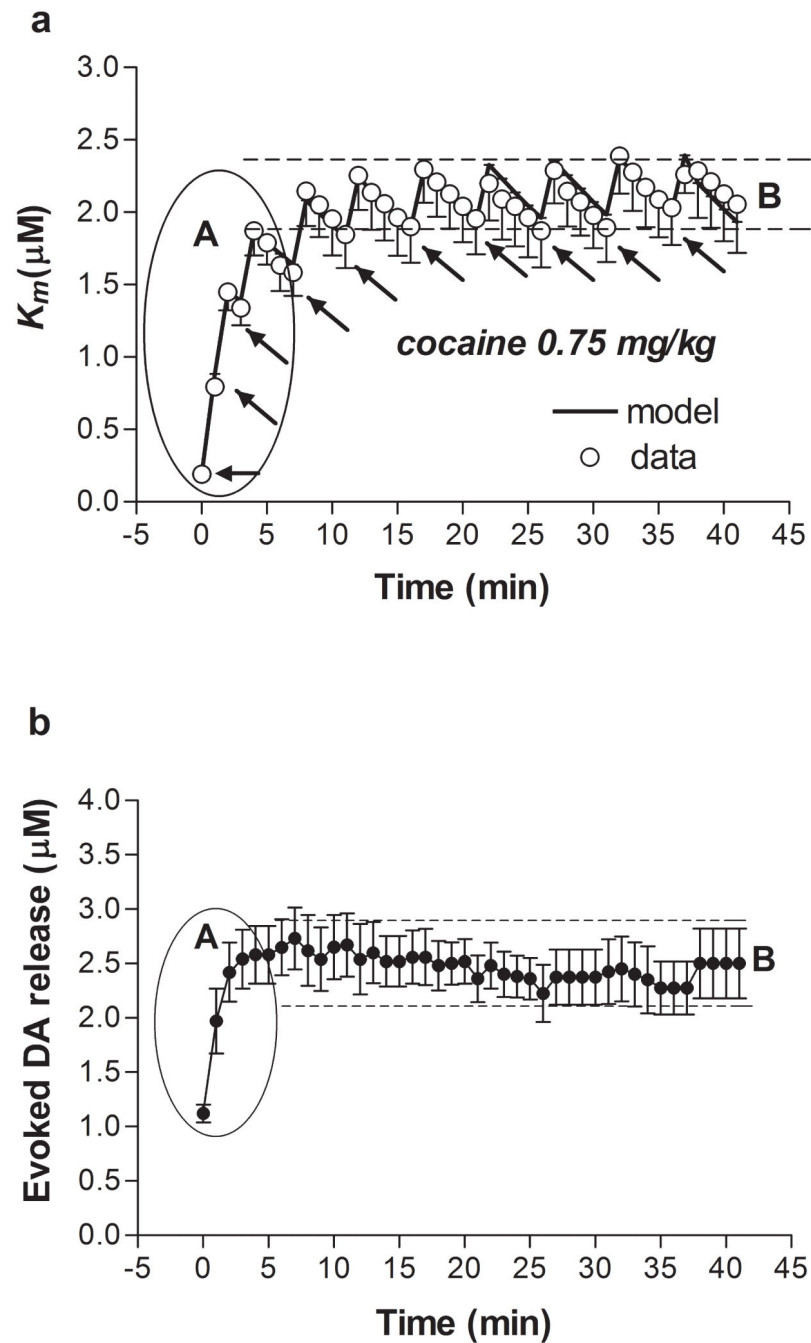


Figure 3.

(a) Dopamine uptake changes obtained with inter-infusion intervals predetermined from rats self-administering cocaine. Dopamine uptake changes, which are measured as alterations in $K_m(\text{app})$ are indicated by empty circles. The solid line shows modeled changes in dopamine uptake inhibition. The time points when cocaine (0.75 mg/kg, i.v.) was injected over 4 s are depicted as arrows. Dopamine uptake changes associated with responding during the loading phase are indicated by the oval (A). A steady-state-oscillation of dopamine uptake inhibition that is observed throughout the maintenance phase (B) is indicated by dashed lines. (b) The

time course of cocaine on the peak evoked dopamine concentration. Data are means \pm SEM of 5 rats per group.

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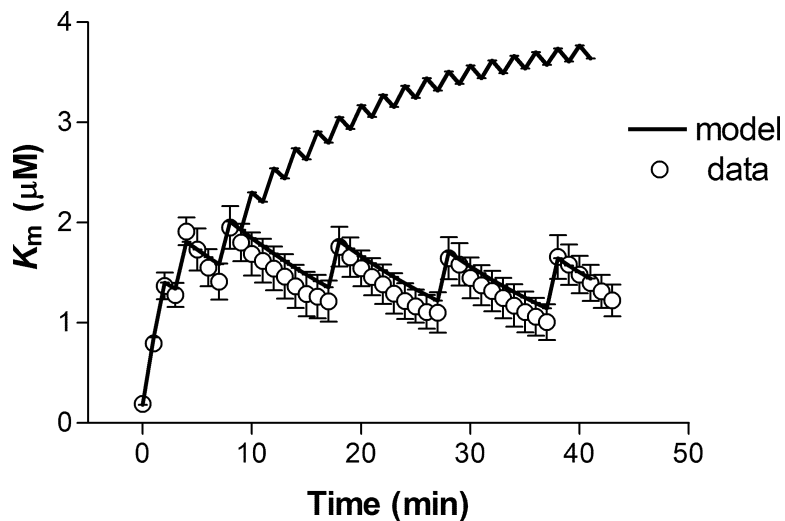


Figure 4. Changes in cocaine-induced dopamine uptake inhibition can be predicted. Two lines show predicted changes in dopamine uptake inhibition using two different inter-infusion intervals. The two curves correspond to blind predictions for the case where the inter-infusion intervals for the maintenance phase are 2 min apart (upper curve) and for an inter-infusion interval of 10 min (lower curve). Dopamine uptake changes using 10 minute inter-infusion intervals, which were measured in animal experiments, are indicated by empty circles. Data are means \pm SEM of 5 rats per group. The experimental measurements agree well with the blind prediction from the model.

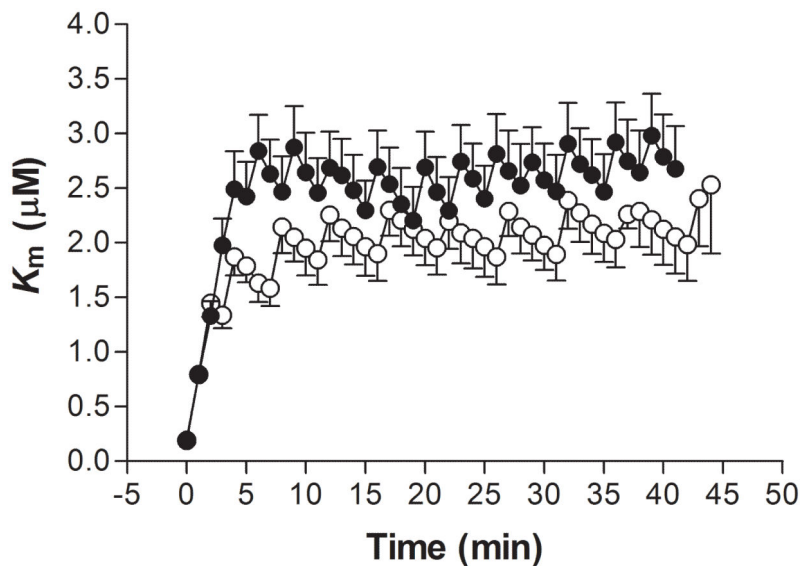


Figure 5. DAT inhibition thresholds are shifted upward during escalated cocaine self-administration. Dopamine uptake changes ($K_m(app)$) obtained using inter-infusion intervals that were predetermined from rats with escalated rates of cocaine self-administration are indicated by solid black circles. Empty circles show cocaine-induced $K_m(app)$ alterations, which were observed with non-escalated intervals. All of the experiments displayed in this graph were conducted using cocaine naïve rats. Data are means \pm SEM of 5 rats per group.

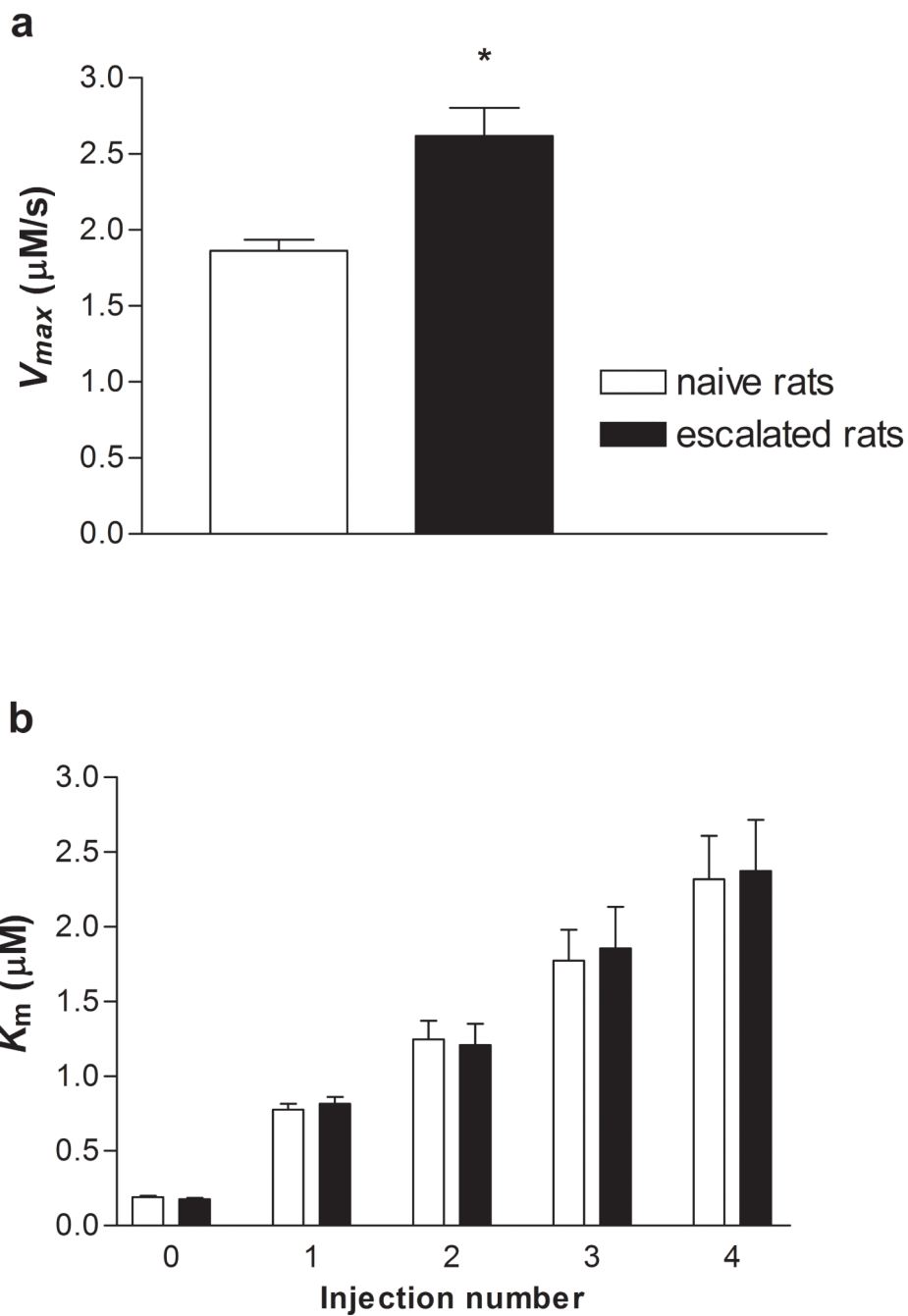


Figure 6. Consequences of escalated cocaine self-administration on the dopamine uptake in rat nucleus accumbens. (a) V_{max} is significantly increased following escalated cocaine intake (black bar) in comparison with naïve control (empty bar). Data are means \pm SEM of 7 rats per group. *, $P < 0.005$. (b) No significant changes in the affinity of DAT for cocaine ($K_m(app)$) are found in rats following escalation in cocaine self-administration (black bars)

compared with naïve control (empty bars). Four cocaine injections (0.75 mg/kg, i.v.) were performed to mimic loading phase. Data are means \pm SEM of 5 rats per group.

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