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Intermittent cocaine self-administration produces sensitization of stimulant effects at the dopamine transporter

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Abbreviations: Dopamine transporter, DAT; Methylphenidate, MPH; FR1, fixed-ratio 1; LgA, long-access; ShA, short-access; IntA, intermittent-access

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Abstract:

Previous literature investigating neurobiological adaptations following cocaine self-administration has shown that the development of pharmacodynamic tolerance, characterized by reduced cocaine potency at the dopamine transporter (DAT), results from high, continuous levels of intake (long-access; LgA), while sensitization of cocaine potency is caused by intermittent patterns of cocaine administration (intermittent-access; IntA). Here we aimed to determine if the changes observed following cocaine self-administration were specific to cocaine, or translated to other psychostimulants as well. Potency was assessed by fast scan cyclic voltammetry in brain slices containing the nucleus accumbens following control, IntA, short-access (ShA), and LgA. We assessed the potency of amphetamine, a releaser, and methylphenidate (MPH), a DAT blocker that is functionally similar to cocaine and structurally related to amphetamine. Changes in MPH potency can give information as to the importance of functional or structural aspects of compounds as related to the expression of tolerance/sensitization effects. MPH and amphetamine potencies were increased following IntA, while neither was changed following LgA. Here we demonstrate that while LgA-induced tolerance at the DAT is specific to cocaine, the sensitizing effects of IntA are conferred to cocaine, MPH, and amphetamine. The unchanged potency of MPH following LgA suggests that the expression of tolerance does not rely on the function of the compound as blocker/releaser. This demonstrates that the pattern with which cocaine is administered is important in determining the neurochemical consequences of not only cocaine effects, but cross-sensitization/cross-tolerance effects of other psychostimulants as well.

Introduction

The rewarding effects of stimulants have been directly linked to their actions at the dopamine transporter (DAT) in experiments that show abolished cocaine-induced conditioned place preference in transgenic mice with cocaine-insensitive DATs (Tilley et al., 2009). Further, the DAT is a critical mediator of cocaine reinforcement and is necessary for self-administration behaviors (Ritz et al., 1989; Roberts et al., 1977). Thus, differences in the potency of cocaine and other stimulants to inhibit the DAT and elevate dopamine levels are particularly relevant for understanding the reinforcing and rewarding effects of the compound. Changes in cocaine potency at the DAT (Calipari et al., 2013a) and concomitant behavioral changes (Zimmer et al., 2012) following a history of cocaine self-administration depend on the specific self-administration paradigm being used.

For example, we have previously demonstrated that the development of cocaine tolerance or sensitization at the DAT is a function of temporal pattern of administration (Calipari et al., 2013a). Long-access (LgA) self-administration results in high and sustained cocaine levels over daily 6-hour self-administration sessions, and it is well documented that this pattern of cocaine exposure results in reduced potency of cocaine at the DAT (Mateo et al., 2005; Ferris et al., 2011, 2012; Calipari et al., 2012, 2013a, b) and concomitant reductions in cocaine-induced increases in extracellular dopamine levels (Hurd et al. 1989; Ferris et al., 2011; Calipari et al., 2013b). Conversely, intermittent-access (IntA) self-administration, where animals are given time-outs in order to force self-administration patterns that result in sharp rises in cocaine levels followed by rapid decreases, results in sensitized cocaine potency at the DAT. Sensitization and tolerance of cocaine potency following intermittent and continuous administration have been demonstrated using self-administration (Calipari et al., 2013a) and with non-contingent administration of intermittent (i.p. injections) and continuous (mini-pumps) regimens of cocaine exposure (Addy et al., 2008). Although the effects of cocaine at the DAT are well established following a number of paradigms, how these cocaine self-administration protocols affect the potency of other psychostimulants, and what factors dictate the expression of tolerance/sensitization, remains to be determined.

Therefore, we determined how the potencies of methylphenidate (MPH; a dopamine uptake inhibitor) and amphetamine (a dopamine releaser) were affected by IntA, short-access (ShA), and LgA cocaine self-administration. Previously published work from our lab has shown that although MPH is a DAT blocker, it is affected by changes at the DAT that alter releaser potency, possibly due to the amphetamine-like structure of the compound (Calipari et al., 2012, 2013c; Ferris et al., 2012). DAT binding is highly dependent on structural components; accordingly, work has shown that MPH binds to the DAT in a fashion that is similar to amphetamine (Wayment et al., 2005; Dar et al., 1999). Thus, MPH shares functional properties with cocaine but some structural properties with amphetamine and therefore can give insight into whether changes in potency at the DAT are due to a compound's function, or due to other factors, such as specific DAT-stimulant structure interactions. If the compensatory effects of cocaine self-administration are conferred to compounds of the same dopamine uptake inhibitor class, MPH potency will change in a similar fashion to cocaine; however, if the changes are specific to some shared aspect of MPH and amphetamine structure, then outcome for MPH will be similar to amphetamine. In order to be consistent with previous cocaine self-administration studies using extended-access paradigms (Calipari et al., 2013a; Ferris et al., 2012), we hypothesized that MPH and amphetamine potency would be unaffected by LgA as compared to ShA controls. Alternatively, although IntA self-administration effects on drugs other than cocaine have not been studied previously, non-contingent administration of cocaine has been demonstrated to result in cross-sensitization for the releasers methamphetamine (Hirabayashi et al., 2001) and amphetamine (Lett 1989). Thus, we hypothesized that the IntA cocaine self-administration would result in the sensitization of MPH and amphetamine.

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Methods and Materials:

Animals: Male Sprague-Dawley rats (375–400 g; Harlan Laboratories, Frederick, Maryland) were maintained on a 12:12 hour reverse light/dark cycle (3:00 am lights off; 3:00 pm lights on) with food and water *ad libitum.* All animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine.

Self-Administration: Rats were anesthetized and implanted with chronic indwelling jugular catheters as previously described (Calipari et al., 2013d). Animals were singly housed, and all sessions took place in the home cage during the active/dark cycle (9:00 am–3:00 pm). After a 2-day recovery period from surgery, animals underwent a training paradigm within which animals were given access on a fixed ratio one (FR1) schedule to a cocaine-paired lever, which, upon responding, initiated an intravenous injection of cocaine (0.75 mg/kg, infused over 4s). After each response/infusion, the lever was retracted and a stimulus light was illuminated for a 20 second timeout period. Training sessions were terminated after a maximum of 20 infusions or 6h, whichever occurred first. Acquisition occurred when an animal responded for 20 injections for two consecutive days and a stable pattern of infusion intervals was present. Following training, animals were assigned to IntA, LgA, or ShA groups. All self-administration was 14 consecutive sessions, after which animals were sacrificed and brains were prepared for voltametric recordings.

LgA Group: After training, subjects completed daily 6h sessions during which they had unlimited access to cocaine (0.75 mg/kg; infused over 4s; for structure see Kinsey et al., 2010) on an FR1 schedule during 6h daily sessions for 14 consecutive days. At the start of each infusion, a stimulus light signaled a 20-s timeout period during which the lever was retracted.

ShA Group: After training, subjects were given unlimited access to cocaine (0.75 mg/kg; infused over 4s) on an FR1 schedule during 2h daily sessions for 14 consecutive days. At the start of each infusion, a stimulus light signaled a 20-s timeout period during which the lever was retracted.

IntA Group: A separate group was given access to cocaine on an intermittent schedule of administration described previously (Zimmer et al., 2012). During each 6h session animals had access to cocaine for 12 five minute trails separated by 25-minute timeout periods. Within each five-minute session, there were no timeouts other than during each infusion, and the animal could press the lever on an FR1 schedule to receive a 1-sec infusion of cocaine (0.375 mg/kg/inf).

Calculating Brain Concentrations: Brain-cocaine concentrations were estimated using equations employed by Pan *et al* (1991). The equation used was $c = \frac{dk}{v(\alpha - \beta)}(e^{-\beta t} - e^{-\alpha t})$ which calculates the brain-cocaine concentration in the brain compartment at time (t). The variables account for the dose of cocaine (d), the transfer of drug between the blood and brain compartments ($k = 0.233\ \text{min}^{-1}$), the apparent volume of the brain compartment ($v = 0.15\ \text{LKg}^{-1}$), and the removal of cocaine from the system via redistribution ($\alpha = 0.642\ \text{min}^{-1}$) and elimination ($\beta = 0.097\ \text{min}^{-1}$). This equation has been widely used to correlate estimated brain-cocaine levels with behavioral (Ahmed and Koob, 2005), electrophysiological (Peoples et al, 2007; Peoples et al, 2004), microdialysis (Wise et al, 1995) and voltammetric (Hermans et al, 2008; Stuber et al, 2005a; Stuber et al 2005b) measures. Estimates in brain cocaine levels in the literature are highly variable, spanning from the nM to the µM range. The aim of this study was not to determine brain levels of cocaine, which we did not directly measure, but rather, to show the relative brain level fluctuations over time within a session. Therefore we chose to present these data as arbitrary units.

Ex Vivo Voltammetry: Fast scan cyclic voltammetry (FSCV) was used to characterize the potency of psychostimulants to inhibit dopamine uptake in the nucleus accumbens (NAc) core. Voltammetry experiments were conducted during the dark phase of the light cycle 18 hours after commencement of the final self-administration session. A vibrating tissue slicer was used to prepare 400 µm thick coronal brain sections containing the NAc. The tissue was immersed in oxygenated artificial cerebrospinal fluid (aCSF) containing (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), L-ascorbic acid (0.4) and pH was adjusted to 7.4. Once sliced, the tissue was transferred to the testing chambers containing bath aCSF (32°C), which flowed at 1 ml/min. A carbon fiber microelectrode (100–200 µM length, 7 µM radius) and bipolar stimulating electrode were placed into the core of the NAc, which was selected because of its role in the reinforcing and rewarding actions of cocaine. Dopamine release was evoked by a single electrical pulse (300 µA, 4 msec, monophasic) applied to the tissue every 5 minutes. Extracellular dopamine was recorded by applying a triangular waveform (−0.4 to +1.2 to −0.4V *vs* Ag/AgCl, 400 V/s). Once the extracellular dopamine response was stable, MPH (0.03–30 µmol/L; for structure see Froimowitz et al., 1995) or amphetamine (0.1–10 µmol/L; for structure see Santagati et al., 2002) were applied cumulatively to the brain slice.

Data Modeling and Analysis: Demon Voltammetry and Analysis software was used for all acquisition and modeling of FSCV data (Yorgason et al., 2011). To evaluate drug potency, evoked levels of dopamine were modeled using Michaelis-Menten kinetics. Recording electrodes were calibrated by recording responses (in electrical current; nA) to a known concentration of dopamine (3 µM) using a flow-injection system. This was used to convert electrical current to dopamine concentration. For MPH and amphetamine dose-response curves, a measure of apparent affinity (app. K_m) for the DAT was used to determine changes in the potency of the psychostimulants to inhibit dopamine uptake. As app. Km increases, the affinity of dopamine for the DAT decreases. Increasing concentrations of MPH or amphetamine decrease the affinity of dopamine for the DAT, such that shifts in app. Km across treatment groups indicate shifts in the potency of the drug directly at the DAT.

Calculating K_i Values: Inhibition constants (K_i) were determined by plotting the linear concentration-effect profiles and determining the slope of the linear regression (Jones et al., 1995; Calipari et al., 2013a). The K_i was calculated by the equation K_m/slope. K_i values are reported in µM and are a measure of the drug concentration that is necessary to produce 50% uptake inhibition.

Statistics: Graph Pad Prism (version 5, La Jolla, CA, USA) was used to statistically analyze data sets and create graphs. Release data and data obtained after perfusion of MPH or amphetamine were subjected to a two-way analysis of variance (ANOVA) with experimental group and concentration of drug as the factors. When main effects were obtained ($p < 0.05$), differences between groups were tested using a Bonferroni *post hoc* test.

Results:

IntA cocaine self-administration resulted in "spiking" brain cocaine levels, while ShA and LgA resulted in sustained brain cocaine levels. In order to determine the factors that influence psychostimulant potency, we manipulated temporal pattern of cocaine administration and daily cocaine intake (Fig 1). LgA (Fig 1A) and ShA (Fig 1B) resulted in a similar pattern of self-administration, which is characterized by sustained brain cocaine levels over a 6hr and 2hr session, respectively. Given the shorter session length, ShA rats administer significantly less cocaine per session (Calipari et al., 2013a). Thus, the comparison of ShA vs LgA was used to determine if the neurochemical adaptations that that occurred were due to differences in total daily intake. IntA animals were given 5 minute unlimited access periods to a cocaine-paired lever followed by 25 minute time-out periods. This resulted in a "spiking" pattern of brain cocaine levels characterized by rapid increases, which return near baseline during the time-out period (Fig 1C). Although the temporal profile of brain cocaine levels differs between ShA and IntA, both groups have comparable levels of cocaine intake (Calipari et al., 2013a). Thus, ShA vs IntA was used to determine the effect of different patterns of consumption on psychostimulant potency, given the same amount of daily cocaine intake.

Amphetamine potency was increased following IntA, but not LgA or ShA cocaine self-administration. ANOVA revealed a main effect of self-administration paradigm on amphetamine potency (app. K_m) ($F_{3, 84} = 6.213$, $p < 0.001$; Fig 2A, B). Bonferroni post hoc analysis revealed that there was a significant increase in amphetamine's effect on the DAT ($p< 0.001$) at the highest dose of the dose-response curve following IntA. K_i values for amphetamine ($t_9 = 2.765$, $p < 0.05$; Table 1) were reduced in the IntA group as compared to naïve control animals indicating increased potency.

ShA resulted in no change in amphetamine potency as measured by app. K_m (Fig 2) or K_i (Table 1) as compared to control animals. LgA, which also resulted in sustained cocaine brain levels, but with higher daily intake levels, resulted in no change in amphetamine potency as measured by app. K_m or K_i (Fig 2; Table 1) as compared to naive control animals or animals with a history of ShA cocaine self-administration.

MPH potency was increased following IntA, but not LgA or ShA cocaine self-administration. For the IntA group, ANOVA revealed a main effect of paradigm on app. K_m for MPH ($F_{3, 86} = 6.408$, $p < 0.001$; Fig 3A, B). Bonferroni post hoc analysis revealed that there was an increase in MPH potency at the highest dose of the dose-response curve ($p< 0.001$) as well as the 10 µM dose ($p < 0.05$) following IntA. Also, K_i values for MPH ($t_{10} = 2.474$, $p < 0.05$; Table 1) were reduced in the IntA group indicating an increased potency. ShA, which results in sustained cocaine levels at the DAT, resulted in no change in MPH potency as measured by app. K_m (Fig 3) or K_i (Table 1) as compared to control animals. LgA resulted in no change in

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MPH potency as measured by app. K_m or K_i (Fig 3; Table 1) as compared to naive control animals or animals that had undergone ShA cocaine self-administration.

MPH and amphetamine-induced dopamine alterations in peak amplitude of dopamine release is not determined by amount or pattern of cocaine intake. To determine how LgA, ShA, and IntA affected dopamine release in the presence of drug, we measured the effects of MPH and amphetamine on the peak height of evoked dopamine release across a dose-response curve for each of these drugs. First, we assessed the release profile of MPH to determine how it was affected by a prior cocaine self-administration history. Dopamine uptake occurs continuously in the presence of dopamine, thus, the peak height of evoked dopamine release is a balance between vesicular release and dopamine uptake via the DAT. Because of their ability to inhibit the DAT and slow uptake, blockers result in inverted “U” shaped dose-response curves, where they increase peak height at lower doses, due to their uptake-inhibition effects. However, at the higher doses, peak height is reduced, likely due to the increased uptake inhibition resulting in an inability of the terminal to effectively repackage dopamine into vesicles. MPH exhibits an inverted “U” shaped profile that is indicative of a dopamine transporter blocker (Fig 4A; Ferris et al., 2012). ANOVA revealed a main effect of MPH on dopamine release ($F_{3, 80} = 11.89$, $p < 0.05$). However, there were no differences in stimulated dopamine release in the presence of MPH between naïve control, LgA, ShA, and IntA (Fig 4A).

Amphetamine has a different profile than MPH due to its mechanism of action as a dopamine releaser. Because amphetamine is releasing dopamine at all times, independent of stimulated release, it dose-dependently depletes dopamine releasable pools leading to decreased evoked dopamine release over the dose-response curve (Fig 4B; Ferris et al., 2012; Calipari et al., 2013c). ANOVA revealed a main effect of amphetamine on dopamine release ($F_{3, 75} = 78.36$, $p < 0.0001$). There were no differences in stimulated dopamine release in the presence of amphetamine between naïve control, LgA, ShA, and IntA (Fig 4B).

Discussion

The present results show that the temporal pattern of cocaine intake during self-administration determines the changes in stimulant potency that occur following repeated administration of cocaine. Here we propose that the changes in psychostimulant potency following LgA are dictated by psychostimulant interactions with altered motifs on the DAT protein that only affect the potency of a select group of stimulants, while IntA induces non-specific increases in the potency of all psychostimulants. This is supported by the fact that LgA affects some drugs (cocaine) and not others (amphetamine, MPH), while IntA increases the potency of all of the compounds that were tested (cocaine, amphetamine, MPH). Previous work has demonstrated that LgA cocaine self-administration results in decreased cocaine potency; however, here we show no change in MPH or amphetamine potency, indicating that LgA-induced DAT changes do not affect the function of amphetamine-like compounds. This is consistent work using a limited intake extended access self-administration paradigm (5 days; 40 injections; 1.5mg/kg/inj) which showed reduced potency of cocaine and other blockers (nomifensine, bupropion) at the DAT, but no change in the potency of any releasers (amphetamine, phentermine, methamphetamine, benzypiperidine; methylenedioxymethamphetamine) or MPH (Calipari et al., 2012; Calipari et al., 2013b; Ferris et al., 2011; Ferris et al., 2012; Ferris et al., 2013). In addition, we show that IntA causes a sensitization of amphetamine and MPH, which is consistent with previous studies examining cocaine potency following IntA (Calipari et al., 2013a). Thus, unlike LgA, the effects of IntA cocaine self-administration on psychostimulant potency generalized to all stimulants tested, whereby we observed increases the potency of amphetamine (releaser) and MPH (amphetamine-like blocker) as compared to both ShA and naïve control groups.

The unique properties of MPH may give insight into the factors driving the changes in drug potency following LgA and IntA. Although classified as a blocker, binding studies show that MPH binds to the DAT in a fashion that is similar to amphetamine, likely due to certain structural similarities between the two compounds (Wayment et al., 2005; Dar et al. 1999). Studies using DAT mutants that are stabilized in either an outward-facing or inward-facing conformation have elucidated two distinct classes of psychostimulant compounds that are not dictated by their function as a “blocker” or “releaser”. Some compounds preferentially interact with outward-facing conformations as compared to inward-facing conformations. Outward-facing mutants increase the affinity for both MPH and cocaine, while leaving the affinity of the blockers benztropine, GBR12909, bupropion, or releasers such as amphetamine unaffected (Schmitt et al., 2008, 2010). However, changes in the inward/outward-facing selectivity of the DAT are likely not the mechanism for the changes observed following LgA within the current study, because these changes would likely affect cocaine and MPH in a similar fashion. Previous work has shown that cocaine potency is reduced following LgA (Calipari et al., 2013a), while the current study shows that MPH potency at the DAT is unchanged. Furthermore, IntA produces a sensitized effect for all psychostimulants tested to date, and one would expect differential effects of cocaine and MPH as compared to amphetamine if inward versus outward selectivity explained the effects reported within the current study. Therefore, the changes are likely not global changes to the conformation of the transporter, but rather a specific site that only alters the potency of a select group of stimulants.

It is tempting to postulate that LgA does not change the potency of amphetamine-like compounds because shifts in MPH potency closely resemble the profile of the dopamine releaser amphetamine; however, it is more likely that the differences between blockers and releasers and MPH are not only due to structure *per se*, but rather the way in which the compounds interact with the DAT directly, possibly at specific sites on the transporter protein. Giving further support to this idea is the fact that the structurally dissimilar releaser benzylpiperidine was also unaffected following extended-access cocaine self-administration (Ferris et al., 2012). Although structurally dissimilar from the other releasers tested, benzylpiperidine is transported via the DAT, and thus interacts with the transporter in a way that is similar to other structurally dissimilar releasers. Thus, although structural components are integral to determining unique conformational interactions with the DAT, a number of different structures can interact with the same motifs on the DAT protein (Schmitt, Rothman & Reith, 2013). Consistent with this hypothesis, recent studies comparing DAT inhibitors have demonstrated that the effects of DAT ligands are not based on class (blocker vs releaser), but rather specific to how each compound interacts with the transporter (Schmitt, Rothman & Reith, 2013). Here we suggest that particular structural components of the DAT are altered following LgA cocaine self-administration and that compounds that bind to the transporter at that site, regardless of structure, are affected by the alterations.

In contrast to LgA, IntA resulted in increased potency for MPH and amphetamine. Previous work has also shown that IntA results in increased cocaine potency (Calipari et al., 2013a), suggesting that the effects are not specific to class or interactions of each compound with the DAT, but rather affect all DAT-interacting compounds. The increased potency of MPH and amphetamine following IntA self-administration could be due to the fact that IntA results in increased DAT levels in these animals (Calipari et al., 2013a). Previous work has demonstrated that increased DAT levels leads to an increase in the potency of both amphetamine and MPH (Salahpour et al., 2008; Calipari et al., 2013c). If this is the case, then the increase in cocaine potency would have to be via a different mechanism, as numerous previous studies have demonstrated that increases in DAT levels do not increase cocaine potency. If anything, increased DAT levels have been demonstrated to decrease cocaine potency (Chen & Reith, 2007; Rao et al., 2013). Thus, it is possible that the increase in amphetamine and MPH potency is via increased DAT levels, while increased cocaine potency manifests via an intrinsic change to the DAT, both of which are occurring simultaneously.

While the mechanism driving the IntA-induced DAT changes is unclear, the fact that IntA results in increased potency for all of the psychostimulant drugs tested suggests that many drugs may have an elevated abuse liability / addiction potential following intermittent cocaine administration. Indeed, for cocaine, it has been demonstrated that the reinforcing efficacy of cocaine is increased following an IntA cocaine self-administration paradigm compared to LgA and ShA (Zimmer et al., 2012). Although this work has not been extended to other psychostimulants, it is likely that the reinforcing efficacy of the drugs tested here may be increased as well. These questions could be answered by conducting a comprehensive structure-function analysis a wide range of compounds that bind to the DAT and alter its function. One particularly interesting avenue would be the utilization of many structurally distinct DAT inhibitor compounds in order to determine if a specific structural component of the molecule is predictive of the changes in psychostimulant potency directly at the DAT following each of these cocaine self-administration paradigms.

Here we demonstrate that access conditions and the pattern of intake dictate not only the effects on cocaine potency as seen previously, but also whether these changes are conferred to other psychostimulants. We suggest that changes in psychostimulant potency following LgA are dictated by altered motifs on the DAT protein, which decrease the potency of a select group of stimulants, while intermittent patterns of administration induce non-specific increases in the potency of psychostimulants. The current work supports the hypothesis that the binding of DAT ligands to the transporter is not based on psychostimulant class, but rather specific to interactions with the DAT. Additionally, the divergent effects of LgA and IntA highlight the importance of considering pattern and total intake when modeling the behavioral and neurochemical processes involved in addiction. The aim of future studies should be to identify the specific DAT motifs that are altered following LgA cocaine self-administration. The identification of the specific DAT changes that occur following clinically relevant models of cocaine abuse could lead to targeted therapies that may allow for the reduction in cocaine potency without altering other critical aspects of DAT protein function.

Conflict of Interest: The authors have no conflicts to report.

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Authorship Contributions:
Participated in research design: Calipari, Ferris, Jones
Conducted experiments: Calipari, Ferris, Siciliano, Zimmer
Performed data analysis: Calipari, Ferris, Siciliano
Wrote or contributed to the writing of the manuscript: Calipari, Ferris, Siciliano, Jones

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Table/Figure Legends:

Figure 1. Differential effects of intermittent (IntA)-, short (ShA)-, and long (LgA)-access self-administration on presynaptic dopamine system kinetics. Work from Justice and colleagues (1991) was used to model brain levels of cocaine from representative animals within a self-administration session over time. LgA IntA, and ShA paradigms, expressed as arbitrary units. Each panel shows the modeled brain levels of cocaine (y-axis) versus time (x-axis) throughout the entire session for individual rats self-administering cocaine to highlight the fluctuation of cocaine levels within the brain over each representative session. (A) LgA results in high, sustained brain cocaine over the six hour session. Tick marks (below) represent infusions/responses on the lever (B) IntA is achieved by giving 5 minutes access followed by 25 minute forced time-outs. This results in “spiking” brain levels where animals load up to reach levels equivalent to LgA, but cannot maintain, thus cocaine is cleared from the brain and levels return near baseline. Spacing between clusters of tick marks is due to a forced time-out period. (C) ShA results in high, sustained brain cocaine over the two-hour session.

Figure 2. Intermittent access (IntA) self-administration results in sensitization to the neurochemical effects of amphetamine. (A) Representative traces from control (black), IntA (blue), long-access (LgA; red), and short-access (ShA; green) animals. Traces are represented as concentration in dopamine over time and are normalized to peak height. (B) Cumulative amphetamine (0.1-10 µM) dose response curves in slices containing the nucleus accumbens core. Amphetamine potency is unchanged following LgA and ShA and increased following IntA. *, $p < 0.05$ vs control; ***, $p < 0.001$ vs control; AMPH, amphetamine; DA, dopamine

Figure 3. Intermittent access (IntA) self-administration results in sensitization to the neurochemical effects of methylphenidate (MPH) while long-access (LgA) results in n change. (A) Representative traces from control (black), IntA (blue), LgA (red), and ShA (short-

access; green) animals. Traces are represented as concentration in dopamine (DA) over time and are normalized to peak height. (B) Cumulative MPH (0.3-30 µM) dose response curves in slices containing the nucleus accumbens core. MPH potency is unchanged following LgA and ShA and increased following IntA. *, $p < 0.05$ vs control; ***, $p < 0.001$ vs control

Figure 4. Long-access (LgA) and intermittent access (IntA) cocaine self-administration have no effect on dopamine release in the presence of amphetamine or methylphenidate (MPH). There were no differences in MPH or amphetamine-induced dopamine elevations between control, intermittent-access (IntA), long-access (LgA), or short-access (ShA) cocaine self-administration groups. (A) Normalized stimulated dopamine release measured across a dose-response curve for MPH. (B) Normalized stimulated dopamine release measured across a dose-response curve for amphetamine. AMPH, amphetamine

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Table 1: Summary of K_i values across drugs and paradigms

Treatment	Drug	K_i (µM)	$p < 0.05$ vs control
Control	Cocaine	0.397	- #
	Methylphenidate	0.258	-
	Amphetamine	0.080	-
Intermittent-Access	Cocaine	0.312	↑** #
	Methylphenidate	0.217	↑*
	Amphetamine	0.048	↑*
Long-Access	Cocaine	0.501	↓* #
	Methylphenidate	0.271	↔
	Amphetamine	0.076	↔
Short-Access	Cocaine	0.392	↔ #
	Methylphenidate	0.245	↔
	Amphetamine	0.077	↔

Note: K_i inhibition constant; ↑, increase; ↓, decrease; ↔, no change; *, $p < 0.05$ vs control; **, $p < 0.01$ vs control; #, data from Calipari et al., 2013a.

Figure 1

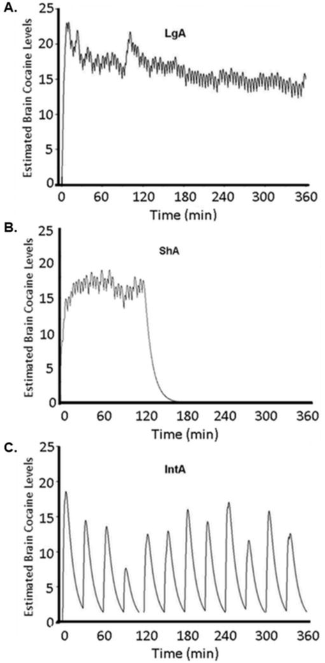

A.
LgA
Estimated Brain Cocaine Levels
0
5
10
15
20
25
0
60
120
180
240
300
360
Time (min)
B.
ShA
Estimated Brain Cocaine Levels
0
5
10
15
20
25
0
60
120
180
240
300
360
Time (min)
C.
IntA
Estimated Brain Cocaine Levels
0
5
10
15
20
25
0
60
120
180
240
300
360
Time (min)

Figure 2

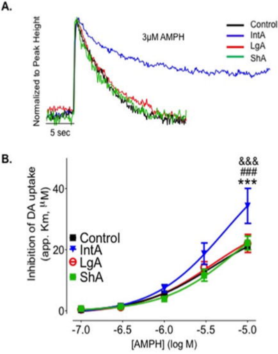

A.
Normalized to Peak Height
3μM AMPH
Control
IntA
LgA
ShA
5 sec
B.
Inhibition of DA uptake (app. Km, μM)
&&&
###
***
40
20
0
Control
IntA
LgA
ShA
-7.0
-6.5
-6.0
-5.5
-5.0
[AMPH] (log M)

Figure 3

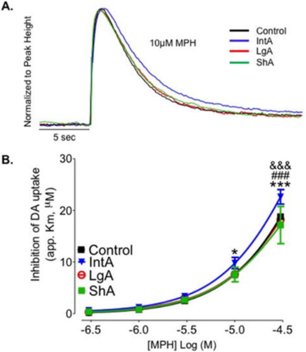
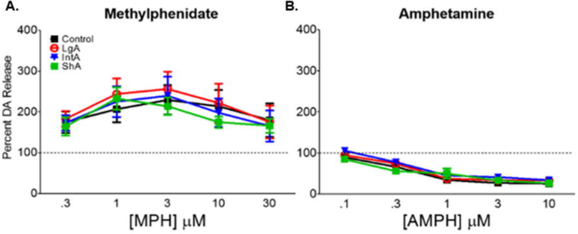

A.
Normalized to Peak Height
10μM MPH
Control
IntA
LgA
ShA
5 sec
B.
Inhibition of DA uptake (app. Km, μM)
30
20
10
0
&&&
###
***
*
■ Control
▼ IntA
⊖ LgA
■ ShA
-6.5
-6.0
-5.5
-5.0
-4.5
[MPH] Log (M)

Figure 4


A.
Methylphenidate
B.
Amphetamine
Control
LgA
IntA
ShA
Percent DA Release
400
300
200
100
0
.3
1
3
10
30
[MPH] μM
400
300
200
100
0
.1
.3
1
3
10
[AMPH] μM