Extended Access to Cocaine Self-Administration Enhances Drug-Primed Reinstatement but not Behavioral Sensitization

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Non standard abbreviations: ShA: Short Access; LgA: Long Access; FR-1: Fixed Ratio-1; IP: intraperitoneal; TO: time out; ANOVA: Analysis of Variance; D2: Dopamine 2 Receptor

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ABSTRACT

Cocaine addicts increase the frequency and amount of drug use over time. This characteristic has been modeled by escalating drug intake in rats given extended access to cocaine. However, it remains unclear if the behavior of escalating drug intake or simply increased drug dose is the relevant aspect of this model. The present study investigated whether the phenomenon of escalated drug-taking enhances cocaine-induced sensitization and reinstatement. Rats were trained to self-administer cocaine during daily 1 hr sessions for 7 days. A short access (ShA) group continued to receive 1 hr sessions for 10 days while a group of rats was switched to 6 hr of drug access (ShA-LgA) for 10 days. In addition, a long access only (LgA-only) group was added that was not pre-trained but was only given extended access for 10 days. ShA subjects maintained stable drug intake; ShA-LgA subjects escalated intake while LgA-only subjects decreased drug intake. All groups displayed an inverted-U shaped cocaine dose-response curve in both the sensitization and reinstatement tests. There was no difference in the expression of behavioral sensitization between groups. While both long access groups showed reinstatement at more doses of cocaine than the ShA group, the presence of escalation did not affect reinstatement. These results indicate that extended access to cocaine self-administration produces behavioral differences relative to traditional short access animals in reinstatement, but not sensitization. Furthermore, the differences in reinstatement are predicted more by length of cocaine access than by escalation of drug intake.
INTRODUCTION

A major challenge for the field of neuropsychopharmacology is developing predictive animal models of neuropsychiatric disorders. Among the most well-characterized models is the drug self-administration model in which subjects learn to perform an operant response to achieve intravenous infusions of drug (Weeks 1962). Initial work conducted with drug self-administration allowed subjects unlimited access to the drug, which oftentimes resulted in death by overdose (e.g. Johanson, Balster, & Bonese, 1976). Thus, many researches moved to a “short access” model which permits limited (one or two hour) access to drug infusions. In this situation, animals typically attain a stable level of responding for drug. However, human addicts do not maintain steady intake, but instead increase the frequency and/or amount of drug administration over time (Gawin and Kleber, 1988; Gawin, 1991). In order to more closely mirror the human condition, some researchers have proposed using a “long access” model of drug self-administration in which rats are given six or more hours of drug access. The gradual increase in drug-taking which typically results from extended access is termed “escalation” and is thought to be analogous to the loss of control over drug-taking demonstrated by addicted humans (Ahmed and Koob, 1998; Ahmed et al., 2000; Mantsch et al., 2001; Paterson and Markou, 2003; Ben-Shahar et al., 2004; Ferrario et al., 2005).

Given that extended access protocols are more costly in terms of time and resources, it is important to validate the involvement of escalation in classic behavioral procedures thought to model different aspects of drug addiction. Both behavioral sensitization and reinstatement of drug seeking in animals withdrawn from drug self-administration have been suggested to model aspects of addiction. The sensitization of motor behaviors after repeated drug is proposed to be analogous to human addicts attributing progressively greater salience to drugs and the cues that
predict drugs (Robinson and Berridge, 2000; Robinson, 2004), and reinstatement is thought to model relapse (de Wit and Stewart, 1981). It should be noted that while the reinstatement model is viewed as having adequate criterion validity, many would argue that it has weak construct validity (Epstein et al., 2006; Katz and Higgins, 2003).

Previous studies comparing the degree of sensitization after long (LgA) or short access (ShA) to cocaine have yielded conflicting results with some finding that LgA subjects display tolerance (Ben-Shahar et al., 2004), sensitization (Ferrario et al., 2005), and identical locomotion (Ahmed and Cador, 2006) in comparison with ShA animals. However, it should be noted that Ahmed and Cador performed sensitization testing only 24 hours after the last self-administration session. While Ferrario et al. (2005) report a failure of both LgA and ShA animals to sensitize to traditional estimates of locomotion, they identified enhanced sensitization of head movements in the LgA group.

A variety of results have also been reported when comparing LgA and ShA subjects on the reinstatement of drug-seeking. This paradigm involves extinction of a previously reinforced response (i.e. lever presses) and subsequent, non-contingent presentation of the reinforcer itself (i.e. drug) or reinforcer-related cues (Stewart and deWit, 1987). Ferrario et al. (2005) reported enhanced reinstatement of cocaine-seeking behavior in LgA animals relative to ShA animals. However, these authors omitted the extinction portion of the reinstatement paradigm and tested animals which had been in withdrawal for 47 days and were thus measuring abstinent-relapse. Similarly, Ahmed & Cador (2005) reported greater reinstatement in LgA animals while testing after only 24 hours of withdrawal and permitting animals just one 45-min extinction session. When the traditional two to three weeks of extinction training was implemented, De Vries et al. (2005) found no differences in reinstatement behavior between LgA and ShA animals while
Mantsch et al. (2004) found that LgA animals displayed enhanced reinstatement relative to ShA subjects. Although Kippin, Fuchs, and See (2006) did not find escalation of drug-taking in their LgA subjects, they found that this group nonetheless displayed enhanced drug-primed reinstatement of cocaine-seeking.

The present study was designed to examine the effects of long access and the relevance of escalating drug intake in the sensitization and reinstatement models of cocaine addiction. In addition to short access (ShA) and long access (ShA-LgA) treatment groups, a novel long access group was included that was not pre-trained using short access to cocaine (LgA-only). It was predicted that this group would fail to escalate, and thus serve as a control for the length of access given to the traditional ShA-LgA group. Thus, by examining a dose-response curve for cocaine-induced sensitization and reinstatement of drug seeking, it was shown that access duration and escalation were without effect on behavioral sensitization. In contrast, long access resulted in reinstatement to more doses of cocaine, but the increase was independent of the presence of escalated drug intake.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats (Charles River Laboratories, Indianapolis, IN) weighing 250-275g were housed individually in a temperature controlled vivarium on a 12h/12h light-dark cycle (lights on at 0700h). All experiments were conducted during the light cycle. Animals were provided with ad libitum food and water with the exception of the five day food-training procedure during which animals only received food during operant training. All experiments were conducted according to specifications of the National Institute of Health Guide for the Care
and Use of Laboratory Animals. A total of 96 rats were used for this study, of which 24 rats were excluded for the following reasons: 8 for failing to acquire cocaine self-administration, 6 for failing to meet extinction criteria, and 9 for catheter failure.

Drugs

Cocaine hydrochloride was generously donated by the National Institute on Drug Abuse (Bethesda, MD) and was dissolved in 0.9% physiological saline for injection. For the purposes of self-administration, a 5 mg/ml solution was prepared and subjects received 0.25 mg/infusion. Doses of 3, 10, and 30 mg/kg cocaine (IP) were administered for both the sensitization and reinstatement studies.

Self-administration Procedures

After one week of acclimatization to the colony room and handling procedures, animals were food-deprived and given five one-hour sessions (one session/day) of food training in a two-lever operant chamber (Med Associates, St. Albans, VT). For complete description of operant chambers see (McFarland et al., 2003). Animals were trained on an FR-1 schedule of reinforcement in which each press on the active lever (always the right lever) resulted in the delivery of one 45 mg food pellet (Noyes) and the illumination of the stimulus light above the active lever. The light signified the length of the time-out (TO) period during which presses on the active lever were recorded but did not result in delivery of the reinforcer. During food training, the TO period was initially 1 s until 100 pellets were earned in one session. After reaching this criterion, subjects experienced one session in which the TO was 10 s, and then the remaining sessions used a TO of 20 s. Three to seven days following food training, subjects
underwent surgery for the implantation of jugular catheters for the delivery of intravenous cocaine as described previously (McFarland et al., 2003). Upon recovery from surgery (3 to 7 days), animals began cocaine self-administration (FR-1, 20 s TO) training for one hr/day during which active lever presses resulted in a cocaine infusion (0.25 mg in 0.05 ml over 2.7 s). A subset of animals (n=12) were yoked-saline controls and received an infusion of saline (0.05 ml over 2.7 s) when their yoked counterpart received a cocaine infusion. This acquisition period lasted 7 days, at which point cocaine self-administering subjects were divided into two groups: a short access (ShA, n=21) group which continued to receive 1 hr of access to cocaine and a long access (ShA-LgA, n=22) group which was permitted 6 hrs of access to cocaine for the next ten days (the escalation period). Another group was added at this point, a long-access only (LgA-only, n=23) group which never experienced the 1 hr training sessions that the ShA-LgA group received but instead only experienced 10 days of 6 hr access to cocaine self-administration.

Animals underwent self-administration sessions every day of the week, with no drug-free days until the conclusion of the escalation period. Upon conclusion of the 10 day “escalation period,” a subset of animals (n=36) was returned to their home cages for a 2 week withdrawal period while a separate group of animals (n=42) underwent extinction procedures.

**Reinstatement testing**

Subjects underwent extinction of the lever-pressing response during which presses on the active lever no longer resulted in cocaine infusions. Animals received one daily 1 hr extinction session for a minimum of two weeks. After pressing reached 20% of self-administration levels, animals were tested with 0, 3, 10, or 30 mg/kg cocaine (IP). Animals were again subjected to extinction procedures a minimum of 3 sessions or until the previous extinction criterion was met, and were
tested with a second dose of cocaine different from the dose utilized in the first reinstatement trial. The order of doses was randomized.

**Behavioral Sensitization Testing**

Behavioral activity was measured in a photocell apparatus (Omnitech Electronics Inc., Columbus, OH). On the day prior to the first cocaine sensitization test subjects were placed into the apparatus for a 60 min acclimation period and then received a saline injection (0.25 mL, IP) and behavioral activity was recorded for 120 minutes. The following day, animals were placed in the apparatus for the 60 min acclimation period before receiving an injection of one of three doses of cocaine (3, 10, or 30 mg/kg, IP). Behavior was recorded in 10 min increments for the 120 min following the cocaine injection as well as for the habituation period. Each rat was tested with all doses of cocaine in random order; each test was separated by 4 to 6 days. Motor activity was quantified as total distance traveled (estimated by breaking of adjacent photobeams), total horizontal beam breaks, vertical movements, and stereotypy counts (estimated by repetitive breaking of the same photobeam).

**Data Analyses**

The behavioral data were compared using Analyses of Variance (ANOVA) or when comparing only two groups, a two-tailed Student’s t-test was used. Because subjects in the reinstatement portion of the experiment were tested with only two challenge doses of cocaine and did not receive all treatment doses, these analyses were done assuming independent groups, because repeated-measures analyses were not possible. When a statistically significant interaction (p < 0.05) was obtained with an ANOVA, the Least Significant Differences (LSD) post-hoc test was
used to assess specific group differences (Milliken and Johnson, 1984). For a one-way ANOVA, a Students’ t-test with a Bonferroni adjustment was used for post-hoc comparisons.

RESULTS

Self-administration

A change in cocaine intake during the 10 day escalation period was quantified by comparing the average of the mean number of infusions obtained during the first 3 days (days 1-3) with the last 3 days (days 8-10). This was done to account for the variance that was observed in the day-to-day cocaine intake of both long access groups. Most previous studies employing long access paradigms find that the escalation of drug-taking is most prominent during the first hour of the self-administration session (Ahmed and Koob, 2004; Ahmed and Cador, 2006). Accordingly, Figure 1 shows that escalation of cocaine infusions in the ShA-LgA group occurred during the first hour (t(18)=2.510, p=0.022; Figure 1B and 1D), but not when averaged over the entire 6 hour session (t(18)=1.267). In contrast, the ShA subjects maintained stable levels of cocaine intake (t(18)=0.490; Figure 1A and 1D), while the LgA-only group significantly decreased intake during the first hour (t(20)=2.128, p=0.045), as well as over the 6 hour access period (t(20)=3.053, p=0.006; Figure 1C and 1D).

Reinstatement of Drug Seeking

While the ShA group initially displayed greater responding on the active lever during extinction training, all groups decreased their active lever pressing in a similar manner during the first 10 extinction sessions (Fig 2A), yielding a significant effect of Time (F(9,18)=5.236, p=0.001) but not Group (F(2,36)=0.808). It should be noted that Fig 2A only displays the first 10 days of
extinction training since all animals experienced a minimum of 10 extinction sessions (5 trials/week for two weeks) but the majority of subjects required additional sessions to meet extinction criteria (20% of active lever pressing achieved during the last three days of self-administration). There was no significant difference in the mean number of days to reach extinction criteria between the three groups (F(2,33)=0.862; ShA: 12.7±3.4; ShA-LgA: 11.7±2.7; LgA-only: 11.5±1.6). Six animals were excluded for failing to meet criteria by week 3 of extinction training (ShA: 2; ShA-LgA: 3; LgA-only: 1).

Responses on the active lever during a reinstatement test were compared to the average number of responses made during the last three days of extinction training. All three groups showed an inverted U shaped dose-response curve for reinstated cocaine-seeking behavior (Fig 2B). A two-way ANOVA yielded a significant effect of Group (F(2,6)=5.626, p=0.005) and Dose (F(3,6)=10.507, p<0.001) but no Group X Dose interaction (F(6,100)=0.868). A LSD post-hoc comparison between groups revealed that the LgA-only and the ShA-LgA groups were not different from each other (p=0.498), but that the ShA group differed significantly from both the ShA-LgA group (p=0.048) and the LgA-only group (p=0.007). Although a significant interaction was not measured by the overall ANOVA, if a one-way ANOVA was conducted within each group, all groups displayed significant reinstatement in comparison with extinction responding (ShA: (F(3,31)= 6.628, p= 0.001); ShA-LgA: (F(3,32)= 4.570, p= 0.009); LgA-only: (F(3,40)= 3.77, p=0.018)), and the ShA group (p< 0.001) displayed significant increases in active lever presses relative to extinction only after the 10 mg/kg injection of cocaine, while the LgA-only group reinstated to both 10 mg/kg dose (p= 0.012) and 30 mg/kg (p= 0.022). The ShA-LgA group also significantly reinstated to two challenge doses: the 3 mg/kg (p=0.021) dose and the 10 mg/kg dose (p< 0.001). Fig 2C displays the mean inactive lever responding during extinction
and reinstatement testing. A two-way ANOVA revealed no significant Group x Dose interaction (F(6,100)=0.459), nor were there effects of Group (F(2,6)=0.099) or Dose (F(3,6)=0.940) on responding for the inactive lever during reinstatement.

Behavioral Sensitization

Four different indices of behavioral sensitization were used: distance traveled, overall horizontal activity, vertical movements and stereotypy counts. There were no significant effects measured in vertical movements so these data are not illustrated. Figure 3 displays the time-course and dose-response function for all the behavioral measures that shared a general biphasic character and a peak effect at 10 mg/kg. Figure 3A shows the temporal pattern of distance traveled when rats were injected with 10 mg/kg, and a two-way ANOVA with repeated measures over time revealed a significant Group X Time interaction (F(33,352)=1.959, p=0.002) and a significant effect of Time (F(11,33)=41.484, p< 0.001), but not Group. While all three self-administration groups show significant sensitization compared with the yoked-saline controls during the first 40 min after cocaine administration, they did not differ between each other. The dose-response function for total distance traveled over the 30 minutes following the injection reveals that all self-administration groups displayed similar behavior relative to yoked-saline controls. A two-way ANOVA with repeated measures indicates that there is a significant effect of Dose (F(2,6)=17.701,p<0.001) but not Group, and no significant Dose x Group interaction. Although there was not a significant interaction, a one-way ANOVA conducted between treatment groups at the 10 mg/kg dose revealed a significant effect (F(3,32)=2.906, p=0.05), and a post-hoc analysis (LSD) revealed that this effect was due to the saline group being significantly different from all cocaine groups.
A similar pattern was observed for horizontal activity (Fig 3B). A two-way ANOVA with repeated measures over time revealed a significant Group X Time interaction (F(33,352)=2.040, p=0.001) and a significant effect of Time (F(11,33)=48.915, p<0.001), but not Group. All three self-administration groups showed significant sensitization compared with the yoked-saline controls during the first 40 min after cocaine administration. Analysis of the dose-response function for horizontal activity over the 30 minutes following the injection revealed a significant effect of Dose (F(2,6)=17.607, p<0.001) but not Group, and no significant Dose x Group interaction. Although there was not a significant interaction, a one-way ANOVA conducted at the 10 mg/kg dose revealed a significant difference between the saline group and all the cocaine groups (F(3,32)=3.439, p=0.028).

While both the time-course and dose-response function of the mean number of stereotypies (Fig 3C) resembled the measures of distance traveled and total horizontal activity, a two-way ANOVA with repeated measures over time revealed only an effect of Time (F(11,33)=48.137, p<0.001), and no Group or Time x Group interaction. However, a one-way ANOVA conducted at the 10 mg/kg dose revealed a significant difference between the saline control group and the three cocaine groups (F(3,32)=4.117, p=0.014).

Escalated vs. Non-escalated subjects

To further evaluate sensitization and reinstatement relative to escalating drug intake during the training period, a median split on was conducted on the first hour self-administration data to divide the ShA-LgA subjects into “escalated” and “non-escalated” subjects. Again, the mean number of infusions attained in days 1-3 were compared with the mean number attained in days 8-10. The non-escalated subjects either decreased mean infusions over the course of the
experiment or only increased a maximum of 3 infusions (1.3, -15 to 3; median, range). All escalated subjects increased the number of infusions (13.7, 3.3 to 20.7; median, range). As shown in Figure 4A, escalated subjects clearly increased the mean number of infusions obtained in the first hour of the self-administration session over the course of the escalation period (t(9)=4.137, p=0.003) while the non-escalated subjects did not (t(8)=0.430).

Re-examination of the data shown in figures 2 and 3 after dividing the groups into escalated and non-escalated ShA-LgA subjects revealed no effect of Group and no Group x Dose interaction on the measures of sensitization and reinstatement (Figures 4B and C). A significant effect of Dose was measured in the sensitization experiment (F(1,6)=4.607, p=0.033)

DISCUSSION

When given the opportunity to self-administer cocaine for an extended period of time, the ShA-LgA subjects significantly escalated the intake of drug in the first hour of the self-administration session over the course of 10 days. This was in contrast with the ShA subjects that remained stable in their drug consumption, and the LgA-only subjects which showed a decrease in cocaine self-administration. Thus, three populations of subjects were generated: a stable group, a group which escalated its drug intake, and a “decelerated” group (see Figure 1). It is possible that the deceleration was an artifact of food-training in that this group alone was not initially given 1 hr sessions, but rather its first experience with cocaine self-administration was during a 6 hour session. Thus, the high level of cocaine attained on the first day of self-administration may have been aversive and resulted in deceleration of self-administration. The ShA group displayed significantly different reinstatement from either of the LgA groups, and the LgA groups did not differ from each other in the reinstatement of drug seeking. Thus, regardless of acceleration or
deceleration of cocaine self-administration, training with six hours of cocaine access caused subjects to reinstate to more doses of cocaine than the ShA subjects. Despite the different patterns of self-administration behavior, all groups expressed similar dose-response curves for behavioral sensitization. Further supporting the idea that escalated drug intake itself does not underlie differences in subsequent cocaine-induced behavior, dividing animals into escalating and non-escalating groups revealed no differences in the reinstatement of drug-seeking or expression of sensitization. Additionally, the modest escalation seen in these rats and the fact that only 50% of ShA-LgA subjects escalated over the course of the experiment raises the question of whether escalation is truly a robust phenomenon in Sprague-Dawley rats.

**Reinstatement of Drug Seeking**

The present data show that long-access training caused animals to reinstate to more doses of cocaine, but that the augmentation was independent of escalating drug intake. This was revealed both by using a cocaine dose-response comparison between self-administration groups, as well as by dividing animals into escalating and non-escalating subgroups. To date, four publications report testing for reinstatement of drug seeking in long access versus short access subjects (Mantsch et al., 2004; Ferrario et al., 2005; Ahmed and Cador, 2006; Kippin et al., 2006). Akin to the present experiment, all four studies found enhanced reinstatement in long access relative to short access subjects.

Comparing the experimental details and results of these four reinstatement studies in combination with the present manuscript points to a conclusion that the main factors influencing reinstatement behavior is the length of access to self-administration and potentially the dose of cocaine attained. The presence of escalated drug intake, strain of rats, withdrawal period, and
extinction training has not been shown to enhance reinstatement in rats trained on a long access regimen of cocaine self-administration. For example, while the long access animals in the Kippen et al. (2006) study did not show escalated intake, these subjects nonetheless demonstrated the same enhancement in reinstatement displayed by the subjects in the other three published studies (Mantsch et al., 2004; Ferrario et al., 2005; Ahmed and Cador, 2006). Mantsch et al. (2004) showed that long access training at higher doses of cocaine was more likely to induce escalating drug intake, and found that augmented reinstatement to a cocaine priming injection was dependent on the long access training dose. Thus, while the long access rats trained on a low dose of cocaine showed increased reinstatement compared to the short access group, long access rats trained on the high dose demonstrated even more robust reinstatement. While strain may play a role in propensity to escalate cocaine-seeking (C. Ferrario, personal communication), it has no apparent effect on the augmented reinstatement in the published studies since both Wistar (Ferrario et al., 2005; Ahmed and Cador, 2006) and Sprague-Dawley (Mantsch et al., 2004; present data) rats show increased reinstatement after long access training. Finally, the augmented reinstatement measured by Ferrario et al. (2005) was found in animals that did not undergo extinction training, but were placed in abstinence for 45 days after discontinuing daily training sessions. Similarly, Ahmed & Cador (2006) tested for reinstatement after only 24 hours of withdrawal and used only one extinction training session which took place immediately prior to reinstatement testing. Thus, even though extinction training is known to induce neurobiological adaptations in cocaine trained animals (Sutton et al., 2003), this factor has not been shown to significantly influence the augmented reinstatement accompanying long access training.
Behavioral Sensitization

Akin to previous studies (Hooks et al., 1994; Phillips and Di Ciano, 1996), animals trained to self-administer cocaine using a short access paradigm demonstrated behavioral sensitization. However, similar to observations by Ahmed and Cador (2006), sensitization was not augmented by long access training. Moreover, there was no difference in sensitization between escalating and non-escalating long access subjects. In contrast, other studies report enhanced (Ferrario et al., 2005) and diminished (Ben-Shahar et al., 2004; Ben-Shahar et al., 2005) expression of behavioral sensitization in escalated subjects when testing at least 2 weeks after the last self-administration session. Although many parameters (e.g. the strain of rats used and the dose of cocaine self-administered) in the present study were identical to those in the Ben-Shahar et al. studies, over a range of challenge doses, sensitization was equivalent between the short access and long access subjects. However, it should be noted that the report of decreased sensitization in long access animals may be due to the intravenous administration of the challenge dose of cocaine (Ben-Shahar et al., 2004). When these investigators administered a 15 mg/kg challenge injection via the IP route 14 days after discontinuing cocaine self-administration, they no longer found sensitization in either short access or long access subjects relative to saline subjects, and the motor response between the short access and long access groups was equivalent. Interestingly, testing these subjects again at 60 days post-self administration yielded a reduced locomotor response in long access subjects, while the short access subjects showed behavioral sensitization (Ben-Shahar et al., 2005). Akin to Ben-Shahar et al. (2005), Ferrario et al. (2005) reported no differences in the mean number of beam breaks (analogous to the “horizontal activity” measure used in the present paper) between long access, short access, and control subjects administered cocaine after 30 days of withdrawal from cocaine self-administration.
Although these animals did not show evidence of sensitization estimated by standard automated measures of motor activity, when Ferrario et al. (2005) visually quantified “head movements” they found evidence that both short access and long access sensitized relative to saline control subjects, and that the response was augmented in long-access compared with short access subjects. Given that some studies did not find augmented sensitization in rats trained on long access protocols (Ben-Shahar et al., 2004; Ben-Shahar et al., 2005; present data), and that the one study showing enhanced sensitization after long access to cocaine did not find sensitized behavior in measures known to reliably manifest sensitization (Kalivas and Stewart, 1991), escalation of cocaine intake may have less impact on sensitization than other experimental variables such as dose, timing or route of the test injection of cocaine, or how the behavioral profile is quantified.

Conclusions

Taken together with the studies outlined above, the present data make a clear statement that escalation of drug intake is not critical for long access training to augment cocaine-induced reinstatement, nor is it related to changes in behavioral sensitization. It is possible that after other doses of cocaine or durations of self-administration there may be a greater correlation between the presence of escalated intake and reinstatement or sensitization. However, the present study clearly shows dissociation between these behaviors and casts doubt on the utility of escalating drug intake in rats as an important influence on traditional models of cocaine-induced behavioral plasticity. Nonetheless, it remains important to contrast the biological effects of higher doses of self-administered cocaine, regardless of whether escalating drug intake is a meaningful correlate of addiction in rodents. Studies to date which make neurochemical or
morphological comparisons between animals trained on different cocaine access paradigms find that both access conditions produce the same direction of changes but the long-access subjects show an enhancement of these changes relative to the short-access subjects, including increased D2 and preproenkephalin mRNA in the striatal complex (Mantsch et al., 2004). Increases in dendritic spine density in both the shell and core of the nucleus accumbens of short access rats relative to yoked-saline controls (Robinson et al., 2001) are augmented by long access training only in the core (Ferrario et al., 2005). Other changes, including relatively reduced activation of c-fos in a number of brain regions in long access subjects, indicate that in some measures, higher drug intake is resulting in tolerance (Ben-Shahar et al., 2005). Based upon present findings, these comparisons between long and short access in terms of biological changes may be more readily interpreted as correlates of access duration and dose and not escalated intake.

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REFERENCES


FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. The number of cocaine infusions taken during the first hour of self-administration and during the entire self-administration session.  A) The mean ± sem number of cocaine infusions taken by the ShA group remained stable over the 10 day escalation period.  B) The ShA-LgA group significantly escalated their drug-taking in the first hour of the session but not over the entire session.  C) The LgA-only group significantly decreased their first hour and entire session drug-taking.  D) When the average of the first hour intake over the first 3 days of self-administration was compared with the first hour intake in the last 3 days, the ShA-LgA subjects escalated while the LgA-only subjects decreased drug intake, while the ShA subjects remained stable.

* p< 0.05, paired two-tailed Student's t-test

Figure 2. Active and inactive lever presses during extinction and reinstatement testing.  A) The mean active lever presses during the first 10 days of extinction training.  B) Mean ± sem responses made on the active lever during reinstatement induced by an injection of cocaine (3, 10 or 30 mg/kg IP). Extinction responding is shown as the mean ± sem of the last three days of extinction training.  C) Responses made on the inactive lever during extinction and reinstatement testing. Number of determinations shown in the bar.

* p< 0.05, comparing each dose to extinction within each group, using a one-way ANOVA
+ p< 0.05, comparing ShA with ShA-LgA and LgA-only, using a two-way ANOVA

Figure 3. Cocaine-induced motor behavior. Each animal was injected with 3, 10 or 30 mg/kg (IP) in random order separated by a 4-6 day inter-trial interval.  A) Time course data for distance
traveled during the 60 minutes prior to the 10 mg/kg cocaine injection and the 60 minutes following the injection (left). The dose-response curve for the mean ± sem distance traveled over the first 30 min after injection is also shown (right). **B**) Time course and dose-response curve for horizontal photobeam breaks. **C**) Time course and dose-response curve for stereotypy.

* p< 0.05, compared to yoked saline at each time point using a two-way repeated measures ANOVA followed by LSD (Milliken and Johnson, 1984).

+ p< 0.05, compared to yoked saline at each dose, using a one-way repeated measures ANOVA

**Figure 4.** Using a median split to separate the ShA-LgA group into escalated and non-escalated subjects shows no effect of escalation on reinstatement or sensitization. **A**) Mean ± sem average number of infusions on the first 3 versus last 3 days of self-administration training; derived from data shown in figure 1D. **B**) Distance traveled between the escalated and non-escalated subjects derived from figure 3A. **C**) Active lever presses induced by a cocaine priming injection derived from data in figure 2B. Number of determinations shown in the bar, and all data are shown as mean ± sem.

* p< 0.05, comparing days 1-3 with days 8-10, using a two-tailed paired Students t-test.
Figure 3
Figure 4

- Panel A: Infusions (first hr)
  - Days 1-3: Non-escalated (9) vs. Escalated (10)
- Panel B: Distance Traveled (cm)
  - Non-escalated: 4, Escalated: 4
- Panel C: Active Lever Presses
  - Extinction
  - 3 mg/kg
  - 10 mg/kg
  - 30 mg/kg
  - Non-escalated: 5, Escalated: 6