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Dose preference and dose escalation in extended-access cocaine self-administration in Fischer and Lewis rats

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Abstract

Rationale—Drug addiction is a disease with a genetic component that may be involved in different stages of its progression. Cocaine users escalate unit doses and frequency of self-administration events in naturalistic settings. Rats that self-administer drugs of abuse over extended sessions increase the number of infusions over days.

Objectives—Comparison of two genetically different inbred rat strains, Fischer and Lewis, in a new self-administration paradigm whereby rats select between different unit doses of cocaine, thus potentially escalating the unit dose and the number of infusions.

Methods—Extended (18 h/day) self-administration sessions lasted for 14 days. Rats had access to two active levers associated with two different unit doses of cocaine. If a rat showed preference for the higher unit dose, then the available doses were escalated in the following session. Four cocaine unit doses were available (0.2, 0.5, 1.25, and 2.5 mg/kg/infusion).

Results—Lewis rats showed a clear preference for the two higher doses of cocaine (70% of rats), with a high percentage (35%) of the individuals escalating to the highest unit dose, and escalated the total amount of cocaine taken over days. Fischer rats, however, preferred the two lower doses (63%) and did not escalate the amount of cocaine taken over days. Fischer, but not Lewis, rats showed an activated hypothalamic–pituitary–adrenal axis in acute withdrawal (24 h).

Conclusion—This work shows the power of a model of extended-access self-administration that allows for the subject-controlled dose-escalation of the unit dose of cocaine, and underlines the genetic differences that modulate cocaine intake.

Keywords

Cocaine; Self-administration; Escalation; Fischer; Lewis; HPA

Introduction

Genetic factors may contribute to the development of drug addiction, with an estimated heritability of 30%–60% in humans (Tsuang et al. 1999, 1998). These factors may be involved in all stages of the progression from the initial use of drugs of abuse to the establishment of regular use, to addiction and relapse.

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Inbred rat strains are used to examine genetic factors modulating the effects of drugs, having the advantage over outbred strains of a more homogeneous genotype (George and Goldberg 1989; Hedrich 2006). Among the inbred strains, Lewis and Fischer rats are typically compared because of behavioral and neuronal molecular differences in responding to drugs of abuse. Lewis rats are more responsive to a number of drugs in several behavioral tests than Fischer rats; e.g., Lewis rats more readily acquire morphine (Ambrosio et al. 1995) and cocaine (Kosten et al. 1997) self-administration. Lewis rats show stronger cocaine conditioned place preference (CPP) (Kosten et al. 1994), and only Lewis, but not Fischer, rats develop nicotine CPP (Horan et al. 1997). Ethanol is a stronger positive reinforcer in Lewis than in Fischer rats (Suzuki et al. 1988).

The mesolimbic dopaminergic system is central for the rewarding effects of drugs. Naïve Lewis and Fischer rats differ in a number of cellular characteristics within this system (Kosten and Ambrosio 2002), which responds differently when challenged with various drugs of abuse (Cadoni and Di Chiara 2007; Cadoni et al. 2009; Haile et al. 2001; Sanchez-Cardoso et al. 2009).

Fischer and Lewis strains also differ in the responsivity of the hypothalamic–pituitary–adrenal (HPA) axis to stress stimuli (Chaouloff et al. 1995; Dhabhar et al. 1997; Kosten and Ambrosio 2002). Basal corticosterone level in male Lewis rats is lower than in Fischer rats at any time point during 24 h (Griffin and Whitacre 1991). Fischer rats maintain higher levels of adrenocorticotrophic hormone (ACTH) after a prolonged stress than Lewis rats (Dhabhar et al. 1997). Corticosterone response to an acute restraint stress is also higher in Fischer than Lewis rats (Dhabhar et al. 1993). The corticosterone system in Fischer rats shows little habituation in response to prolonged restraint stress or repeated stress stimuli (Dhabhar et al. 1997). However, in Fischer rats chronically treated with “binge” cocaine injections for 14 days, the corticosterone system shows habituation (Zhou et al. 1996).

Animal models that mimic certain aspects of human drug self-administration have been available for years and are valid tools for studying pre-clinical treatments of cocaine and opiate abuse (Haney and Spealman 2008; Mello and Negus 1996). In these models, animals are typically allowed to self-administer a drug during short sessions (up to 3 h/day), and this results in a stable pattern of drug intake. However, this experimental design does not reproduce compulsive and uncontrolled drug-seeking and -taking behaviors, which are characteristics of addictive diseases. When intravenous self-administration (IVSA) sessions are extended to 6 h or more, animals exhibit escalation of cocaine intake (Ahmed and Koob 1998, 1999; Kruzich et al. 2003; Mantsch et al. 2001, 2003, 2004; Roth and Carroll 2004; Tornatzky and Miczek 2000; Wee et al. 2007).

Commonly, in rodent studies, animals are allowed to self-administer only one unit dose of drug throughout the study, while humans may escalate the unit dose of cocaine over time (Kreek et al. 2009; Zernig et al. 2007). Indeed, the outbred Sprague–Dawley rats escalate the dose of morphine when four different unit doses are available (Kruzich et al. 2003).

In this study, we compared cocaine self-administration in Fischer and Lewis rats exposed to cocaine for 18 h/day. Importantly, in this new model, rats could choose and escalate the unit dose of cocaine per infusion received. Thus, we studied whether the two strains of rats would self-administer a different overall amount of cocaine during the exposure, and whether they would escalate the amount of cocaine taken across the 14 days of the experiment. We also wanted to compare the propensity of these two strains to escalate the unit dose of cocaine received at each infusion. These results are particularly relevant since two recent papers (Freeman et al. 2009; Kosten et al. 2007) reported that Fischer rats are

more susceptible to self-administration than Lewis rats, thus challenging the previous model claiming the opposite.

Cocaine self-administration and withdrawal from cocaine administration are stressors that modulate the activity of the HPA axis (Koob and Kreek 2007). We, therefore, measured levels of ACTH and corticosterone 24 h after the last self-administration session in both rat strains.

Materials and methods

Animals and drug

Self-administration experimental procedures were conducted in male Fischer and Lewis rats (Charles River, Wilmington, MA). Fischer and Lewis rats weighed between 250 and 300 g (corresponding to 9–10 weeks of age), respectively, at the time of surgery. Normal chow and water were available ad libitum in both testing chambers and home cages. Rats were singly housed in a temperature- and humidity-controlled environment in the Comparative Bioscience Center of The Rockefeller University, which is an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. The experimental protocol was approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

Rats were maintained on a 12-h reversed light–dark cycle (lights on at 0100 hours, off at 1300 hours). Cocaine hydrochloride purchased from Sigma (St. Louis, MO) was dissolved in physiological saline.

Intravenous self-administration apparatus

Experimental chambers consisted of 12 standard self-administration chambers from Med Associates Inc. (St. Albans, VT). Operant conditioning chambers were equipped with three retractable levers with stimulus lights (28 VDC, 100 mA, 2.5-cm diameter) located above each lever. The two active levers were mounted at the back of the chambers on opposing walls (one on the right and the other on the left), while the inactive lever was mounted at the front. Each chamber was equipped with a multiple tone generator (Med Associates Inc.). Water bottles mounted on the ceiling of the chambers and food-containing porcelain dishes (90-ml capacity, 77×32 mm) were available during the sessions.

Sound-attenuating cubicles were equipped with exhaust fans that provided ventilation and white noise to mask extraneous sounds. Programming and data collection were performed using a computer system running MED-PC® IV software (Med Associates Inc.).

During an IVSA session, drug doses were delivered from syringes mounted on syringe pumps (PHM-100, Med Associates Inc.) placed outside the cubicle. Each syringe was connected via a Tygon tube to a swivel (Instech, Plymouth Meeting, PA). Another Tygon tube connected the swivel to the external portion of the cannula of the intravenous catheter.

Surgery

Surgery was conducted as described in Caine et al. (2002), with the following modifications. Each catheter consisted of a 9-cm long Silastic tubing with an underlying Dacron mesh (Textile Development Associates, Inc., Franklin Square, NY), and was inserted 3 cm into the vein.

Once the catheter was implanted, it was flushed with 500 µl of heparin 30 U/ml to prevent catheter clogging and gentamycin 1 mg/kg in saline to prevent infections. The same mixture was used to flush the catheters during the first 4 days after surgery.

Catheter evaluation was performed by flushing about 100 μ l of a xylazine/ketamine (8/80 mg/kg) cocktail through the catheter. Rats not showing immediate signs of sedation were removed from the study. This test was performed on the day before the first self-administration session, and then once a week, or when the drug self-administering behavior deviated dramatically from that observed previously.

Cocaine self-administration

Acquisition—Four to five days after surgery, the rats were placed in the IVSA chambers for 2 h/day starting at least 1 h after the onset of the dark cycle, 5–6 days/week and connected to the cocaine delivery pump. Pressing of either active lever resulted in the delivery of one 0.5-mg/kg cocaine infusion (FR1, time out (TO) 40 s applied to both levers). Volume and duration of infusion depended on the rat's body weight. As a reference, for a rat weighing 300 g, the infusion delivery time was 1.66 s ($\sim$ 40 μ l). A light cue above the pressed lever was illuminated during the infusion and the TO. Responding on the inactive lever was recorded, but had no consequences.

Training was complete when self-administration behavior stabilized, i.e., when rats had three consecutive sessions with <10% variation in the total number of cocaine infusions per session.

Dose-response curve—After the acquisition training, responding to different doses of cocaine was assessed using multiple-component sessions, during which each rat was tested with ascending doses of cocaine each session (Caine et al. 2002).

Briefly, multiple-component sessions started about an hour after the onset of the dark cycle. Each session was comprised of five 25-min-long components separated by 5-min-long intervals during which levers were retracted and the chamber was illuminated. Five cocaine doses (0.02, 0.2, 0.5, 1.25, and 2.5 mg/kg/infusion) were presented in ascending order to prevent sensitization effects (Caine et al. 2002). Different unit doses were administered by changing the duration of the infusion. In addition to the illumination of the light cue above the pressed lever, responding for the 0.2, 0.5, 1.25, and 2.5 mg/kg doses was associated with a tone cue of 1, 1.5, 2.5, 5 kHz, respectively. The duration of the tone cue equaled the duration of the infusion. Each component started with a non-contingent infusion of the appropriate dose. Rats were exposed to four multi-component sessions on consecutive days.

Responding for saline alone was tested in three separate sessions before the day of exposure to the multi-component sessions because when a saline component immediately preceded the ascending cocaine components, responding to subsequent doses of cocaine was blunted in both strains (Picetti, pilot studies).

Extended access with subject-controlled dose escalation—After the dose-response curve determination, rats were placed in the self-administration chambers for 18 h starting approximately 3 h after the onset of the dark cycle. The overall exposure lasted for 14 days. Each session was divided into three cycles defined by the doses of cocaine associated with the active levers. Every 18-h session started with a 1-h exposure to the active lever associated with the lower dose plus the inactive lever, followed by a 5-min TO with the levers retracted, followed by a 1-h exposure to the active lever associated with the higher dose plus the inactive lever. After a 5-min TO, the animal was exposed to the three levers for the remainder of the time. As with determination of the dose-response curve, different unit doses were administered by changing the duration of the infusion. Auditory and visual cues were the same as described for the dose-response curve.

From day 1 to day 4 (Fig. 1), one active lever delivered 0.2 mg/kg/infusion, while the other delivered 0.5 mg/kg/infusion (FR1, TO 40 s applied to both levers). Pressing an active lever was associated with the illumination of the light cue above the same lever during the infusion and TO, and the onset of a dose-specific tone cue (see above) during the infusion. Half of the rats in each strain started with the highest dose on the left, and the other half on the right. Higher doses of cocaine were not always associated with the same lever. For instance, if, on day 1, the 0.5-mg/kg/infusion dose was associated with the left lever, and the 0.2-mg/kg/infusion dose was associated with the right lever, then, after escalation, the 1.25-mg/kg/infusion dose was on the right, with the 0.5-mg/kg/infusion dose still on the left. Therefore, the rat could not escalate simply by perseverating on a preferred lever and had to select levers. If the animal pressed the lever associated with the higher dose 70% of the time on days 3 and 4, then from day 5, the available doses became 0.5 and 1.25 mg/kg/infusion (Fig. 1).

For rats that did not fulfill this requirement, the doses were not changed until they showed 70% preference for the higher dose on two consecutive sessions. Similarly, rats that preferred the 1.25-mg/kg/infusion dose on days 7 and 8 were shifted to 1.25 and 2.5 mg/kg/infusion from day 9 to day 14 (Fig. 1). Again, for rats that did not meet this criterion immediately, there was no change unless the criterion was met for two consecutive days.

Cocaine unit doses and experimental design were based on preliminary experiments in which more doses were tested.

The light/dark cycle inside the testing chambers was synchronized to the cycle in the home cages (lights on at 0100 hours, off at 1300 hours). After each IVSA session, the rats were weighed and returned to their home cages for 6 h.

Saline-yoked rats—Saline-yoked rats, ten per strain, had surgery as described above. They were randomly paired to their cocaine self-administering counterparts. They went through the same complete procedure as their cocaine self-administering partners starting from the acquisition phase, but received saline (0.9%) when their paired partner pressed an active lever.

Quantitation of plasma hormones

The rats were decapitated 24 h after the end of the last self-administration session (1000 hours). Trunk blood from each animal was collected in tubes placed on ice, and spun at 3,500 rpm for 20 min. Plasma was collected and stored at -80°C for further analysis.

ACTH immunoreactivity levels were measured by using the ACTH kit (DiaSorin, Stillwater, MN). Corticosterone levels were assayed by using the Corticosterone ^{125}I -RIA kit (MP Biomedicals, Solon, OH). ACTH and corticosterone values were determined in duplicates in a single assay.

Statistical analysis

The dose-effect curve was analyzed with a two-way ANOVA (strain $\times$ dose) followed by Newman-Keuls post-hoc test. The total daily cocaine intake was analyzed with a two-way ANOVA (strain $\times$ day). The comparison between blocks of days in Fig. 3b was performed with a two-way ANOVA (strain $\times$ block of days) followed by planned comparisons. The difference between the number of presses on the active and inactive levers was analyzed with a three-way ANOVA (strain $\times$ lever $\times$ day).

Body weight (Online Resource 2) analysis was carried out with a three-way ANOVA (strain $\times$ condition $\times$ day).

ACTH and corticosterone levels were analyzed with a two-way ANOVA with two between factors, strain and condition, followed by Newman–Keuls post-hoc test. Statistical analysis was performed with the program Statistica (StatSoft, Inc., Tulsa, OK) and GraphPad Prism 5 (La Jolla, CA).

Results

Response to different doses of cocaine prior to extended session exposure

Acquisition of cocaine self-administration behavior (2 h/session, 0.5 mg/kg/infusion during the dark phase of the light–dark cycle) was initiated without prior training for food-reinforced responding. There was no significant difference in days between the two strains to meet the acquisition criteria. During the last 3 days of acquisition, Fischer rats took a greater amount of cocaine (27.3 ± 2.1 mg/kg/session) than Lewis rats (22.2 ± 1.2 mg/kg/session), but this difference did not reach statistical significance (Student's *t* test $p=0.0524$; data not shown).

After acquisition was completed, the rats were tested for their responses to a range of unit doses of cocaine (0.02, 0.2, 0.5, 1.25 and 2.5 mg/kg/infusion tested in an ascending order on the same day. Saline tested separately). The two strains showed a dose-dependent modulation of the number of infusions, $F(5,180)=30.22$, $p<0.001$, and the dose-related response curves were superimposable (Fig. 2). Fischer and Lewis strains were virtually indistinguishable across different doses of cocaine, with the main effect $F(1,36)=0.026$, $p=0.87$ between strains prior to exposure to extended access self-administration sessions.

Subject-controlled dose-escalation and extended-access to cocaine self-administration

Once the dose-response study was completed, self-administration sessions were then extended to 18 h/day, starting approximately 3 h into the dark cycle. Fischer and Lewis rats showed a remarkable difference in their daily cocaine intake across the 14-day exposure (Fig. 3a). Notably, Fischer rats did not vary their average level of cocaine intake throughout the 14-day exposure. In contrast, Lewis rats began to escalate their cocaine intake starting from the fourth day of exposure and had not reached a plateau by the 14th day of exposure. A two-way ANOVA (strain $\times$ day) showed a significant main effect of days of exposure, $F(13,520)=7.74$, $p<0.001$, and a significant interaction between strain and day of exposure, $F(13,520)=6.96$, $p<0.001$.

From Fig. 3a, it is evident that the two strains were different in their cocaine intake at the beginning and at the end of the 14-day exposure. Although the mean daily intake of cocaine across 14 days was similar in both strains (116.8 ± 3.5 mg for Fischer and 124.8 ± 13.8 mg for Lewis), Fischer rats did not significantly vary their daily intake from days 1 to 14. In contrast, Lewis rats had a lower cocaine intake at the beginning of the exposure to the extended-access IVSA, and they increased the daily amount of cocaine taken over time. Indeed, Fig. 3b shows that the average total amount of cocaine taken during the first 4 days was significantly higher in Fischer than Lewis rats (planned comparison, $F(1,40)=7.26$, $p<0.01$), whereas the situation was reversed during the last 4 days of exposure (planned comparison, $F(1,40)=10.07$, $p<0.003$). Moreover, whereas Fischer rats did not show any difference between these two 4-day time blocks, Lewis rats showed a robust increase in cocaine intake on days 11–14 vs. days 1–4.

In the context of this study, the four-unit doses available were divided into two groups: lower doses (0.2 and 0.5 mg/kg/infusion) and higher doses (1.25 and 2.5 mg/kg/infusion). The analysis of the unit dose preferred by each subject on day 14 revealed that 63.2% of Fischer rats preferred the two lower-unit doses (Table 1), while 69.6% of the Lewis rats

preferred the two higher-unit doses (Table 1), with 50% of this group preferring the highest unit dose of cocaine available.

Details of subject-controlled dose escalation

Figure 4 details daily activity on the active levers of the rats during the 14-day extended exposure to cocaine self-administration. Figure 4a and c show that during the first 4 days, Fischer rats received most of the cocaine from the 0.5-mg/kg/infusion-associated lever. Starting from day 5 onward, a total of 18 Fischer rats were exposed to the 0.5 and 1.25 mg/kg/infusion doses (see Fig. 1 for experimental design). As a consequence, the amount of cocaine received from the 0.5-mg/kg/infusion-associated lever (Fig. 4a) and the number of presses on this lever (Fig. 4c) decreased, but with the amount of cocaine received from the 1.25-mg/kg/infusion-associated lever, the total amount of drug obtained remained approximately the same. From day 9, four of 19 Fischer rats escalated the unit dose to the highest combination of doses (1.25 and 2.5 mg/kg/infusion) and, interestingly, these rats again titrated the quantity of cocaine received so that the total amount did not vary significantly compared to previous days (Fig. 3a). Indeed, Fig. 4a and c show that in this case, both the amount of cocaine and lever presses slowly decreased.

Lewis rats showed a different pattern of behavior. While their initial preference for the 0.5-mg/kg/infusion lever was less than Fischer rats (Fig. 4a and b, days 1–4), during the following days, Lewis rats never decreased the amount of cocaine received at any given dose. This behavior is even more evident when analyzing the lever presses, where Lewis rats increased the number of lever presses across days (Fig. 4d), whereas Fischer rats did not (Fig. 4c). Thus, Lewis rats steadily escalated the daily cocaine intake across daily sessions (Fig. 3b). Starting from day 5, 20 Lewis rats were exposed to the 0.5–1.25-mg/kg/infusion choice, and from day 9, a total of 11 of 23 Lewis rats had access to the highest doses of cocaine.

The mean number of presses on the inactive lever across the 14-day exposure to extended sessions was 30 ± 7.8 by Fischer rats and 44 ± 10.3 by Lewis rats, whereas the mean number of presses on both active levers was 72.1 ± 7.1 by Fischer rats and 97.8 ± 5.3 by Lewis rats. The number of presses on the active vs. inactive levers across the 14-day exposure was significantly higher, $F(1,40) = 37.02$, $p < 0.001$.

Further details on the hourly pattern of infusions during the extended sessions on days 1 and 14 are available in Online Resource 1.

ACTH and corticosterone levels

Rats were euthanized 24 h after the last self-administration session and their plasma was collected. There was an apparent slightly higher ACTH level in cocaine-exposed Fischer rats compared to saline-yoked controls (Fig. 5a), but two-way ANOVA revealed that this difference was not significant. Also, no difference was found between Lewis rats of the saline-yoked and the cocaine groups. However, ANOVA showed that, overall, the ACTH levels of the Fischer strain were significantly higher than those of the Lewis, $F(1,38) = 13.47$, $p < 0.001$ (Fig. 5a).

Corticosterone levels were increased in Fischer rats that self-administered cocaine compared with saline-yoked controls (Fig. 5b). However, no such difference was detected in Lewis rats. A two-way ANOVA revealed that corticosterone levels in Fischer rats were significantly higher than in Lewis, $F(1,31) = 24.59$, $p < 0.01$. Moreover, there was a significant interaction of condition $\times$ strain, $F(1,31) = 24.59$, $p < 0.05$. A Newman–Keuls post-hoc test also showed a significantly higher level of corticosterone in cocaine self-administering Fischer rats than their saline-yoked controls ($p < 0.01$).

Discussion

In this paper, we used a new self-administration model to simulate critical aspects of the behavior of humans that escalate the self-administered unit dose of cocaine over time (Kreek et al. 2009; Zernig et al. 2007), and to create a model of self-administration that parallels the cocaine escalating “binge” administration pattern (Schlussman et al. 2005). Our main goal was to allow individual rats to select the unit doses of cocaine to be self-administered, and to escalate these doses across days. We selected two rat strains, Fischer and Lewis, whose behavior is very different when challenged with drugs, in order to analyze the importance of the genetic background under the new model of self-administration shown here. The animals could self-administer cocaine for 18 h/day, and then were returned to their home cages for the rest of the day to simulate the acute withdrawal period (“crash”) experienced by cocaine abusers. The exposure lasted for 14 days to reproduce a chronic experience, while other protocols are more focused on acute (24 h) binge exposures (Covington et al. 2008; Quadros and Miczek 2009). One novel aspect of our model is that rats were not experimentally forced to increase the unit dose of cocaine; rather, each rat regulated its dose escalation. A similar paradigm was successfully used in our laboratory with rats having to select different doses of morphine (Kruzich et al. 2003).

The major finding of this paper concerned Fischer and Lewis rats' behavior during extended access IVSA sessions to cocaine. One initial finding was that Fischer rats did not escalate the overall amount of cocaine taken across 14 days, while Lewis rats started by self-administering less cocaine than Fischer rats, but escalated across time. In fact, by the end of the 14-day exposure, Lewis rats self-administered significantly more cocaine than Fischer rats. This is striking since it was previously shown that when these two strains self-administer cocaine at a fixed dose (0.75 mg/kg/infusion) for 6 h across 20 sessions, Fischer rats obtain significantly more infusions than Lewis rats (Freeman et al. 2009). Interestingly, even though four Fischer rats escalated to the highest combination of unit doses available, they decreased the amount of cocaine taken so that the total amount of cocaine taken across days did not change.

Our results show also that the total mean daily intake of cocaine over the 14-day exposure to cocaine is similar in the two strains. Thus, when Lewis rats have longer access to cocaine and are allowed to self-administer high-unit doses of the drug, they increase their intake to the point of reaching and exceeding the level of self-administration of Fischer rats after 14 days. Importantly, even over the 14-day exposure, Lewis rats did not reach a plateau in cocaine intake. Notably both in the present paper and in Freeman et al. (2009), Fischer rats did not escalate the total amount of cocaine taken across the exposure period despite the very different protocols of self-administration used. The findings herein are intriguing since they show that Fischer rats maintain a constant daily cocaine intake even when they select increasing unit doses of cocaine.

The dose-response curve in self-administration assays is the result of a complex interaction of several factors such as titration, satiety, and toxicity (Calabrese 2008; Mello and Negus 1996; Zernig et al. 2007). In the present study, the cocaine unit dose-response curves of the two rat strains examined were very similar prior to extended-access exposure to cocaine. This result is substantially in agreement with a previous study (Kosten et al. 1997), although in that study, a small but significant difference was found for one of the three doses tested. This small discrepancy between our study and that of Kosten et al. (1997) may derive mainly from the time of day when the animals were tested (at least 1 h into the light phase of the light/dark cycle in Kosten et al. (1997), and at least 1 h into the dark phase in the study described here). The curve shows only the descending limb for both strains. This is in agreement with Kosten et al. (1997) for the dose of 0.2 mg/kg/infusion and higher. Also, the

lowest unit dose lies on the descending limb, possibly because either lower doses of cocaine or a higher fixed ratio as in Kosten et al. (2007) are needed to capture the ascending limb.

Fischer rats have been considered to be less vulnerable than Lewis rats to the rewarding effects of drugs because of their lower levels of cocaine-induced CPP (Kosten et al. 1994) and their decreased ability to acquire cocaine self-administration (Kosten et al. 1997). However, two recent papers suggest that Lewis rats may be more sensitive to cocaine (Freeman et al. 2009; Kosten et al. 2007). In both published studies, rats could self-administer cocaine for up to 6 h/day, although in one study (Kosten et al. 2007), rats had two sessions under a progressive ratio schedule, while in the other (Freeman et al. 2009) they were studied for 20 days under a fixed ratio design. It can be argued that the length of self-administration sessions is one important factor leading to the escalation of drug intake which may model compulsive addictive use. Cocaine self-administration sessions lasting 12 h result in a greater escalation of drug intake than shorter sessions (i.e., 6 h) (Wee et al. 2007). High-unit doses of cocaine are also important for the establishment of compulsive drug intake (Mantsch et al. 2001; Wee et al. 2007). Results presented here are in agreement with previous studies (Freeman et al. 2009; Kosten et al. 2007) during the first 3 days of exposure to the extended sessions of self-administration, which is when rats are exposed to lower unit doses of cocaine, but are different with higher unit doses. The difference between our results obtained with high-unit doses of cocaine and earlier papers (Freeman et al. 2009; Kosten et al. 2007) may be partially explained by the duration of the sessions in combination with the possibility for rats to escalate the unit dose of cocaine up to 2.5 mg/kg/infusion.

Escalating the total amount of cocaine self-administered has been considered a key element in the transition from drug use to drug addiction in Wistar rats (Ahmed and Koob 1998, 1999), which is hypothesized to result from the loss of subject control over drug-taking (Koob and Le Moal 1997, 2008a, b). Repeated extended drug intake dysregulates the reward system leading to an allostatic state (Koob and Le Moal 2008a). In the present study, Fischer rats do not escalate the total amount of cocaine intake even when they escalate the unit dose, while Lewis rats escalate the intake of cocaine as the unit dose increases. Thus, interestingly, cocaine, under the self-administration paradigm described here, may not be able to induce a deviation of the reward threshold from the set point in Fischer rats, but it induces an allostatic state in Lewis rats by dysregulating the brain reward circuits. Therefore, we suggest that Lewis rats are more susceptible than Fischer rats to addiction-like behaviors and adaptations when high doses of cocaine are available. This hypothesis is supported by experiments showing that Lewis rats have higher rates of delay discounting for a food reward (i.e., higher impulsivity) than Fischer rats (Anderson and Woolverton 2005; Garcia-Lecumberri et al. 2010). Impulsivity is an important behavioral trait involved in the vulnerability to drug addiction (Koob and Volkow 2010). Moreover, a very recent paper (Garcia-Lecumberri et al. 2010) studying morphine self-administration in Lewis and Fischer rats supports the hypothesis that Lewis rats are more vulnerable to the rewarding effects of drugs of abuse and are more impulsive than Fischer rats.

Fischer and Lewis rats differ in the expression of a number of genes in the brain, and it has been suggested that naïve Fischer rats share some characteristics with naïve outbred Sprague–Dawley rats, while naïve Lewis rats are more similar to Sprague–Dawley rats chronically exposed to cocaine or morphine (Kosten and Ambrosio 2002; Nestler 1992). Major examples of these differences include a higher level of Δ FosB in the nucleus accumbens (NAc) of Lewis rats (Haile et al. 2001). Lewis rats have lower levels of D2-like dopamine receptors in the striatum and NAc core, and D3 receptor in the NAc shell and olfactory tubercle (Flores et al. 1998). In microdialysis experiments, the dopaminergic system in the NAc of Lewis rats is more responsive to cocaine, amphetamine, and morphine injections than that of Fischer rats (Cadoni and Di Chiara 2007). We speculate that the lower

basal levels of D2-like receptors in NAc core in Lewis rats may account for the lower initial intake of this strain compared to Fischer rats. The prolonged exposure to increasing unit doses of cocaine, together with a higher responsivity of the dopaminergic system, and a higher accumulation of Δ FosB in the NAc in Lewis rats may contribute to the escalation of their cocaine intake. Thus, future studies will determine the expression of these and other relevant genes in the brain areas collected from the animals described here.

A second important result of this study concerns the different distribution of the preference of cocaine doses between the two strains studied by the last day of exposure. Most Fischer rats preferred to self-administer lower unit doses of cocaine, while most Lewis preferred higher unit doses. The unit doses of cocaine selected by Lewis rats are higher than the doses usually tested in earlier papers. This factor, together with 18 h-long sessions, may account for the escalation in Lewis rats. Indeed, the escalation of the daily total amount of cocaine taken became evident in Lewis rats starting from the fifth day of exposure, when rats began to be exposed to the 1.25-mg/kg/infusion dose. This analysis is supported by a more detailed examination of the lever-pressing activity associated to each dose and the amount of cocaine taken at each dose. Whereas Fischer rats clearly preferred the 0.5-mg/kg/infusion dose during the first 4 days, the lever-pressing activity and, as a consequence, the amount of cocaine taken at this dose declined as higher unit doses were tested. However, the lever-pressing activity associated with the higher unit doses also slowly declined over time. In contrast, Lewis rats began by showing little preference for the 0.5- vs. 0.2-mg/kg/infusion-associated levers, but the activity on the active levers never declined at any of the doses tested. In fact, the number of lever presses on the 0.5- and 2.5-mg/kg/infusion-associated levers tended to increase across days, thus escalating the total amount of cocaine self-administered. We speculate that the reason for this difference between the two strains may be that most Fischer rats find high-unit doses of cocaine aversive. In cocaine-induced CPP experiments, Fischer rats spent less time in the drug-paired compartment than the saline-paired compartment when they were conditioned with a high dose of cocaine (30 mg/kg), while Lewis rats showed robust CPP up to and including that dose (Kosten et al. 1994). Additional details on the hourly cocaine intake during the 18-h-long sessions are provided in Online Resource 1.

Cocaine pharmacokinetics in Fischer and Lewis strains after an intravenous (i.v.) infusion (1 mg/kg) or an intraperitoneal (i.p.) injection (15 mg/kg) are similar (Guitart et al. 1992; Kosten et al. 1997), although according to one report in castrated rats, cocaine levels (10 mg/kg, i.p.) are higher in Lewis than Fischer rats (Camp et al. 1994). Thus, although the behavioral differences reported here are not likely due to differences in cocaine pharmacokinetics, we cannot rule out this possibility because the plasma determinations reported above followed acute injections, not the chronic i.v. administration reported here.

Fischer and Lewis rats were also different in their HPA response to the acute withdrawal (24 h) from chronic cocaine self-administration. Saline-yoked control Fischer rats had a higher ACTH level than Lewis rats, while the level of corticosterone was similar, although it was slightly lower in Lewis rats. However, 24 h after the last exposure to cocaine, Fischer rats had higher levels of ACTH and corticosterone, while Lewis rats did not show any change. These results are consistent with the literature showing lower basal levels of HPA axis activity in Lewis rats (Griffin and Whitacre 1991), and impaired response to stressors in the same strain (Kosten and Ambrosio 2002). Moreover, our results obtained here with Fischer rats in the self-administration paradigm are consistent with the results obtained with chronic steady-dose “binge”-pattern cocaine administration (14 days) (Zhou et al. 2003).

We have constantly monitored body weight, food and water consumption, and found a reduction of all three-parameters across sessions (Online Resource 2).

In conclusion, we describe here a model of subject-controlled dose-escalating self-administration that allows the animal to select higher unit doses of cocaine over the course of 14 days of exposure. These results are important for the design of behavioral models that are closer to human behavior, and underline the importance of environmental factors (e.g., session length and drug doses available) and genetic factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Experimental design. Flow chart with the experimental design of the 14-day exposure to cocaine extended sessions of self-administration. The escalation criterion consisted of 70% of lever presses for the higher available unit dose on two consecutive sessions. The *asterisks* indicate the only two steps where rats were exposed to the indicated doses for 4 days minimum. No constraints in the number of days were used in the other cases. Flowchart symbols: **a** Beginning and end of exposure to 18-h-long sessions, **b** Procedure including unit doses (mg/kg/infusion) and minimum number of days for escalation criterion to be met (if any), **c** Decision node

Fig. 2. Dose-response curve. Dose-response curves were determined as the mean of four 5-component sessions. Each component lasted 25 min. Fischer $n=19$; Lewis $n=23$

Fig. 3. Self-administration escalation and dose preference. **a** Total daily intake of cocaine during the extended IVSA sessions. The *asterisks* above the first and last 4 days refer to the analysis of **b**. **b** Average cocaine intake across the first 4 days of exposure (columns F 1–4 and L 1–4), and across the last 4 days of exposure (columns F 11–14 and L 11–14). ** $p < 0.01$, *** $p < 0.001$ Fischer $n = 19$; Lewis $n = 23$

Fig. 4.

Daily activity on active levers. Quantity of cocaine self-administered (**a-b**), and number of lever presses (**c-d**) divided according to the four unit doses used, respectively

Fig. 5. Hormonal levels 24 h after the last extended self-administration session. ACTH (a) and corticosterone (b) levels in rats sacrificed 24 h after the last self-administration session. Fischer CTRL $n=10$; Fischer coc $n=11$; Lewis CTRL $n=10$; Lewis coc $n=11$. ** $p<0.01$, *** $p<0.001$, ## $p<0.01$

Percentages of rats preferring the different unit doses of cocaine at day 14. *Bold numbers* are the sums of percentages of each strain preferring the two lower (0.2 and 0.5) or higher (1.25 and 2.5) unit doses of cocaine. Fischer $n=19$; Lewis $n=23$

Table 1

Cocaine dose (mg/kg/infusion)	0.2	0.5	Total low doses	1.25	2.5	Total high doses
Fischer (%)	5.3	57.9	63.2	31.5	5.3	36.8
Lewis (%)	8.7	21.7	30.4	34.8	34.8	69.6