

Alcohol-preferring (P) rats are more sensitive than Wistar rats to the reinforcing effects of cocaine self-administered directly into the nucleus accumbens shell

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ARTICLE INFO

Article history:

Received 15 March 2011

Received in revised form 16 June 2011

Accepted 18 June 2011

Available online 25 June 2011

Keywords:

Intracranial self-administration

Nucleus accumbens shell

Reinforcement

Alcohol-preferring rats

Ethanol

Cocaine

ABSTRACT

Wistar rats will self-administer cocaine directly into the nucleus accumbens shell (AcbSh), but not into the nucleus accumbens core. In human and animal literature, there is a genetic association between alcoholism and cocaine dependency. The current experiment examined whether selective breeding for high alcohol preference is also associated with greater sensitivity of the AcbSh to the reinforcing properties of cocaine. P and Wistar rats were given cocaine (0, 100, 200, 400, or 800 pmol/100 nl) to self-infuse into the AcbSh. Rats were given cocaine for the first 4 sessions (acquisition), artificial CSF for sessions 5 and 6 (extinction), and cocaine again in session 7 (reinstatement). During acquisition, P rats self-infused 200–800 pmol cocaine (59 infusions/session), whereas Wistar rats only reliably self-infused 800 pmol cocaine (38 infusions/session). Furthermore, P rats received a greater number of cocaine infusions in the 200, 400 and 800 pmol cocaine groups compared to respective Wistar groups during acquisition. Both P and Wistar rats reduced responding on the active lever when aCSF was substituted for cocaine, and reinstated responding in session 7 when cocaine was restored. However, P rats had significantly greater infusions during session 7 compared to session 4 at all concentrations of cocaine tested, whereas Wistar rats only displayed greater infusions during session 7 compared to session 4 at the 400 and 800 pmol cocaine concentrations. The present results suggest that, compared to Wistar rats, the AcbSh of P rats was more sensitive to the reinforcing effects of cocaine. The reinstatement data suggest that the AcbSh of P rats may have become sensitized to the reinforcing effects of cocaine. Overall, the findings from this study support a genetic association between high alcohol preference and greater sensitivity to the reinforcing effects of cocaine.

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1. Introduction

Cocaine and alcohol are frequently co-abused. The majority of cocaine users (up to 90%) report co-administering EtOH during cocaine binges (Brookoff et al., 1996; Magura and Rosenblum, 2000). The high prevalence of co-abuse of alcohol with cocaine in humans has been postulated to be predicated upon both a common genetic factor that predispose an organism to abuse multiple substances, including alcohol, and the interaction of the drugs within the organism (Uhl, 2004, 2006; Uhl et al., 2008). Individuals predisposed to abuse alcohol and other drugs of abuse are disproportionately reactive to alcohol and other drugs of abuse when given alone, and co-administration of alcohol and other drugs of abuse result in further divergence (Schuckit, 1994a,b; Kareken et al., 2010; Uhl et al., 2008; Piazza and Le Moal, 1996).

In addition to previously mentioned literature, a number of studies have focused at directly assessing the genetic influence on alcohol dependency (AD) and cocaine dependency (CD) in humans. In a detailed COGA study, the rate of CD was approximately 2.5 fold higher in individuals with a genetic predisposition for alcoholism than the general population (Nurnberger et al., 2004). Similar findings were reported in a study that examined the effects of a family history of alcohol-related problems. For example, if a strict DSM-IV AD diagnosis was used for family history positive, the odds ratio was 1.6 for comorbid CD and AD in family history positive individuals, or if the criterion for family positive was reduced to alcohol abuse and not AD, the odds ratio increased (Compton et al., 2002). In a twin study, there was strong linkage for familial factors between major depression, AD, and CD (Lin et al., 1996).

In humans, cocaine use increases the amount of alcohol consumed in polydrug users (Williamson et al., 1997). Conversely, alcohol consumption is associated with greater cocaine usage (Magura and Rosenblum, 2000). The rate of alcoholism in high-frequency cocaine users was approximately 60% compared to 37% in low-frequency cocaine users (Fox et al., 2005). Subjects with the diagnosis of alcohol dependence are more likely to become cocaine misusers and experience more adverse

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consequences of cocaine use (Heil et al., 2001; Staines et al., 2001). In addition, alcohol abuse is a common problem among cocaine dependent patients (Miller et al., 1989). Co-administration of alcohol during cocaine binges allows the user to prolong the euphoric effects and diminish the anxiogenic effects of cocaine (Williamson et al., 1997). Additionally, the likelihood to relapse to cocaine and/or alcohol use was greater in individuals who co-abused (Fox et al., 2005).

In animal studies, FAST and SLOW mice, selectively bred for their differential locomotor responses to ethanol, displayed parallel divergence for ethanol and cocaine (Bergstrom et al., 2003). Cocaine increases extracellular dopamine (DA) levels to a greater extent in the nucleus accumbens (Acb) and caudate-putamen in AA (Alko, Alcohol) rats compared to ANA (Alko nonalcohol) rats (Mikkola et al., 2001). In Wistar rats selected for high and low alcohol-preference, locomotor stimulation induced by cocaine was positively correlated with alcohol preference (Stromberg and Mackler, 2005). Additionally, high alcohol consuming Wistar rats were more sensitive to the reinforcing effects of cocaine than low alcohol consuming Wistar rats as measured by conditioned place preference (Stromberg and Mackler, 2005). Alcohol-preferring (P) rats are more resistant to extinguish cocaine self-administration and are more sensitive to a priming dose of cocaine to elicit cocaine-seeking behaviors than alcohol-nonpreferring (NP) rats (Lê et al., 2006).

The intracranial self-administration (ICSA) technique has been used to identify specific brain regions involved in the initiation of response-contingent behaviors for the delivery of a reinforcer (Bozarth and Wise, 1980; Goeders and Smith, 1987; McBride et al., 1999). The ICSA procedure has successfully isolated discrete brain regions where opioids (Bozarth and Wise, 1981; Devine and Wise, 1994), amphetamine (Hoebel et al., 1983; Phillips et al., 1994), acetaldehyde (Rodd-Henricks et al., 2002), and ethanol (Gatto et al., 1994; Rodd-Henricks et al., 2000) produce their reinforcing effects. Previous ICSA research indicated that cocaine was self-administered into the medial prefrontal cortex (mPFC; Goeders and Smith, 1983) and posterior, but not anterior, ventral tegmental area (VTA; Rodd et al., 2005). Cocaine is self-administered by Wistar rats directly in the AcbSh, but not in the AcbC (Rodd-Henricks et al., 2002). In addition, ICSA studies have found that P rats have a greater sensitivity to the reinforcing actions of ethanol compared to Wistar rats in the AcbSh (Engleman et al., 2009).

The goal of the present study was to compare the dose-response effects for the self-infusion of cocaine into the AcbSh of selectively bred alcohol preferring (P) and Wistar rats. The Wistar rat is the founding stock of the P rat. The hypothesis to be tested is that selective breeding for high alcohol preference is also associated with increased sensitivity to the reinforcing effects of cocaine in the AcbSh.

2. Methods

2.1. Animals

Female P rats, from the 52nd and 53rd generations, and Wistar rats (Harlan, Indianapolis, IN) weighing 250–320 g at time of surgery were used. Female rats were used in the present study because (a) female rats were used in previous studies involving the ICSA of cocaine (Rodd et al., 2005; Rodd-Henricks et al., 2002; McKinzie et al., 1999), and (b) female rats appear to maintain their body weights and head size better than male rats for more accurate stereotaxic placements (Ikemoto et al., 1997a,b; Rodd-Henricks et al., 2000, 2002 and 2003). Rats were double-housed upon arrival and maintained on a 12-h reverse light–dark cycle (lights off at 0900 h). Although not systematically studied, the estrus cycle did not appear to have a significant effect on ICSA behavior in the present study, or in previous ICSA studies (Gatto et al., 1994; Ikemoto et al., 1997a,b; Rodd-Henricks et al., 2000; Rodd-Henricks et al., 2002; Rodd-Henricks et al., 2003), as indicated by no obvious fluctuations in self-administrations by

female rats given the same dose over several consecutive sessions. Animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for the Care and Use of Laboratory Animals (National Research Council 2011).

2.2. Drug and vehicle

The artificial cerebrospinal fluid (aCSF) consisted of (in mM): 120.0 NaCl, 4.8 KCl, 1.2 KH₂PO₄, 1.2 Mg SO₄, 25.0 NaHCO₃, 2.5 CaCl₂, and 10.0 d-glucose. Cocaine (Sigma) was dissolved in the aCSF solution. When necessary, 0.1 N NaOH was added to adjust the pH to 7.4 ± 0.1.

2.3. Animal preparation

While under isoflurane anesthesia, a unilateral 22-gauge guide cannula (Plastics One) was stereotaxically implanted in the right hemisphere of each subject, aimed 1.0 mm above the target region. Coordinates (Paxinos and Watson, 1998) for placements into the AcbSh were 1.2 mm anterior to bregma, 2.1 mm lateral to the midline, and 8.0 mm ventral from the surface of the skull at a 10-degree angle to the vertical. Between experimental sessions, a 28-gauge stylet was placed into the guide cannula and extended 0.5 mm beyond the tip of the guide. Following surgery, all rats were individually housed and allowed to recover 7–10 days. Animals were handled for at least 5 min daily following the fourth recovery day. Subjects were not acclimated to the test chamber prior to the commencement of data collection, nor did they receive any prior operant training.

2.4. General test condition

Testing was conducted in standard two-lever operant chambers as previously described (Ikemoto et al., 1997b; Rodd-Henricks et al., 2002; Rodd et al., 2005). The electrolytic microinfusion transducer (EMIT) system has also been described in detail (Bozarth and Wise, 1980). For testing, subjects were brought to the testing room, the stylet was removed, and the injection cannula screwed into place. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5 s during insertion of the injector, which resulted in a single non-contingent administration of infusate at the beginning of the session. Injection cannulae extended 1.0 mm beyond the tip of the guide. The test chamber was equipped with two levers. Depression of the 'active lever' (FR1 schedule of reinforcement) caused the delivery of a 100-nl bolus of infusate over 5 s followed by a 5-s time-out period. During both the 5-s infusion period and 5-s time-out period, responses on the active lever did not produce further infusions. Responses on the 'inactive lever' were recorded, but did not result in infusions. The assignment of active and inactive lever with respect to the left or right position was counterbalanced among subjects. The active and inactive levers remained the same for each rat throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the active and inactive lever were recorded. The duration of each test session was 4 h and sessions occurred every other day.

2.5. Dose response

P (n = 7–8/dose) and Wistar (n = 8–12/dose) rats were randomly assigned to one of five groups. A vehicle group received infusions of aCSF for all seven sessions. The other groups received infusions of 100,

200, 400 or 800 pmol/100 nl cocaine for the first four sessions. During the fifth and sixth sessions, all animals received infusions of aCSF (extinction sessions). On the seventh session (reinstatement), rats were allowed to respond for their originally assigned infusate. In the learning field, reinstatement by definition is the return of the availability of a reinforcer following a period of unavailability, typically following extinction training (Flaherty, 1985). Reinstatement should not be confused with 'reinstatement of responding', the animal model of drug-induced seeking developed by Stewart and Shaham (Shaham et al., 2003). In the current experiment, reinstatement as defined by the learning field was examined during session 7. A previous study indicated that stable responding on the cocaine lever was attained by sessions 3 and 4, extinction was reached within 2 sessions, and responding on the active lever was reinstated within one session when cocaine was restored (Rodd-Henricks et al., 2002). A total of 37 P rats and 52 Wistar rats completed the ICSA dose-response experiment into the AcbSh. In addition, 8 P rats had cannulae implanted aimed at the nucleus accumbens core (AcbC). These rats were allowed to self-infuse either 400 or 800 pmol cocaine.

2.6. Histology

At the termination of the experiment, 1% bromophenol blue (0.5 μ l) was injected into the infusion site. Subsequently, the animals were given a fatal dose of Nembutal and then decapitated. Brains were removed and immediately frozen at -70°C . Frozen brains were subsequently equilibrated at -15°C in a cryostat microtome and then sliced into 40 μ m sections. Sections were then stained with cresyl violet and examined under a light microscope for verification of the injection site using the rat brain atlas of Paxinos and Watson (1998).

2.7. Statistical analysis

Data analysis consisted of a line \times concentration \times session mixed ANOVA, with a repeated measure of 'session', performed on the number of infusions. Additionally, for each individual group, lever discrimination was determined by type (active or inactive) \times day mixed ANOVA with a repeated measure of 'session'.

3. Results

The AcbSh and AcbC were defined by Paxinos and Watson (1998), and the injection sites are illustrated in Fig. 1. For the P rats with cannula aimed at the AcbSh, there were 3 misses (all in the AcbC), and two (1–400 pmol, 1–800 pmol) were added into the control condition. For the P rats with cannula aimed at the AcbC, there was one placement in the AcbSh (1–400 pmol); these data were included in the AcbSh group. Misses in Wistar rats tended to be ventral to the AcbSh ($n=4$), and no self-administration was observed. Data for the P rats self-administering cocaine into the AcbC were not included in the statistical analyses (average responses/session were 13 ± 5 and 11 ± 7 on the active and inactive levers, respectively, which is in agreement with findings previously reported by Rodd-Henricks et al., 2002). Placements within the AcbSh of P rats overlapped with and were similarly located to the placements within the AcbSh of the Wistar rats.

The number of animals indicated for each experiment represents 96% of the total number that underwent surgery; about 4% of the animals were not included for analyses mainly due to the loss of the guide cannula before completion of all experimental sessions. The data for these animals were not used because their injection sites could not be verified.

Cocaine concentrations of 100, 200, 400 and 800 pmol/100 nl were tested in the present study to determine the response-contingent behaviors of P and Wistar rats (Fig. 2). The overall analysis examined the number of infusions received across all 7 sessions and indicated that

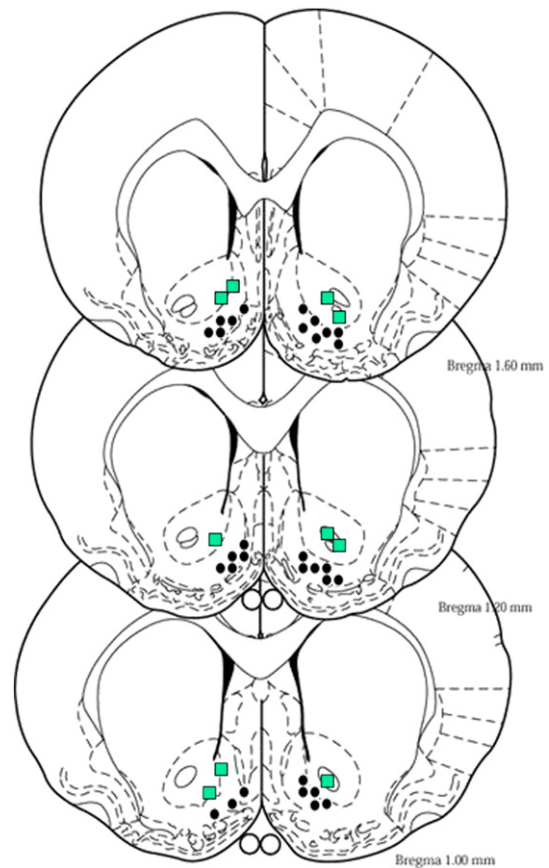


Fig. 1. Representative injection sites in the AcbSh and AcbC of P and Wistar rats (overlapping sites are excluded). Circles represent sites of injection within the AcbSh and squares represent sites of injection within the AcbC. For illustrative purposes, injection sites for P and Wistar rats are depicted on the left and right sides of the figures, respectively.

there was a significant effect of line ($F_{1,79} = 110.6$; $p < 0.0001$), concentration ($F_{4,79} = 64.6$; $p < 0.0001$), session ($F_{6,74} = 77.6$; $p < 0.0001$), and a line \times concentration \times session interaction ($F_{24,308} = 3.8$; $p < 0.0001$). Reducing the analysis to the average number of infusions received during the initial 4 sessions (acquisition; Fig. 2) revealed that there was a significant effect of line ($F_{1,79} = 84.5$; $p < 0.0001$), concentration ($F_{4,79} = 58.0$; $p < 0.0001$), and a line \times concentration interaction ($F_{4,79} = 12.9$; $p < 0.0001$). In the P rats, there was a significant effect of

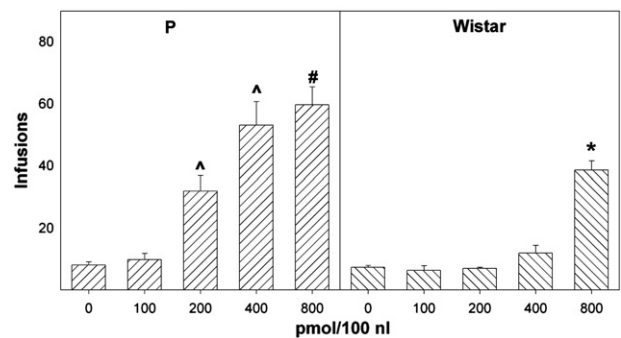


Fig. 2. The effects of the concentration of cocaine infused into the nucleus accumbens shell on the mean (\pm SEM) number of infusions received over the initial 4 sessions (acquisition) by P (left panel) and Wistar (right panel) rats. ^ indicates a significantly higher number of infusions than the 0 or 100 pmol/100 nl groups by P rats. # indicates significantly more infusions compared to the 200 pmol/100 nl group by P rats. * indicates significantly higher infusions compared to the 0, 100, 200 and 400 pmol/100 nl groups by Wistar rats. ^ also indicates significantly greater infusions by P rats compared to Wistar rats for the 200, 400 and 800 pmol/100 nl concentrations of cocaine.

cocaine concentration ($F_{4,32} = 24.5$; $p < 0.0001$), with post-hoc comparisons (Tukey; $p < 0.05$) indicating that P rats self-administering 200, 400 or 800 pmol cocaine received significantly more infusions than P rats self-administering 0 or 100 pmol cocaine, and that P rats self-administering 400–800 pmol cocaine self-infused more than P rats self-administering 200 pmol cocaine.

For Wistar rats, there was a significant effect of cocaine concentration ($F_{4,47} = 48.9$; $p < 0.001$), with post-hoc comparisons (Tukey; $p < 0.05$) indicating that Wistar rats self-administering 800 pmol cocaine received significantly more infusions than Wistar rats self-administering all other concentrations. Additionally, the interaction term was further examined by comparing the number of infusions received for each cocaine concentration between the P and Wistar rats. There were no significant differences between P and Wistar rats self-administering 0, or 100 pmol cocaine (F values $_{1, 16} < 2.0$; p values > 0.18). However, for rats self-administering 200, 400, or 800 pmol cocaine, there were significant differences between P and Wistar rats (F values $_{1, 16} > 11.5$; p values < 0.005), with P rats having higher self-administrations (Fig. 2).

Throughout the sessions, the number of lever presses by the aCSF group for both P and Wistar rats did not differ ($p = 0.46$) between the active and inactive lever (Fig. 3). Overall, statistical analysis of the active lever responses indicated there was a significant effect of line ($F_{1,79} = 83.1$; $p < 0.0001$), concentration ($F_{4,79} = 54.5$; $p < 0.0001$), session ($F_{6,74} = 66.8$; $p < 0.0001$), and a line \times concentration \times session interaction ($F_{24,308} = 4.1$; $p < 0.0001$). Active lever responses (Figs. 3 and 4; left panels) were higher in P rats self-administering 200, 400 and 800 pmol/100 nl cocaine than in P rats self-administering aCSF or 100 pmol/100 nl cocaine during sessions 1–4 (F values $_{4,32} > 12.1$; p values < 0.001 ; Tukey's b $p < 0.05$). In sessions 5 and 6, when aCSF was substituted for cocaine, P rats in the 200–800 pmol/100 nl cocaine groups displayed reduced responding on the active lever. In P rats self-administering 200–800 pmol/100 nl cocaine (Figs. 3 and 4; left panels), there was a significant effect of session when contrasting the active lever responses among sessions 4, 5, and 6 (F values $_{2,6} > 12.6$; p values < 0.007). Post-hoc comparisons indicated that active lever responses were lower during session 5 and 6 than during session 4. In session 7, when cocaine was restored, P rats self-administering 100–

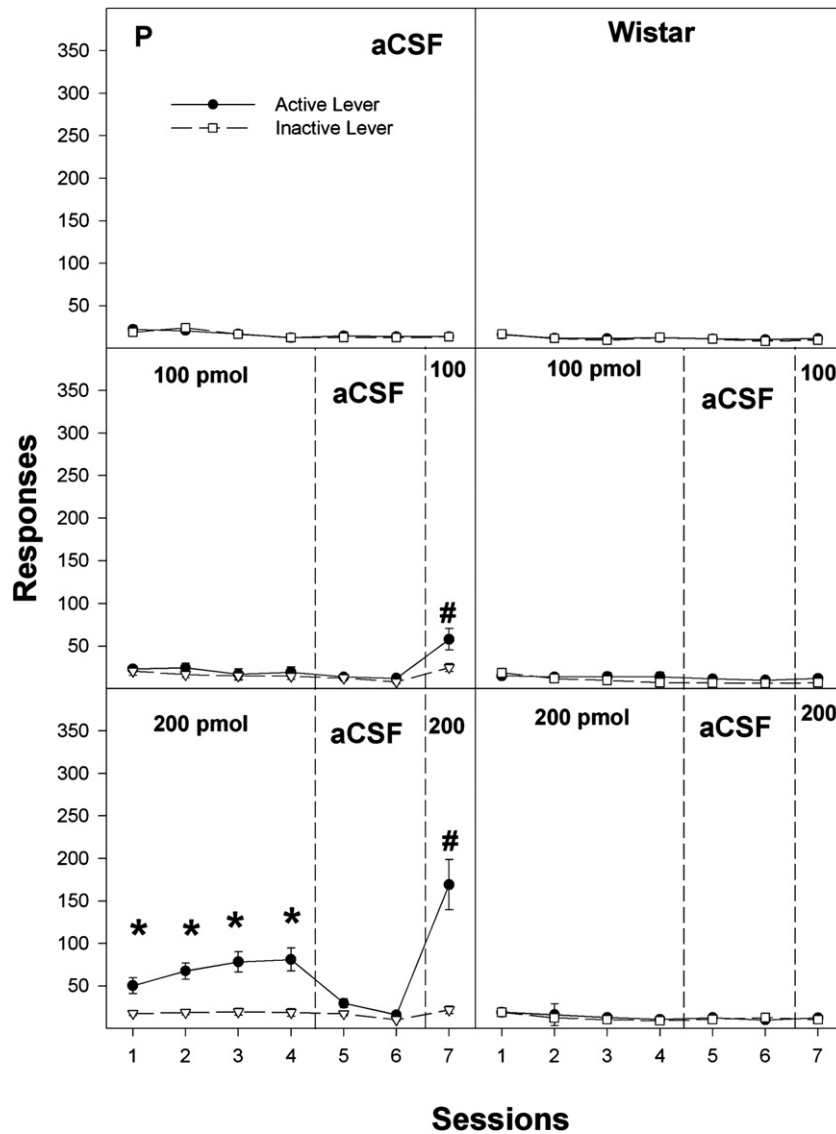


Fig. 3. The effects of the concentration of cocaine infused into the nucleus accumbens shell on the mean (\pm SEM) number of responses on the active and inactive levers by P (left panel) and Wistar (right panel) rats for the 0, 100 and 200 pmol/100 nl groups. * indicates significantly greater active lever responses by P rats for the 200 pmol/100 nl group compared to respective inactive lever responses and compared to active lever responses for the 0 or 100 pmol/100 nl groups during sessions 1–4. # indicates greater responses during session 7 compared to session 4 by P rats for both the 100 and 200 pmol/100 nl groups and greater responses compared to the aCSF (0 pmol/100 nl) group.

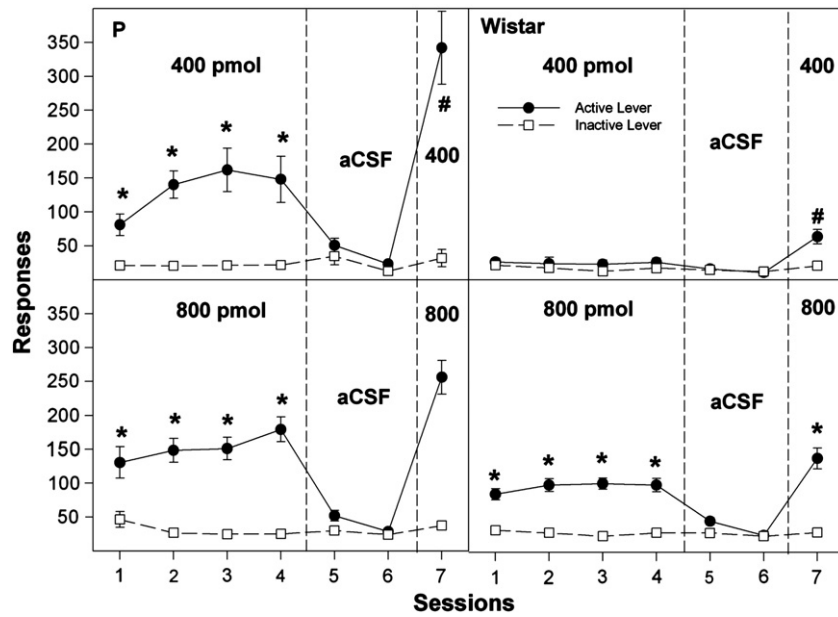


Fig. 4. The effects of the cocaine infused into the nucleus accumbens shell on the mean (\pm SEM) number of responses on the active and inactive levers by P (left panel) and Wistar (right panel) rats for the 400 and 800 pmol/100 nl groups. * indicates significantly greater active lever responses by P rats compared to respective inactive lever responses and greater active lever responses for the 400 and 800 pmol/100 nl group compared to active lever responses for the 0 or 100 pmol/100 nl groups during sessions 1–4. * also indicates significantly greater active lever responses by Wistar rats (right panels) for the 800 pmol/100 nl group compared to the 0, 100, 200 and 400 pmol/100 nl groups during sessions 1–4 and session 7. # indicates significantly greater active lever responses during session 7 compared to session 4 by P rats for the 400 pmol/100 nl group. # also indicates significantly greater active lever responses during session 7 compared to session 4 by Wistar rats for the 400 pmol/100 nl group.

800 pmol/100 nl cocaine increased the number of active lever responses compared to session 6 (F values_{1,6}>22.9; p <0.001). In addition, responses on the active lever for P rats given 100–400 pmol/100 nl cocaine were significantly (F values_{1,7}>17.2; p <0.004) higher in session 7 than session 4 (Figs. 3 and 4; left panels).

Active lever responses (Figs. 3 and 4; right panels) were higher in Wistar rats self-administering only 800 pmol/100 nl cocaine than in Wistar rats self-administering aCSF or any other concentration of cocaine during session 1–4 (F values_{4,51}>28.7; p values<0.0001; post-hoc Tukey's b p <0.05). Additionally, Wistar rats self-administering 800 pmol/100 nl cocaine (Fig. 4; right panels) reduced responding on the active lever when aCSF was substituted for cocaine (session 4 vs sessions 5 and 6, F values_{2,6}>50.8; p values<0.0001). Wistar rats, self-administering 400 or 800 pmol/100 nl cocaine, also reinstated active lever responding when cocaine was returned (session 6 vs 7, F values_{1,7}>

87.8; p <0.0001). In addition, responses on the active lever for Wistar rats given 400 pmol/100 nl cocaine were significantly ($F_{1,11}=24.2$; p <0.001) higher in session 7 than session 4 (Fig. 4).

A line \times concentration \times day mixed ANOVA, with a repeated measure of 'session', performed on the number of infusions self-administered during sessions 4 and 7 revealed a significant effect of line ($F_{1,79}=123.7$; p <0.0001), concentration ($F_{4,79}=56.5$; p <0.0001), session ($F_{1,79}=209.9$; p <0.0001), and a line \times concentration \times session interaction ($F_{4,79}=10.8$; p <0.0001). For Wistar rats (Fig. 5, right panel), the number of infusions received during session 7 was greater than infusions during session 4 in the groups given 400 or 800 pmol/100 nl cocaine (session: F values_{1,11}>21.4; p values<0.001). For P rats (Fig. 5, left panel), the number of infusions was higher in sessions 7 compared to session 4 for all cocaine concentrations (F values_{1,6}>42.3; p values<0.001).

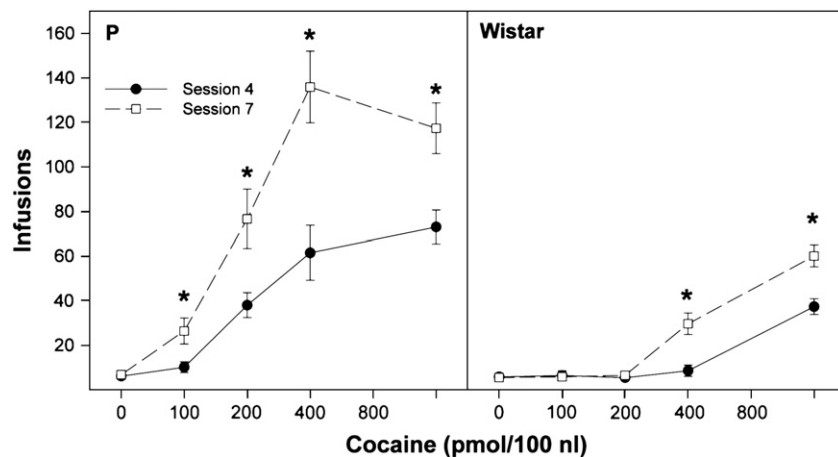


Fig. 5. The effects of the concentration of cocaine (0, 100, 200, 400 and 800 pmol/100 nl) infused into the nucleus accumbens shell on the average (\pm SEM) number of infusions received during sessions 4 (acquisition) and 7 (reinstatement) by P (left panel) and Wistar (right panel) rats. * indicates significantly greater infusions during session 7 by P rats (left panel) for the 100, 200, 400 and 800 pmol/100 nl groups compared to session 4, and greater infusions during session 7 by Wistar rats (right panel) for the 400 and 800 pmol/100 nl groups compared to session 4.

4. Discussion

The present findings indicate that both P and Wistar rats will acquire and maintain the self-administration of cocaine directly into the AcbSh (Figs. 2, 3 and 4), which suggests that the AcbSh is critically involved in the reinforcing effects of cocaine. Furthermore, the current results support previous findings in our laboratory that Wistar rats will initiate and maintain the self-infusion of cocaine into the AcbSh, but not the AcbC (Rodd-Henricks et al., 2002). The self-infusion of cocaine into the AcbSh does not appear to be a result of a general increase in behavioral activity, because rats in this study learned to discriminate the active from the inactive lever for the self-infusion of 200, 400 and 800 pmol cocaine for P rats and 800 pmol cocaine for Wistar rats (Figs. 3 and 4). Regardless of line, animals that demonstrated significant lever discrimination also displayed decreased responding on the active lever when aCSF was substituted for cocaine and reinstated responding when the cocaine was restored (Figs. 3, 4 and 5). A genetic effect was also apparent because P rats demonstrated greater sensitivity to cocaine, as indicated by self-infusion of 200, 400 and 800 pmol into the AcbSh, whereas Wistar rats did not demonstrate self-administration until 800 pmol was given (Fig. 2). In our previous study (Rodd-Henricks et al., 2002), Wistar rats infused 400 pmol cocaine at a slightly higher rate than aCSF (15 ± 4.2 vs 4.4 ± 3.1 infusions), but was significantly lower than Wistar rats self-administering 800 pmol cocaine. The lack of significance for the self-administration of 400 pmol cocaine into the AcbSh in Wistar rats in the current experiment was due to a reduction in the average number of infusions (11.9 ± 2.5) and more infusions in the aCSF group (5.3 ± 1.1). A small meta-analysis (collapsing all groups across the Rodd-Henricks et al., 2002 report and the current data set) indicated that 400 pmol is not significantly self-administered into the AcbSh in Wistar rats (post-hoc Tukey's b). Nevertheless, P rats displayed significantly more responses for cocaine than Wistar rats at the 200 and 400 pmol concentrations (Figs. 3 and 4). Overall, the data suggest that cocaine is reinforcing in the AcbSh and selective breeding for high alcohol intake is associated with increased sensitivity of this brain region to the reinforcing properties of cocaine.

P rats received a greater number cocaine infusions into the AcbSh for the 200, 400 and 800 pmol compared to the 0 or 100 pmol cocaine groups and received a greater number of infusions at the 400 and 800 pmol compared to the 200 pmol cocaine groups (Fig. 2; left panel). In contrast, Wistar rats only received a greater number of infusions of cocaine into the AcbSh in the 800 pmol cocaine group (Fig. 2; right panel). These findings indicate that P, but not Wistar rats, show a concentration-dependent increase in the number of infusions of cocaine self-administered into the AcbSh. Furthermore, P rats received a greater number of cocaine infusions in the 200, 400 and 800 pmol cocaine groups compared to respective Wistar groups (Fig. 2), which suggests that P rats are more sensitive to the reinforcing effects of cocaine self-administered into the AcbSh than Wistar rats during acquisition. These findings are in agreement with previous findings from our laboratory in which Wistar rats self-administered 400 to 1600 pmol cocaine concentrations into the AcbSh, but not AcbC (Rodd-Henricks et al., 2002).

Compared to session 4, the number of self-infusions by P rats during reinstatement sessions was significantly higher at all concentrations of cocaine tested, whereas Wistar rats only received significantly more infusions at the 400 and 800 pmol concentrations. These findings suggest that the AcbSh of P rats, and to a lesser degree the AcbSh of Wistar rats, was associated with neuronal adaptations resulting from prior cocaine self-administration and extinction that modified the reinforcing properties of cocaine within this nucleus. Repeated, intermittent exposure to cocaine has been shown to result in numerous molecular, neurochemical, and behavioral alterations that have been hypothesized to be the basis for cocaine sensitization (for reviews, see Koob and Nestler, 1997; Pierce and Kalivas, 1997).

Repeated administration of cocaine results in a supersensitivity in the efficacy of reuptake blockade by cocaine (Henry and White, 1995). Similarly, repeated injections of amphetamine produced sensitization to the locomotor stimulation effect of a challenge dose of amphetamine administered directly into the Acb (Paulson and Robinson, 1991). Evidence suggests that cocaine-induced psychomotor sensitization involves neuroadaptations in mesocorticolimbic dopamine (DA) and glutamatergic terminal fields, such as the Acb (Nestler, 2002; Wolf et al., 2004; Everitt and Robbins, 2005; Kalivas and O'Brien, 2008). It has been generally accepted that psychomotor stimulants produce sensitization by increasing extracellular concentrations of DA in the Acb (Robinson and Becker, 1986; Kalivas and Stewart, 1991; White and Wolf, 1991). Other dopaminergic mechanisms contributing to the expression of cocaine sensitization may be supersensitivity of DA D1 receptor-mediated responses in the Acb (Henry and White, 1992; White et al., 1992, 1995). For example, repeated cocaine administration enhances the inhibitory efficacy of DA and DA D1 receptor agonists on Acb neurons (Henry et al., 1989; Henry and White, 1991). Neuroadaptations in accumbal glutamatergic neurotransmission have also been implicated in psychomotor cocaine sensitization, as withdrawal from cocaine potentiates glutamatergic neurotransmission and increases α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) glutamate receptor surface expression (Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007). In general, the data indicate that subthreshold concentrations of cocaine produced a "kindling-like" effect after an abstinence period (a total of 6 days from sessions 4 to 7).

The 800 pmol concentration of cocaine appeared to be the most reinforcing in P rats compared to other doses tested, since it maintained high active lever responding during acquisition (179 ± 18) and resulted in a high number of reinstatement responses (342 ± 54 ; Fig. 4). In Wistar rats, the most reinforcing concentration of cocaine was also 800 pmol. However, levels of responding in Wistar rats were lower than P rats at this same concentration (104 ± 9 ; Fig. 4). The infusions of cocaine at significantly higher levels by P than Wistar rats suggest that cocaine could have a greater reward saliency in the P rats. Alternatively, it is possible that P rats are less sensitive than Wistar rats to the aversive effects of cocaine at high doses, since P rats self-infused more cocaine than Wistar rats at the highest concentration tested in the current study.

P rats will self-administer EtOH directly into the AcbSh at a lower concentration (75 mg%; 16.5 mM) than Wistar rats (100 mg%; 22 mM; Engleman et al., 2009). Thus, selective breeding for high alcohol preference resulted in a significant (1.3 fold) alteration in the sensitivity of the AcbSh to the reinforcing actions of EtOH when compared to Wistar rats. In contrast, P rats displayed a four-fold (200 pmol/100 nl vs. 800 pmol/100 nl; 2 mM vs. 8 mM) greater sensitivity to the reinforcing actions of cocaine in the AcbSh compared to Wistar rats (Figs. 2–4). Therefore, selective breeding for high alcohol preference may have produced a greater divergence in sensitivity to the reinforcing actions of cocaine in the AcbSh than EtOH. Burst-firing activity within the VTA has been demonstrated to be greater in P than Wistar rats (Morzorati, 1998), which suggests that alcohol-preferring rats may release more DA in the NAC compared with alcohol-nonpreferring rats under basal conditions. Also, animals with a high propensity to self-administer drugs of abuse (HR rats) exhibit a greater locomotor response to systemically administered cocaine and amphetamine (Hooks et al., 1991; Piazza et al., 1989) and a greater dopaminergic response within the NAC to cocaine (15 mg/kg; ip) than animals with a low propensity to self-administer substances of abuse (LR rats) (Hooks et al., 1992). Furthermore, HR rats display a higher locomotor response than do LR rats to the intra-accumbens infusion of dopamine and cocaine (Hooks et al., 1993, 1994). These data may explain why alcohol-preferring animals are more sensitive to the dopamine-dependent aspects of the reinforcing

properties of ethanol and cocaine. That is, alcohol and cocaine may be more reinforcing in animals exhibiting an enhanced dopaminergic response to these drugs, which may be associated with high-alcohol preference.

P rats displayed a greater sensitivity to the intracranially self-infused cocaine compared to ethanol, which may reflect cocaine having a greater impact on DA neurotransmission than ethanol, which would magnify any potential differences between P and Wistar rats' sensitivity to the reinforcing effects of cocaine. Indeed, cocaine administration has been shown to enhance Acb DA efflux from 200 to 600% of baseline levels (Hemby et al., 1997; Smith et al., 2006; Zocchi et al., 2003), whereas ethanol administration has been shown to enhance Acb DA by only 150 to 200% of baseline levels in rats and mice (Zocchi et al., 2003; Weiss et al. 1993; Yoshimoto et al., 1992; Katner and Weiss, 2001).

The results of the present study provide important information regarding how genetic factors influence a predisposition to high alcohol drinking and abuse of other drugs, and how genetic factors that influence alcohol drinking can also influence the effects of other drugs of abuse. In humans, concurrent use of alcohol and other drugs of abuse have been reported in numerous clinical studies and the high prevalence of alcohol and cocaine co-abuse in humans has been postulated to be predicated upon both a common genetic factor that predisposes an organism to abuse multiple substances and the interaction of drugs within the organism (Uhl, 2004, 2006; Uhl et al., 2008). Furthermore, research indicates that individuals genetically predisposed to abuse alcohol and other drugs of abuse are disproportionately reactive to alcohol and other drugs of abuse when given alone (Schuckit, 1994a,b; Kareken et al., 2010; Uhl et al., 2008; Piazza and Le Moal, 1996). These findings in humans are supported by the present findings in which rats selectively bred for high alcohol preference are also more sensitive to the reinforcing actions of cocaine and, therefore, support the hypothesis that there is a common genetic basis for the abuse potential of alcohol and cocaine.

In summary, the results of the present study support the AcbSh as being a neuroanatomical substrate involved in cocaine self-administration. In addition, the AcbSh of P rats is more sensitive than Wistar rats to the reinforcing effects of cocaine, and a brief history of cocaine self-administration and extinction is accompanied by neuronal adaptations that enhanced the reinforcing properties of cocaine in the AcbSh.

Acknowledgments

This study was supported in part by AA07611, AA07462, and AA012262.

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