Pharmacology, Biochemistry and Behavior 90 (2008) 331–338

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The effects of novelty-seeking phenotypes and sex differences on acquisition of cocaine self-administration in selectively bred High-Responder and Low-Responder rats

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ARTICLE INFO

Article history:
Received 4 December 2007
Received in revised form 28 February 2008
Accepted 10 March 2008
Available online 25 March 2008

Keywords:
Estrous cycle
Drug abuse
Psychomotor stimulants
Sex differences
Novelty-seeking

ABSTRACT

Individual differences in exploratory behavior can predictably influence psychostimulant self-administration behavior. Male rats that exhibit a high degree of locomotor activity in a novel environment (High Responders, HR) will self-administer cocaine more readily than males exhibiting low levels of novelty-induced locomotion (Low Responders, LR). The present experiment investigates the combined influences of the sex of an individual and individual phenotypes in novelty-induced locomotion to predispose animals to acquire cocaine self-administration behavior, in male and female rats selectively bred for the HR–LR phenotypes. We first established that HR females, like their male counterparts, exhibit a dramatically greater locomotor response to novelty and less anxiety-like behavior than do LR females. While locomotor behavior was subtly influenced by estrous stage, with both HR and LR females showing increased activity during metestrus and diestrus compared to proestrus and estrus, the effect did not obscure HR–LR differences. When male and female HR–LR animals were trained to self-administer cocaine (2 h/day, 5 days/wk×3 wk, 0.2 mg cocaine/kg/infusion), HR males and females acquired cocaine self-administration significantly faster than their LR counterparts. Furthermore, HR females self-administered significantly more cocaine than all other groups. In conclusion, female rats, like males, exhibit HR–LR phenotypes that predict rapidity of acquiring cocaine self-administration. Moreover, HR females self-administer more cocaine than HR males and both LR groups.

1. Introduction

Individual differences in novelty-induced locomotion influence psychostimulant self-administration (Piazza et al., 1989, 1991a). When placed into a novel environment, Sprague–Dawley rats exhibit a wide range of behavior, with some animals showing high levels of novelty-induced locomotor behavior (High Responders, HR) and others showing much lower levels of locomotor activity (Low Responders, LR). Numerous studies have used the HR–LR model to demonstrate the relationship between novelty-induced locomotor activity, drug-taking and other risk-taking behaviors, as well as neuroendocrine stress reactivity (Hooks et al., 1991; Kabbaj et al., 2000; Piazza et al., 1989, 1991a). HR rats exhibit exaggerated stress-induced corticosterone secretion (Kabbaj et al., 2000; Piazza et al., 1991a), increased behavioral reactivity to psychostimulants (Hooks et al., 1991; Piazza et al., 1989, 1991a), diminished fear and anxiety-like behavior (Kabbaj et al., 2000; Stead et al., 2006), and increased aggressive behavior (Abraham et al., 2006) compared to their LR counterparts. Neurochemical and neural gene expression differences contribute, at least in part, to the HR–LR phenotype (Hooks et al., 1994a,b; Kabbaj, 2004; Kabbaj et al., 2000; Piazza et al., 1991b).

Many of the behavioral features of the HR–LR phenotype are homologous to novelty-seeking and impulsive behavioral traits in humans that have an enhanced vulnerability for drug abuse (Cloninger, 1987; Zuckerman and Neeb, 1979). Thus, the HR–LR trait may tap into the broad dimension of behavioral disinhibition versus behavioral control—a dimension that has been implicated in the vulnerability versus resilience to numerous psychiatric and addictive disorders (Ball et al., 2005).

In addition to the effects of individual phenotypes in novelty-induced locomotion on psychostimulant intake, sex differences also affect drug intake in humans and rats. Compared to men, women begin using cocaine at earlier ages, enter treatment sooner, have a faster onset of addiction, and have shorter periods of abstinence (Lynch et al., 2002). Women also develop a more severe dependence on cocaine, and when exposed to drug related cues, they report greater craving than

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doi:10.1016/j.pbb.2008.03.008

men (Robbins et al., 1999). Some sex differences may be attributed to fluctuations in estradiol levels during the menstrual cycle, as women subjects report greater pleasure from smoked cocaine when their estradiol levels are elevated (Evans et al., 2002; Sofuoglu et al., 1999).

Similar sex differences have been observed in rodent studies. For example, female rats acquire cocaine self-administration behavior more quickly than males (Lynch et al., 2002; Roth and Carroll, 2004; Roth et al., 2004). Moreover, female rats self-administer different amounts of cocaine across the estrous cycle (Lynch et al., 2000), and consume more drug when estradiol levels are high, either following estradiol treatment in ovariectomized females (Hu et al., 2004; Lynch et al., 2001) or in cycling females in estrus (Roberts et al., 1989). Therefore, sex clearly impacts cocaine self-administration behavior, with estradiol contributing to the magnitude of these sex differences.

We recently began to selectively breed rats according to novelty-induced locomotion, and our results suggest that the HR–LR phenotypes are strongly influenced by genetic factors (Stead et al., 2006). Our selectively bred HR and LR lines show a variety of behavioral differences, including marked differences in anxiety-like behavior, which are consistent with prior studies using commercially purchased HR–LR animals (Kabbaj et al., 2000; Stead et al., 2006). A major goal of the present study was to evaluate cocaine self-administration behavior in our HR–LR bred lines to confirm that selectively bred HRs, like commercially purchased HR animals, will acquire cocaine self-administration behavior more rapidly than LRs (Kabbaj et al., 2001). While the majority of previous studies used male rats to examine HR–LR differences in cocaine and amphetamine self-administration (Dellu et al., 1996; Kabbaj, 2004; Kabbaj and Akil, 2001; Kabbaj et al., 2000, 2004, 2001; Klebaur et al., 2001; Piazza et al., 1989, 1991a), a handful of studies reported similar cocaine-induced behavioral differences in commercially purchased HR–LR females (Klebaur et al., 2001; Sell et al., 2005). A major caveat of this previous work, however, was that HR–LR males and females were never directly compared. Thus, our second major objective was to evaluate the impact of sex on HR–LR differences in cocaine self-administration behavior. We also examine the potential influence of estrous cycle on cocaine self-administration and other aspects of the HR–LR behavioral phenotype in females. Our results show that selectively bred HR females, like their male counterparts, acquire cocaine self-administration more rapidly than LR males and females. Interestingly, we also find that HR females self-administer more cocaine than HR males and LR males and females.

2. Materials and methods

2.1. Animals

Sprague–Dawley male and female rats were acquired from our in-house breeding colony where selectively bred HR and LR lines are maintained. Selectively bred HR and LR females from the 8th generation of our bred lines were used in studies to examine the impact of estrous cycle on novelty-seeking and anxiety-like behavior (N=72 per HR/LR group). Selectively bred HR and LR male and female rats from the 14th generation were used for the cocaine self-administration study (N=15 per group). A description of our breeding strategy and initial behavioral characterization of the selectively bred HR and LR lines has been published (Stead et al., 2006). Male and female rats were reared together in a 14 h light:10 h dark cycle (lights on at 7:00 am) until weaning at 21 days of age. After weaning males were kept in a 12 h light:12 h dark cycle room, and females kept in a 14 h light:10 h dark cycle. Female rats were kept on this extended 14 h light:10 h dark cycle to promote regular estrous cycle and fertility (Everett and Sawyer, 1949). Males were returned to the 14 h light:10 h dark cycle conditions ten days prior to the initiation of self-administration testing. Food and water were always available ad libitum, and all experiments were conducted in accordance with the National Institute of Health (NIH) guidelines on laboratory animal use and care.

2.2. Impact of estrous cycle on novelty-seeking and anxiety-like behavior

Behavioral studies were conducted with selectively bred HR–LR females to evaluate the impact of individual differences and estrous cycle stage on novelty-seeking and anxiety-like behavior. Estrous cycle was monitored daily for 1 week prior to any behavior testing, and was also evaluated immediately following the completion of the three behavioral tests (locomotor response to novelty, elevated plus maze, and light–dark box; described below). Estrous state was determined by microscopic examination of vaginal cells collected by lavage. Behavioral testing was performed between 8:00 and 11:30 am.

2.2.1. Locomotor response to novelty

Animals were handled for three consecutive days prior to testing to familiarize them with the investigator, then screened for locomotor response to a novel environment by placing them in a standard size (43×21.5×24.5 cm) clear acrylic cage in a different room from where the animals had been housed. Test cages were identical to housing cages except that they had a wire mesh floor. Locomotor activity was monitored in 5-minute increments over a 1 h period by two panels of photocells connected to a computer. The first panel of three photocells was placed at ground level (8 cm from the floor) to record horizontal locomotion, with the second panel of 5 photocells located near the top of the cage (16 cm from the floor) to determine rearing behavior. The locomotion testing apparatus and motion recording software were created in-house at the University of Michigan. Final locomotion scores were determined by summing horizontal and rearing activities. At the time of testing, a minimum of 18 HR and 18 LR females were at each of the 4 stages of estrous (proestrus, estrus, metestrus, diestrus). All testing was performed between 8:00 and 11:30 am.

2.2.2. Elevated plus maze test

One week after locomotor testing, a subset of HR- and LR-bred females (N=27 HR and N=27 LR) were subjected to the elevated plus maze anxiety test. The apparatus was constructed of black Plexiglas, with four elevated arms (70 cm from the floor, 45 cm long, and 12 cm wide). The arms were arranged in a cross, with two opposite arms enclosed by 45-cm-high walls, and the other two other arms open. At the intersection of the open and closed arms, there was a central 12×12 cm square platform giving access to all arms. The test room was lit at approximately 30 lx, and behavior was monitored using a computerized videotracking system (Noldus Ethovision, Leesburg, VA). At the beginning of the 5-minute test, each rat was placed in the central square facing a closed arm. The computerized tracking system recorded the latency to first enter the open arm, the amount of time spent in the open arm, closed arm, or center square, and the total distance traveled over the course of the 5 min test. Testing was performed between 8:00 and 11:30 am.

2.2.3. Light–dark box test

One week after locomotor testing, a separate group of HR- and LR-bred females (N=39 HR and N=38 LR) were subjected to the light–dark box anxiety test. The test apparatus was a 30×60×30 cm Plexiglas shuttle-box divided into two equal-sized compartments by a wall with a 12-cm-wide open door. One compartment was painted white and brightly illuminated (70 lx), and the other compartment was painted black with very dim light (5 lx). Rows of five photocells located 2.5 cm above the stainless steel grid floor monitored the rats' locomotor activity and time spent in each compartment. A microprocessor recorded the latency to first exit the dark compartment where the rats were initially placed, the number of photocell beams

interrupted, and the time spent in each compartment during the 5-minute test. Testing was performed between 8:00 and 11:30 am.

2.3. Cocaine self-administration

Selectively bred HR and LR male and female (N=15 per group) rats from the 14th generation of our bred lines were screened for locomotor response to novelty as described above. Male and female rats were tested on separate days after careful cleansing of the apparatus to devoid it from odors. All testing was performed between 8:00 and 11:30 am. Approximately 1 week after locomotor testing, male and female rats were transferred to a common housing room set on a reversed light:dark cycle (14:10, lights on at 5:30 pm).

2.3.1. Surgical procedures

Approximately 5 days following the room transfer, male and female HR–LR rats received implants of indwelling intravenous jugular catheters connected to a back port. Jugular catheter construction and implantation were based on previously described procedures (Crombag et al., 2000; Kalivas and Duffy, 1993; Weeks, 1972). In short, catheters were constructed by gluing silastic tubing (Silastic tubing, 0.51 mm I.D.×0.94 mm O.D., Dow Corning, Midland, MI) to an external guide cannula (22 Gauge guide cannula; Plastics One, Roanoke, VA) using cranioplastic cement. A polypropylene mesh was secured to the bottom of the cannula using this same cement. Rats were anesthetized with a combination of ketamine (40 mg/kg per female; 55 mg/kg per male; i.p.) and medetomidine (0.3 mg/kg per female; 0.4 mg/kg per male; i.p.). The free end of the silastic tubing of the catheter apparatus was inserted into the right jugular vein of the animal and secured using 4.0 silk sutures around the tubing and the venous tissue. The catheter port exited dorsally from the animal. After successful implantation, the animal's catheter was flushed with 0.2 ml of heparin (30 U/ml in 0.9% sterile saline) and 0.2 ml of gentamicin (0.08 mg/ml) to prevent clotting and infection, respectively. A dummy stylet was then inserted into the port opening, and an injection of antisedan (1.5 mg/kg per female; 2.0 mg/kg per male; i.p.) was given to revive the animal. Two days after surgery, catheters were flushed with 0.2 ml of heparin (30 U/ml in 0.9% sterile saline) and 0.2 ml of gentamicin (0.08 mg/ml). Prior to the beginning of each self-administration session, catheters were flushed with 0.1 ml of sterile saline, and following self-administration, catheters were flushed with gentamicin (0.08 mg/ml). Female estrous cycle was monitored via daily vaginal lavage and microscopic examination of vaginal cells collected.

2.3.2. Cocaine self-administration procedures

Five days after surgery, rats were placed into standard operant chambers (Med Associates, Inc., Georgia, VT) for 2 h once a day during which time they could nose poke into the active hole for cocaine on an FR1 schedule of reinforcement. A maximum of 100 infusions could be administered during the 2-hour test period. Each active hole nose poke resulted in a 50-μl infusion of 0.2 mg/kg of cocaine HCL delivered over 2.8 s accompanied by a stimulus light in the active hole and a tone (85 dB). This compound stimulus occurred simultaneously and was followed by a 5 s timeout, during which time nose pokes were recorded but had no consequences. Nose pokes into the inactive hole were recorded but had no consequences. Rats were connected to the infusion syringe via a swivel mounted to a counter balanced arm, which allowed animals to move freely in the testing environment.

Four groups of rats with functional catheters were given the opportunity to self-administer cocaine: female HR (N=7), female LR (N=10), male HR (N=8), and male LR (N=8). Self-administration sessions lasted for 2 h a day, 5 days a week (Monday through Friday), for 3 weeks between 10:00 am and 3:00 pm. Vaginal smears were taken daily for determination of estrous cycle immediately following the self-administration session.

2.4. Data analysis

Behavioral data from the locomotor response to novelty test, the elevated plus maze, and light–dark box tests in 8th generation HR- and LR-bred females were analyzed with a two-way ANOVA (HR–LR phenotype×estrous stage). Data from the locomotor response to novelty test in 14th generation HR- and LR-bred male and female animals were analyzed with a two-way ANOVA (HR–LR phenotype×sex). All ANOVAs were followed by Fisher's post-hoc comparisons when necessary.

Self-administration data were analyzed using SPSS 14.0 for Microsoft (for mixed models analysis) and StatView 4.5 for Macintosh (for one-way and two-way ANOVA's). Data of latency to acquire cocaine self-administration behavior were analyzed using a two-way ANOVA (HR–LR phenotype×sex). Data for the number of infusions were analyzed using a two-way ANOVA (HR–LR phenotype×sex). Data of the impact of estrous cycle on infusions self-administered in females were analyzed using two-way ANOVA (HR–LR phenotype×estrous cycle stage). When necessary, ANOVA's were followed by Bonferroni's multiple comparison test.

3. Results

3.1. Impact of estrous cycle on HR–LR novelty-seeking and anxiety-like behavior

3.1.1. Locomotor response to novelty

Two-way ANOVA revealed a main effect of both HR–LR phenotype (F[1, 158]=271.31, p<0.0001) and estrous cycle stage (F[3,158]=11.10, p<0.0001) on novelty-induced locomotor activity. HR-bred females, regardless of stage of estrous cycle, were much more active in response to novel environment compared to LR-bred females (Fig. 1). Females were slightly but significantly more active during metestrus/diestrus compared to proestrus/estrus, and this effect was apparent in both HR- and LR-bred females (Fig. 1). There was no significant HR–LR phenotype×estrous cycle stage interaction.

3.1.2. Elevated plus maze

Two-way ANOVA revealed a main effect of HR–LR phenotype on two measures of anxiety-like behavior in the elevated plus maze: percent time spent in the open arms (F[1,50]=18.47, p<0.001; Fig. 2A) and latency to initially enter the open arms of the elevated plus maze

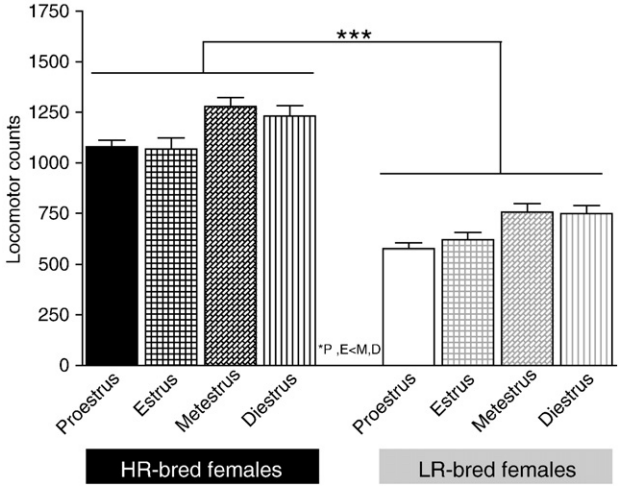

Fig. 1. Impact of estrous cycle on novelty-induced locomotion. When placed in a novel cage, selectively bred HR females (N=18–20 in each stage of estrous), regardless of stage of estrous cycle, were much more active than selectively bred LR females (N=18-20 in each stage of estrous). Overall, females were slightly but significantly more active during metestrus/diestrus compared to proestrus/estrus, but, this effect was apparent in both HR- and LR-bred females. *** indicates p<0.0001; * indicates p<0.05.

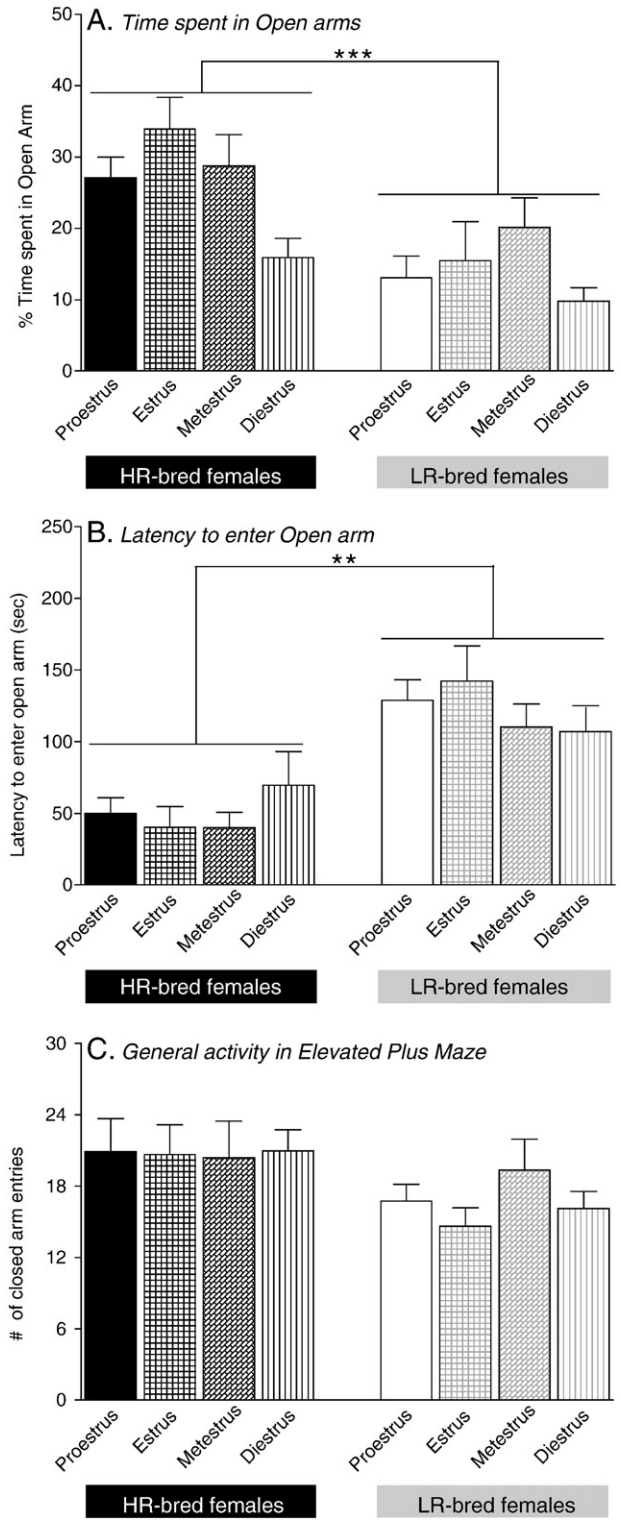

Fig. 2. Impact of estrous cycle on anxiety-like behavior in the elevated plus maze. Selectively bred HR females (N=6–8 in each stage of estrous), regardless of stage of estrous cycle, showed low levels of spontaneous anxiety-like behavior in the elevated plus maze compared to selectively bred LR females (N=6–8 in each stage of estrous). HR-bred females spent more time in the anxiogenic open arm of the elevated plus maze (A), and showed reduced latency to initially enter the open arm (B), but made a similar number of closed arm entries (D) compared to LR-bred females. Stage of estrous also mildly affected behavior in the elevated plus maze, with females in diestrus spending less time in the open arms compared to females in proestrus, estrus, or diestrus. ** indicates $p<0.01$.

($F[1,50]=14.94$, $p<0.01$; Fig. 2B), with HR females spending greater time in the open arm and shorter latency to initially enter the open arm compared to LR females. HR and LR females showed similar numbers of closed arm entries (Fig. 2C). There was a main effect of estrous stage on percent time spent in the open arm ($F[1,50]=5.31$, $p<0.01$), and post-hoc analysis revealed that females in diestrus spent significantly less time in the open arm compared to females in proestrus, estrus, or metestrus. There was no effect of estrous stage on latency to initially enter the open arm or closed arm entries, and no significant phenotype×estrous stage interaction for these variables.

3.1.3. Light–dark box

Two-way ANOVA revealed a main effect of HR–LR phenotype on two measures of anxiety-like behavior in the light–dark box: percent time spent in the light compartment ($F[1,73]=15.33$, $p<0.001$; Fig. 3A) and latency to initially enter the light compartment ($F[1,73]=15.69$, $p<0.01$; Fig. 3B), with HR females spending greater time in the light and shorter latency to initially enter the light compared to females. There was also a main effect of HR–LR phenotype on total locomotor activity in the light–dark box ($F[1,73]=62.23$, $p<0.0001$; Fig. 3C), with HR females showing greater overall activity compared to LR females. There was no main effect of estrous stage on any of these measures, and no significant phenotype×estrous stage interaction for these variables.

3.2. Cocaine self-administration

3.2.1. Locomotor response to novelty

Prior to undergoing cocaine self-administration, selectively bred HR and LR male and female rats were screened for their locomotor response in a novel environment. HR-bred male rats (N=15) had an average locomotor score of 1275±14 counts, and LR-bred male rats (N=15) had an average locomotor score of 185±15 counts. HR-bred female rats (N=15) had an average locomotor score of 1241±93 counts, and LR-bred female rats (N=15) had an average locomotor score of 378±39 counts. Two-way ANOVA revealed a main effect of HR–LR phenotype ($F[1,56]=4200.31$, $p<0.0001$), and a main effect of sex ($F[1,73]=27.45$, $p<0.0001$) on locomotor response to novelty. There was also a significant HR–LR phenotype×sex interaction ($F[1,56]=56.60$, $p<0.0001$). Post-hoc analysis revealed that LR-bred males were significantly less active than LR-bred females ($p<0.0001$), while HR-bred males and females showed similar levels of novelty-induced locomotor activity.

3.2.2. Acquisition of cocaine self-administration behavior

Animals were said to have acquired cocaine self-administration if they received 10 or more infusions in 3 consecutive sessions (Jackson et al., 2006). As was expected, HR animals acquired self-administration more rapidly than did LR animals, independent of the sex of the animals. A two-way ANOVA indicated a main effect of HR–LR phenotype ($F[1,29]=29.63$, $p<0.0001$), but not a main effect of sex ($F[1,29]=0.196$, $p=0.6609$) on acquisition of cocaine self-administration behavior. There was no HR–LR phenotype×sex interaction ($F[1,28]=0.3945$, $p=0.5350$). Post-hoc analyses revealed that HR male and female rats acquired cocaine self-administration behavior in fewer days than LR male and female rats ($p<0.01$).

3.2.3. Number of infusions of cocaine

Female HR rats self-administered significantly more infusions following acquisition of self-administration than any other group (Fig. 4A), even though both male and female HR animals acquired self-administration very rapidly. HR and LR males and females did not differ in the number of inactive pokes following acquisition. Analysis of the mean number of infusions per group using two-way ANOVA (sex×phenotype) revealed main effects of sex ($F[1,403]=4.137$; $p<0.05$), HR–LR phenotype ($F[1,403]=13.973$; $p=0.0002$, and an interaction between

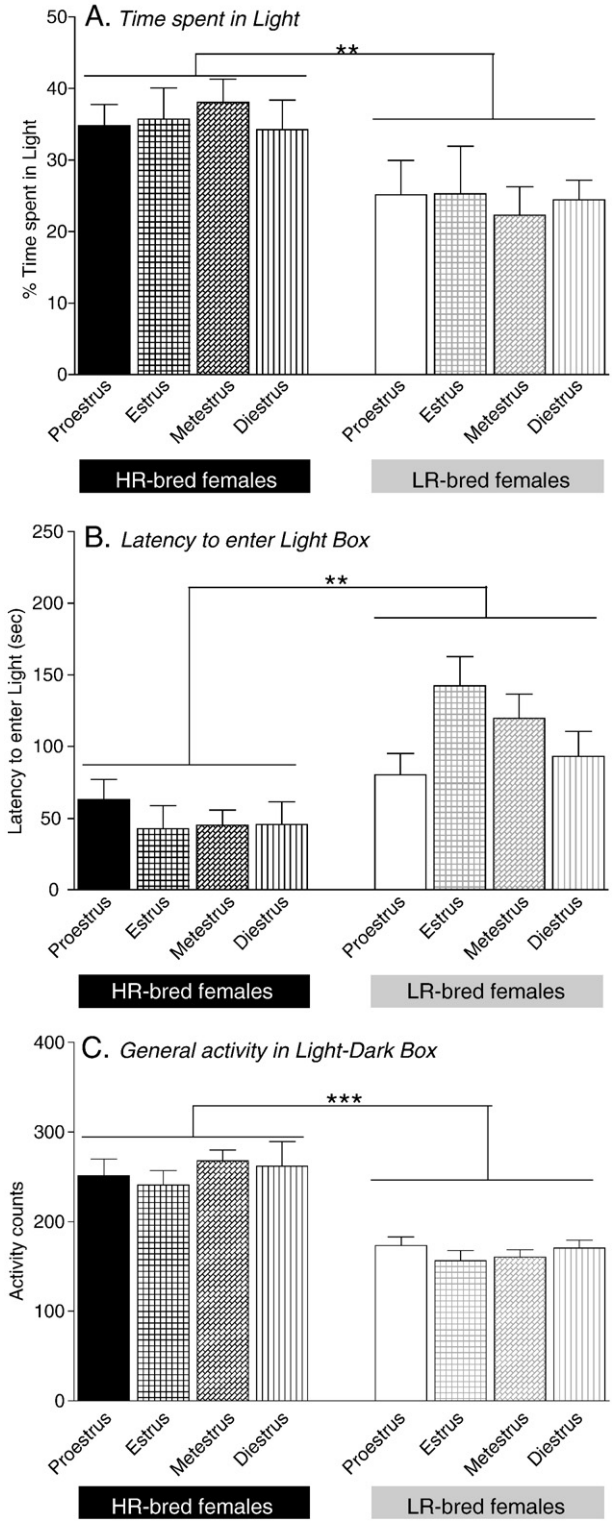

Fig. 3. Impact of estrous cycle on anxiety-like behavior in the light–dark box. Selectively bred HR females (N=8–9 in each stage of estrous), regardless of stage of estrous cycle, showed low levels of spontaneous anxiety-like behavior in the light–dark box test compared to selectively bred LR females (N=8–9 in each stage of estrous). HR-bred females spent more time in the anxiogenic light compartment (A), and showed reduced latency to initially enter the light (B) compared to LR-bred females. HR females were also significantly more active than LR females during the 5-min light–dark test (C). ** indicates $p<0.01$; *** indicates $p<0.0001$.

sex×phenotype ($F[1,403]=73.365$; $p<0.0001$). Post-hoc analysis revealed that HR females received a greater number of infusions than HR males, LR males, and LR females ($p<0.0007$; Fig. 4A), and LR females received more infusions than HR males ($p=0.0077$; Fig. 4A). Analysis of the mean number of inactive pokes per group using two-way ANOVA (sex×phenotype) revealed no effects of sex ($F[1,349]=0.052$; $p=0.8195$) or phenotype ($F[1,349]=0.333$; $p=0.5641$).

When the time course of cocaine self-administration was examined by a mixed models analysis to take into account sex×phenotype×day, there was confirmation of main effects of sex ($F[1,350]=20.872$; $p<0.001$) and HR–LR phenotype ($F[1,350]=10.581$; $p=0.001$), and an interaction between sex×HR–LR phenotype ($F[1,350]=7.781$; $p=0.006$). Additionally, results revealed a main effect of day ($F[14,54]=8.958$; $p<0.001$). Post-hoc analysis indicated that HR females received a significantly greater number of infusions compared to LR females on days 1, 2, and 3 ($p<0.02$, Fig. 4B); HR females received more infusions than HR males on days 11 and 12 ($p<0.05$); HR females received more infusions than LR males on days 1 and 2 ($p<0.004$); and HR males received more infusions than LR males on day 3 ($p=0.0060$, Fig. 4C).

3.2.4. Impact of estrous cycle on the number of infusions of cocaine

No impact of estrous cycle on the number of infusions of cocaine self-administered was observed. Two-way ANOVA revealed a main effect of HR–LR phenotype ($F[1, 149]=11.647$, $p=0.0008$) but not estrous cycle stage ($F[3,149]=1.114$, $p=0.3994$; Fig. 4D) on the number of cocaine infusions.

4. Discussion

In the present study, we first established that our selectively bred HR females, like their male HR counterparts, show exaggerated locomotor response to novelty (Fig. 1) and reduced anxiety-like behavior (Figs. 2–3) compared to LR females, with the stage of estrous cycle exerting only a minor influence on novelty-seeking behavior. Secondly, we report that both selectively bred male and female HR rats acquire cocaine self-administration behavior more rapidly than their LR-bred counterparts of the same sex (Fig. 4), which is consistent with a variety of prior studies using commercially purchased HR–LR animals (Kabbaj et al., 2001; Mantsch et al., 2001; Stead et al., 2006). Furthermore, our direct side-by-side comparison of HR–LR male and female rats revealed that HR-bred females consumed more cocaine than any other group, including the selectively bred HR males (Fig. 4A).

4.1. Impact of estrous cycle on locomotor response to novelty and anxiety-like behavior

The selectively bred HR and LR females displayed dramatic differences in locomotor response to novelty, which is consistent with previous research using selectively bred males (Stead et al., 2006) or outbred HR and LR male rats (Stead et al., 2006), as well as other work with commercially purchased HR–LR female rats (Klebaur et al., 2001; Piazza et al., 1989; Sell et al., 2005). While stage of estrous cycle mildly impacted locomotor response to novelty in the 1 h locomotor screen, with females in diestrus/metestrus showing higher novelty-induced locomotor activity compared to females in proestrus/estrus, this effect was apparent to a similar extent in both HR and LR females, and did not obscure the obvious HR–LR group differences (Fig. 1). These findings are consistent with another recent study which reported that females in diestrus displayed greater novelty-induced locomotor behavior compared to females in estrus (Severino et al., 2004). However, estrous cycle did not appear to affect overall activity in our other behavioral tests (the 5-min elevated plus maze and light–dark tests; Figs. 2C and 3C), possibly due to the shorter test duration or influence of other environmental factors (e.g., light intensity), which may have made these other tests more stressful. Alternatively, the lack of estrous cycle effect on activity in these anxiety tests may have been due to diminished statistical power since the N's per group in these experiments were less than N's used in the locomotor response to novelty test.

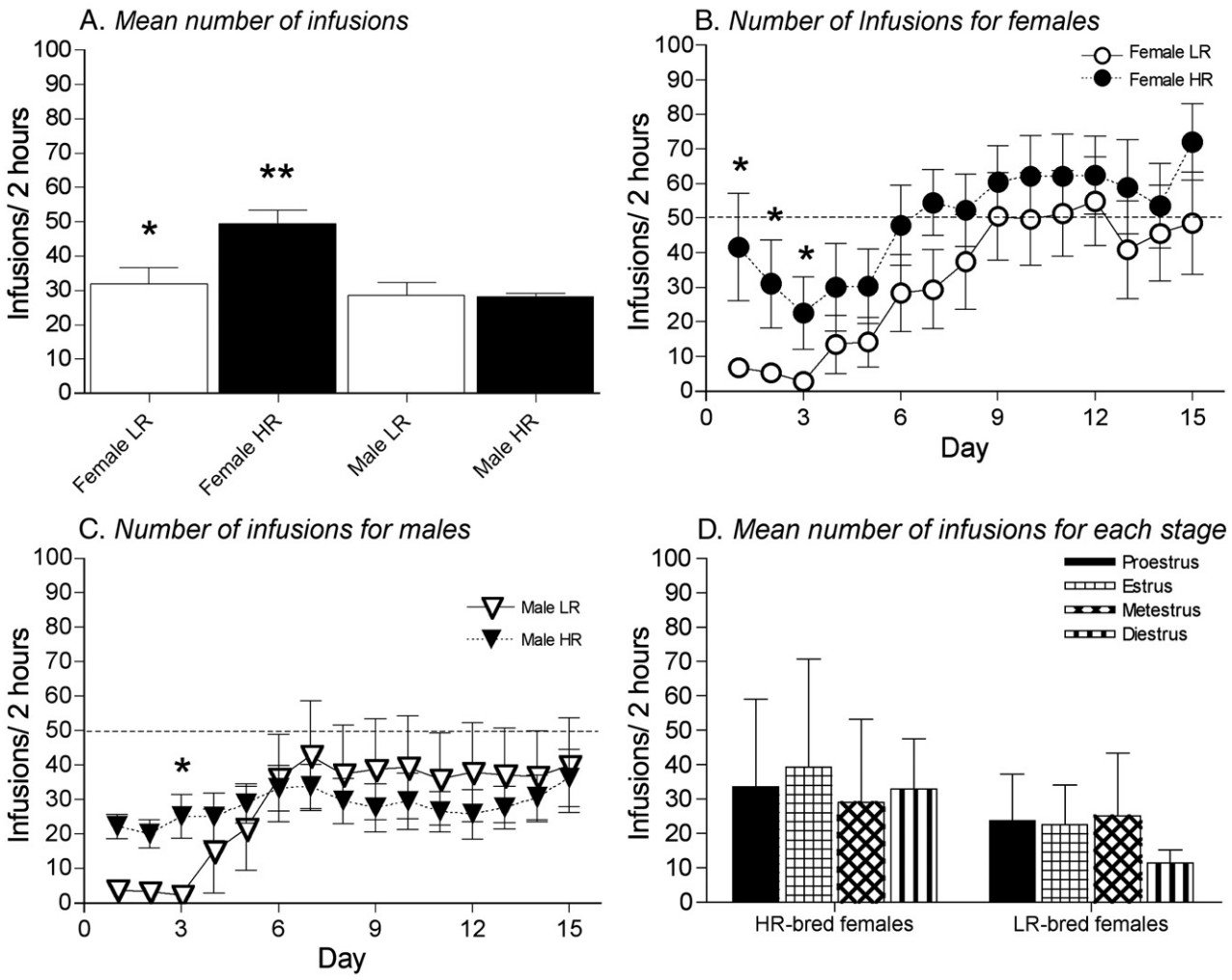

Fig. 4. Impact of sex and individual differences on the number of cocaine infusions self-administered. A) Female HR ($N=7$) rats self-administered a significantly greater number of cocaine infusions than HR females ($N=10$), HR males ($N=8$), and LR males ($N=8$) when the total infusions for the 15 days of testing are considered. Female LR rats received more infusions than male HR rats. * indicates $p<0.05$ compared to HR males; ** indicates $p<0.001$ compared to all other groups. B) HR females self-administered a significantly greater number of cocaine infusions than LR females on days 1–3. * indicates $p<0.05$ HR>LR. C) Male HR and LR significantly differ in the number of cocaine infusions self-administered on day 3. Dashed line denotes 50 infusions per session. *indicates $p<0.05$ HR>LR. D) Estrous cycle had no effect on the mean number of infusions for HR or LR females ($N=4$–7 HR and $N=6$–9 LR in each stage of estrous).

Several studies have shown that the stage of estrous cycle can influence anxiety-like behavior on a variety of tests (Archer, 1975; Bitran et al., 1991; Diaz-Veliz et al., 1997; Fernandez-Guasti and Picazo, 1992; Frye et al., 2000; Gray and Levine, 1964; Johnston and File, 1991; Marcondes et al., 2001; Mora et al., 1996). The majority of these studies report that females in proestrus display reduced anxiety-like behavior compared to females in estrus or diestrus (Diaz-Veliz et al., 1997; Fernandez-Guasti and Picazo, 1992; Frye et al., 2000; Marcondes et al., 2001; Mora et al., 1996). Other studies, however, have failed to detect such behavioral differences, which may be due to a variety of technical factors, including rat strain, individual variation in response to novelty that may enhance or obscure the effects of cycle, the time of day when testing was conducted, and light intensity over the test apparatus (Mora et al., 1996; Nomikos and Spyraki, 1988).

The present set of experiments examined the impact of estrous cycle on anxiety-like behavior in HR–LR females in two tests of anxiety-like behavior: the elevated plus maze and the light–dark box test. Selectively bred HR females, regardless of stage of estrous cycle, showed reduced anxiety-like behavior in both tests compared to LR females (Figs. 2–3). These data are broadly consistent with previous behavioral findings in both selectively bred (Stead et al., 2006) and commercially purchased (Clinton et al., 2007) HR–LR females. Our present results also showed that all females in diestrus, regardless of HR–LR phenotype, showed greater anxiety-like behavior (less open arm time) compared to females in proestrus, estrus, or metestrus, however this effect was most pronounced in HR females. These findings may not be completely congruent with previous work (cited above) due in part to our testing conditions, rat strain, and limited number of animals per group. It is also important to keep in mind that the present studies were conducted in selectively bred HR–LR animals, and the HR–LR phenotypes were rather extreme. Thus, it is conceivable that estrous cycle may play some greater role in shaping behavior of more intermediate animals (i.e., commercially purchased animals).

4.2. Impact of estrous cycle on cocaine self-administration

Prior studies have shown an effect of estradiol on cocaine intake in female rats either during estrus or when given estradiol following ovariectomy (Hu et al., 2004; Jackson et al., 2006; Roberts et al., 1989). We did not, however, see an effect of estrous cycle on self-administration of cocaine in HR and LR females (Fig. 4D). This lack of effect is possibly due to the low dose of cocaine (0.2 mg/kg/infusion) used in this study. Prior studies have found the impact of estradiol on cocaine self-administration in females to be greatest at a dose of 0.4 and 0.5 mg/kg/infusion (Hu et al., 2004; Jackson et al., 2006; Roberts et

al., 1989). We chose a lower dose of cocaine due to our interest in differences in acquisition of self-administration behavior, but this may have made it difficult to see an effect of estrous cycle. It should be pointed out that it took several days for females to acquire self-administration of cocaine, so there were only a few days post-acquisition during which estrous cycle effects could be evaluated, and the low incidence of a complete cycle made it difficult to get data from all animals, thereby decreasing the number of animals that could be included in the analysis and thus statistical power. Consistent with this finding, Lynch et al. (2000) reported that female rats did not always cycle normally during cocaine self-administration, and they observed an effect of estrous cycle on cocaine self-administration behavior only after self-administration behavior had stabilized. Finally, the number of females used for each stage of the estrous cycle was much less in the self-administration study (a minimum of 4 HR and 6 LR) than the number used in locomotor testing (a minimum of 18 HR and 18 LR females) due to the small number of normally cycling rats.

Another important feature of the HR–LR model is the differences between HR and LR animals in hypothalamic–pituitary–adrenal axis function and stress responsiveness, although the majority of studies to date have focused on male animals (Hooks et al., 1991; Kabbaj et al., 2000; Piazza et al., 1989, 1991a). Considering the dynamic interplay between the stress axis and circulating estrogen hormones (Critchlow et al., 1963; Dutriez-Casteloot et al., 2001; Figueiredo et al., 2002; Isgor et al., 2003; Viau and Meaney, 1991), it will be important to carefully examine potential HR–LR differences in stress reactivity across the different stages of the estrous cycle, and the impact of the various behavioral tests on the stress axis. Thus, future work will address these issues, and at the same time, explore neural mechanisms that may contribute to both neuroendocrine and behavioral differences in HR–LR females.

4.3. Impact of individual characteristics on drug use

HR and LR rats clearly exhibit differential behavioral responses to cocaine (Fig. 4) in addition to their dramatic differences in novelty-seeking and anxiety-like behavior. Since the HR and LR lines have been selectively bred for many generations, there may be some concern that while selecting for differences in novelty-induced activity, we have inadvertently co-selected for other differences, such as divergent pharmacokinetic sensitivity or metabolism, that could influence animals' proclivity for psychostimulant self-administration. Several interesting papers have reported differences in neural metabolic capacity (assessed via cytochrome oxidase activity) in rats selectively bred for differences in learned helplessness (Shumake et al., 2000) and behavioral excitability (Gonzalez-Lima and Sadile, 2000) rats (for review see Sakata et al., 2005). These studies suggest that genetic divergence in such rodent models shapes baseline neural metabolism in particular neuroanatomical circuits, setting the stage for animals to behave so differently (Sakata et al., 2005). It is highly possible that HR–LR rats might exhibit neurochemical differences, perhaps in dopaminergic reward circuits, which may underlie their differences in psychostimulant sensitivity. Future studies will explore such possibilities. Regardless, it seems unlikely that selective breeding for novelty-seeking, with less than 1% in-breeding (Stead et al., 2006), has accidentally bred for pharmacokinetic sensitivity differences that drive HR versus LR cocaine responsivity since previous work in commercially available (non-selectively bred) HR/LR rats demonstrated similar HR/LR differences in cocaine sensitivity (Dellu et al., 1996; Kabbaj, 2004; Kabbaj and Akil, 2001; Kabbaj et al., 2000, 2004, 2001; Klebaur et al., 2001; Piazza et al., 1989, 1991a).

This study directly compares cocaine intake among HR-bred and LR-bred males and females. Although all subjects were housed under a 14 h light:10 h dark cycle prenatally and postnatally until weaning (postnatal day 21), after weaning males were moved to a 12 h light:12 h dark cycle for 6 weeks, while females remained on a 14 h light:10 h dark cycle. This change in male photoperiod could be viewed as a confound when directly comparing self-administration behavior between males and females. Prior research in hamsters, however, demonstrates that gestational photoperiod information is communicated to fetuses by the dam (Stetson et al., 1989; Weaver et al., 1987). In our studies, only males are exposed to photoperiod change and that is after weaning. Data have shown some slowing (not inhibition) of development in male hamsters when the dams and pups are transferred from a gestational photoperiod of 16 h light:8 h dark to an intermediate photoperiod of 14 h light:10 h dark immediately after parturition (Prendergast et al., 2004). No effect of post-weaning photoperiod on development has been reported to our knowledge. Therefore, although we note that our male subjects were exposed to photoperiod changes to which females were not exposed, it is unlikely that any resulting effects on development would be substantial enough to make our comparisons invalid.

HR-bred males and females acquired cocaine self-administration behavior more rapidly than did LH-bred males and females. Furthermore, HR-bred females self-administered more cocaine than all other groups. These experiments did not, however, determine motivation for the drug, dose-response functions, or extinction of self-administration behavior. Since phenotype did impact acquisition of self-administration behavior as well as number of cocaine infusions self-administered, future studies will determine if there are effects of phenotype on sex differences in motivation, dose-response differences, and/or effects on extinction and relapse.

In conclusion, HR animals possess many traits that are homologous to novelty-seeking and impulsive behavior in humans, features which can increase an individual's propensity for drug abuse (Cloninger, 1987; Zuckerman and Neeb, 1979). Similar to humans, HR rats show shorter latencies to acquire cocaine self-administration. Moreover, HR females take more cocaine than LR females, HR males, and LR males, suggesting a possible increased risk for women novelty-seekers addicted to cocaine to take more drug than men. In fact, women tend to increase their rate of consumption of a wide range of drugs, including alcohol, marijuana, opioids and cocaine, more rapidly than do men (Brady and Randall, 1999; Hernandez-Avila et al., 2004; Lynch et al., 2002; Mann et al., 2005; Randall et al., 1999). Since individuals who use drugs are more likely to exhibit high novelty-seeking/impulsivity behavioral traits, the clinical data are consistent with our animal findings that the novelty-seeking trait in females may lead to even greater propensity for drug-taking behavior. Therefore, studies that determine the factors and mechanisms that contribute to the greater cocaine intake in HR females will have important clinical implications for understanding sex differences in drug abuse in humans.

Acknowledgements

We are extremely grateful to Sue Miller, Antony Abraham, and Tracy Bedrosian for excellent technical assistance in maintaining the selectively bred HR and LR lines. Thanks also goes to Brandon Luma, Sara Herman, Erin Love, Samir Shah, and Melissa Luma for the help with catheter surgeries and self-administration procedures, Ming Hu, Ph.D. for computer program setup and technical support, and Shelly Flagel, Ph.D. for help with statistics. This study was funded by the Office of Naval Research, grant N00014-02-1-0879 to HA, NIDA RO1 DA13386 to HA and NIMH PO1 MH42251 to SJW, NIDA DA12677 to JBB, and Biology of Drug Abuse Training Grant T32 DA007268.

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