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SEX DIFFERENCES IN PRENATAL STRESS EFFECTS ON COCAINE PURSUIT IN RATS

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Abstract

Disruption of early-life ontogeny has severe and persistent consequences for the health of the developing organism. Both clinical and preclinical findings indicate that such interference can be caused by maternal stress during the gestation period (prenatal stress [PS]). In rats, PS facilitates the rewarding and neurochemical-stimulating effects of drugs, suggesting that PS may represent a risk factor for drug abuse in humans. Very little, however, is known about its effects in females, even though sex differences in drug susceptibility have been well documented in no PS (NPS) controls. Thus, we tested for independent effects and interactions between maternal restraint stress during the last week of gestation and sex on drug use with an extended regimen of drug self-administration. Male and female rats were provided daily access to a large but controlled amount of cocaine for seven weeks. Drug pursuit during the final week was used to indicate susceptibility to developing an addiction-like phenotype, based on reports that drug use becomes increasingly compulsive-like after weeks of testing. Overall, females satisfied more addiction-like criteria than males, and the same was true for PS rats when compared to NPS controls. In addition, sex and PS interacted to disproportionately promote drug pursuit of females with a history of PS. These results indicate that sex differences in drug susceptibility persist with continued drug exposure, and that PS widens this difference by more severely affecting females. In all, PS may be a risk factor for drug addiction in humans, and to a greater extent in women vs. men.

Keywords

self-administration; gestation; dopamine; drug addiction; nucleus accumbens; striatum

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1.0 Introduction

Interference with fetal development has long-lasting consequences for the health of the organism. Perhaps most notably, deficits in prenatal nutrition are linked with chronic, debilitating conditions in both rodents [1–3] and humans [4–7]. Similarly, when development is compromised by maternal stress, mental health issues as well as cognitive deficits have been reported [7–10]. Mothers that experience intense stress and/or anxiety during pregnancy, for example, give birth to children that are at increased risk of developing attention deficit hyperactivity disorder [11–13]. In terms of sex-specific effects of prenatal stress (PS), there is no consistent bias for one sex to be more affected by PS [7].

Work in the preclinical setting suggests that maternal stress may also predispose individuals to become susceptible for drug abuse. Indeed, maternal stress during the final week of the rat gestation period creates a life-long hypersensitivity to psychostimulants [14,15]. PS augments drug-induced dopaminergic signaling in the nucleus accumbens, providing a plausible mechanism for enhanced responsiveness at the behavioral level [13,16–18]. PS also promotes the acute locomotor-activating effects of amphetamine, cocaine and nicotine [13,16,19,20] as well as sensitization to amphetamine and cocaine [21,22]. Given that sensitization is thought to model some of the neuroplastic changes that contribute to drug addiction [23,24], PS may predispose individuals to compulsively pursue drugs later in life.

Consistent with this hypothesis, PS increases drug use when animals are tested using a self-administration paradigm. PS promotes the rate of acquisition for cocaine and amphetamine and augments the reinstatement of cocaine seeking after extinction training [13,19,22]. Thus, when allowed to administer drugs voluntarily, PS rats stabilize their level of intake faster, and are more prone to exhibit behaviors suggestive of relapse, when compared to No PS controls.

Nevertheless, there are significant gaps in the preclinical literature. First, most PS work has excluded females even though profound sex differences in drug responsiveness are evident in humans and non-human animals [25–29]. Briefly, rodent studies indicate that females are more sensitive than males to psychostimulants, and that the difference is not dependent on the presence of gonadal hormones in adulthood [30–32]. This sex difference can be *modulated*, however, by ovarian hormones in females. Pretreatment with estradiol, for example, potentiates the response of ovariectomized females to cocaine [30–32]. Consistent with these findings, the ratio of estradiol to progesterone is thought to mediate estrous cycle-dependent fluctuations in drug sensitivity of females that are reproductively intact [33,34].

Although sex differences in drug taking behavior and the response to psychomotor stimulants have been well documented, very little is known about how they are affected by PS. While one report indicates that PS renders females more sensitive to the motor-altering effects of 3,4-methylenedioxymethamphetamine (MDMA), than is seen with No PS [35]; it is unknown whether these effects are sex dependent. Moreover, it is unclear if and/or how motor alteration is related to MDMA's abuse potential. To date, only one published report assesses the relationship between sex, PS, and drug responsiveness directly. Thomas *et al* (2009) observed sex-dependent effects of PS in 2 models of cocaine abuse: the acquisition of

self-administration and the expression of behavioral sensitization. Interestingly, PS facilitated acquisition only in males, but augmented sensitization exclusively in females. Although it is surprising that PS females' propensity to sensitize did not translate to enhanced acquisition or drug taking, females were already close to the maximum rate of acquisition possible in this paradigm. Thus, these results may indicate that effects of PS in females can only be seen with a different paradigm, or only emerge after *chronic* drug exposure.

To better understand the influence of PS in females, this investigation utilized a self-administration regimen that was intentionally lengthened to evaluate the effect of PS *outside of the acquisition period*. Importantly, this follows in the footsteps of recent work suggesting that the pursuit of cocaine takes on compulsive-like characteristics only after many weeks of testing [36–38]. Consequently, this investigation was expected to provide insight into PS as a potential risk factor for drug addiction. Both males and females were included to test for sex differences independent of and interacting with PS.

2.0 Methods

2.1 Subjects

Male (275–300 grams) and virgin female (200–225 grams) Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN) for breeding purposes and placed in isosexual groups of 3–4 upon arrival. Animals were housed in a temperature-controlled environment on a 14/10 hour light/dark cycle, with ad libitum access to food (2014 Teklad Global 14% protein rodent maintenance diet, Harlan rat chow, Madison, WI) and water. All procedures were performed in accordance to a protocol approved by the University of Michigan Use and Care of Animals Committee.

Based on vaginal lavage, each sexually-receptive female was housed overnight with a randomly-selected male. Successful mating was confirmed the following morning by vaginal lavage inspection for the presence of sperm. The morning of sperm detection was designated as day 0 of pregnancy. Mated females were then housed individually for the entirety of the gestation period.

From gestational days 15–21, a selection of females ($n = 13$) were exposed to repeated daily restraint stress. Restraint stress consisted of removal from the animal colony room and placement in a Plexiglass restrainer for 45 minutes, 3 times per day. Restraint episodes were evenly distributed across the light cycle (0900, 1200, and 1600 hours). Except for cage changes, females not chosen for restraint stress ($n = 12$) were left undisturbed for the duration of pregnancy. We have previously shown sex-specific effect of this stress protocol on cocaine-taking behavior and sensitization to cocaine [22].

Within 24 hours of birth on day 22, pups were briefly separated from their mother and handled by the experimenter for sex identification. Litters were then culled to 10, with an equal or near-equal ratio of males to females. Only 1–2 rats/sex/litter were randomly chosen to participate in the experiment. Litter characteristics are presented in Table 1. We have previously published the birth weights and growth rates from PS and NPS pups who were

obtained under similar conditions and the groups did not differ [22]. Litters were weaned at 21 days of age and housed with same sex littermates. At day 55 animals were transferred to a reversed light cycle (14/10) to mimic conditions animals are exposed when purchased from a breeder.

2.2 Drugs

Cocaine HCL was provided by RTI courtesy of the National Institute on Drug Abuse (Bethesda, MD) and dissolved in 0.9% sterile saline for all procedures.

Surgical procedures

Rats (65–75 days of age) received implants of an indwelling intravenous catheter under isoflurane (2.5% in oxygen) anesthesia [22]. Catheters were then flushed with 0.2 ml heparinized saline (30 U/ml in 0.9% sterile saline) and 0.2 ml gentamicin (3 mg/kg). This dose of gentamicin was administered on a daily basis for the entire experiment.

2.3. Self-administration testing

Self-administration training began 1 week after rats were catheterized and housed under a reverse light/dark (14/10 hour) cycle. There were N=20 in PS females, N=18 in No PS females, N=17 in PS males and N=18 in No PS males. Animals were tested once daily for 5 consecutive days per week across a 7-week period. All testing took place in standard operant chambers (25 × 27 × 30 cm) located within ventilated sound-attenuating cubicles (Med Associates, Inc., Georgia, VT) during the dark period. Shortly before the start of each test session, rats were connected to an infusion syringe and tethered to a swivel. The beginning of each session was signaled by illumination of the house light.

A drug dose of 0.8 mg/kg/infusion was used for all test sessions. Cocaine (50 µl/infusion) was delivered over the course of 2.8 seconds after the required amount of nose pokes in the active hole was reached. The active hole was illuminated with white light during this time and remained so during the subsequent 40-second “time-out” period, during which time nose pokes in both holes were without programmed consequences. In all situations, nose pokes in the inactive hole were recorded, but had no programmed consequences.

The test regimen included a mixture of fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement (Figure 1). Most sessions automatically terminated upon completion of the session’s predetermined infusion criterion (CRIT). Only after reaching the CRIT was an animal removed from the chamber and returned to their home cage.

Training began on a FR1 schedule with a CRIT of 5. The CRIT and response requirements were then steadily increased over the first week, in preparation for the PR schedule used in session 10. An FR1 schedule with a CRIT of 10 and 15 was used for sessions 2 and 3, respectively. Subjects were then moved to an FR2 schedule for sessions 4 and 5, with a CRIT of 15 and 20, respectively. The CRIT then remained at 20 for sessions 6–9. Rats were tested with an FR3 schedule in session 6 and an FR5 schedule for sessions 7–9.

Following the acquisition period (sessions 1–9), most testing was done with an FR5 schedule and a CRIT of 27. Each FR5 session was divided into 3 signaled drug-accessible

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(“drug”) periods, separated by 2 signaled drug-inaccessible (“no drug”) periods (Fig. 1B). “No drug” periods were initiated after the 40-second time-out periods that followed infusions 9 and 18, with the house light being turned off for 15 minutes each time. Nose pokes in both holes were recorded during these times, but were without programmed consequences. Illumination of the house light was then used to signal the subsequent “drug” periods’ onset. The house light remained on until the start of the next “no drug” period or until the end of the time-out period following the 27th infusion. Upon reaching the CRIT, animals were removed from the test chambers and returned to their home cages.

Interspersed among regular FR5 sessions were tests for specific “addiction-like” traits, as in other reports [36,37,39–41]. Each trait was assayed relatively early and late (weeks 2–3 and 6–7, respectively) in the testing regimen (Figure 1).

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Motivation for cocaine was assayed during sessions 10 and 30 with a PR schedule. The responses needed to receive each infusion increased according to the following progression (2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, 1347, 1646, 2012, 2459). The maximal number of responses performed to receive a single infusion constituted the individual’s “breaking point” (BP). PR sessions terminated after 6 hours or after 1 hour elapsed since the last infusion, whichever came first.

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The effect of negative consequences associated with drug use (“punishment”) was assessed with an FR1 schedule during sessions 14 and 35, with each lasting 2 hours in duration. Each drug infusion was paired with a mild electric footshock (0.4 mA, 1 s). Because reliable drug-taking behaviors were not observed during these sessions, however, data from punishment sessions were not included in subsequent analyses.

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Drug seeking in the absence of reinforcement was measured by taking the mean of the total number of nose pokes in the active hole during “no drug” periods of sessions 12–13 and 33–34. Persistent responding in the absence of reinforcement is thought to be one of the hallmarks of addiction [42–44]. In this paradigm the rate of cocaine intake during the period when responding was not reinforced was used as an index of persistent responding. The mean inter-infusion interval (III) during “drug” periods in sessions 13 and 34 was used as an index of persistent responding in the absence of reinforcement.

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The BP score, “no drug” active pokes, and III obtained late in the training regimen were then used to determine each individual’s severity of drug pursuit.

2.4 Statistical analysis

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Acquisition of drug taking was assessed with a mixed model analysis of variance (ANOVA) using the average (1) latency to obtain the first infusion and (2) III, obtained from test sessions 1–9 as dependent measures. Sex and PS were used as between-subject factors, while session was the within-subject factor.

Each addiction-like trait assayed late in training was individually analyzed with ANOVA that also had sex and PS as factors. BP and “no drug” active poke scores, however, were first subjected to natural log and square root transformations, respectively, to normalize the distributions.

Post hoc inspection of data suggested that scores were not evenly distributed across the groups. Specifically, a slightly higher percentage of PS females scored at or above descriptive parameters of the overall rat population, relative to individuals from other groups. Thus, binary logistic regression was used to test for main effects of sex, PS, and a sex X PS interaction, on the probability of scoring at or above these cutoffs. Chi-square tests were then used to compare probability values of PS females to each group individually or all other rats pooled together.

Finally, scores for each trait were independently ranked, and rats scoring in the highest 35% were considered positive for the respective trait and assigned an arbitrary value of 1 [40]. Rats scoring outside of the upper 35% were considered negative and assigned an arbitrary value of 0. Summing newly assigned values then yielded a continuum of persistence in cocaine pursuit, with individuals being positive for 0, 1, 2, or 3 addiction-like criteria [40,44,45]. Sex and PS effects on the number of criteria met after the 7th week were then analyzed with ordinal logistic regression. Correlational analyses of the number of criteria satisfied after 3 and 7 weeks of testing were then used to determine whether the final severity of drug pursuit was predicted by behaviors observed weeks earlier.

For all ANOVAs, significant effects were followed up with Bonferroni post hoc tests. P values < 0.05 were considered statistically significant.

3.0 Results

3.1 Litter characteristics

Table 1 displays a comparison of litter characteristics of PS pups and No PS pups. ANOVAs and chi-square tests indicated that stress had no influence on any parameter measured.

3.2 Acquisition

The number of infusions per session was held constant for all animals so there was no difference among groups in drug exposure. What could vary across animals was the amount of time between infusions or the III. As illustrated in Figure 1, as animals acquired cocaine self-administration the III significantly decreased from session 1 to 9 ($F[8, 100.06] = 16.14$; $p < 0.001$). The III was lower for females than for males ($F[1, 222.08] = 4.96$; $p = 0.03$).

Females also required less time than males to receive their first infusion during each session ($F[1, 160.39] = 4.05$; $p < 0.05$). PS increased the latency to first infusion ($F[1, 160.39] = 3.76$; $p = 0.05$), independent of sex. Overall, latency to self-administer decreased across time ($F[8, 127.55] = 4.26$; $p < 0.001$).

3.3 Rate of drug intake

As depicted in Figure 2, females self-administered cocaine at a faster rate than males ($F[1, 70] = 10.18$; $p = 0.002$). There was no effect of PS, nor an interaction between sex and PS.

3.4 Drug seeking during periods of signaled drug inaccessibility

Figure 3 displays a scatterplot of active nose poke scores when drug was not available, for the entire rat population. The distribution of drug seeking by the PS females was different from that of the other groups. Indeed, a significantly greater percentage of PS females scored at least 1 standard deviation above the overall population mean, relative to all other rats ($\chi^2 = 4.23$ DF=1; $p = 0.04$; Figure 3b). ANOVA revealed no effect of sex or PS on the mean responding during “no drug” periods. In addition, no interaction was observed.

Lastly, correlational analyses were used to determine whether drug seeking and III could be considered independent traits. The correlation coefficients indicate that III did not predict “no drug” active pokes in any of the 4 groups: PS Female $r = -0.16$, DF=18, $p = 0.5$; No PS Female $r = -0.12$, DF=16, $p = 0.64$; PS Male $r = 0.18$, DF=15, $p = 0.5$; No PS Male $r = -0.03$, DF=16, $p = 0.9$.

3.5 Breaking point

Figure 4a displays a scatterplot of BP scores from the entire rat population. Binary logistic regression indicated that the likelihood of scoring at or above the overall median BP value was subject to an interaction that approached significance (Sex X PS; Wald $\chi^2 = 3.56$, DF=1; $p = 0.059$). Subsequent chi-square tests indicated that more PS females met this criterion than individuals from any other group (all $p < 0.05$; Figure 4b).

Females averaged higher BP scores than males ($F[1, 70] = 4.6$; $p < 0.05$; data not shown), and PS produced a trend of overall facilitation of motivation for cocaine ($F[1, 70] = 3.69$; $p = 0.06$). No significant interaction was observed.

3.6 Addiction-like criteria satisfied

Females met more addiction-like criteria than males (Wald $\chi^2 = 9.64$, DF=3; $p < 0.01$) and PS rats satisfied more addiction-criteria than controls (Wald $\chi^2 = 4.22$, DF=3; $p < 0.05$). In addition, the effect of PS was stronger in females than in males (Sex X PS; Wald $\chi^2 = 4.54$, DF=1; $p < 0.05$; Figure 5).

Consistent with previous findings [37,40], rats that developed a “bursting” pattern of drug intake (6 or more infusions earned within a 5-minute time period) by the 7th week of testing satisfied more criteria than those that did not (Wald $\chi^2 = 15.35$, DF=3; $p < 0.001$; Figure 6). Follow-up analyses indicated that the development of “bursting” itself was not group dependent.

Lastly, correlational analyses were used to determine how the number of addiction-like traits that were met changed over time. The sum of addiction-like criteria satisfied after the 3rd week did not predict the number of traits met after week 7 for either of the female groups (PS: Spearman’s $\rho = 0.22$, $p = 0.34$; No PS: Spearman’s $\rho = 0.23$, $p = 0.36$). Nor was a correlation evident in PS males (Spearman’s $\rho = 0.34$, $p = 0.18$). Only in males without a history of PS did the relationship between scores early and late in testing reach significance (Spearman’s $\rho = 0.53$, $p = 0.03$).

4.0 Discussion

The present findings indicate that the sex of the individual and PS have independent but interacting effects to influence development of compulsive-like patterns of drug pursuit. Females expressed a more severe profile of cocaine use than males and, as predicted, the difference was exacerbated by PS. Indeed, PS tended to shift the distribution of females' scores to more severe proportions, relative to the rest of the population. To exemplify, PS increased the percentage of females (but not males) expressing a BP score above the overall population median. When the 3 traits were analyzed together, PS females were relatively underrepresented in the most drug-resistant (0-criteria rats) subpopulation, and represented 60% of the most drug-vulnerable (3-criteria rats) subpopulation. Consequently, sex and PS interact to disproportionately promote compulsive-like drug pursuit developing in females with a history of PS.

It is well established that there are robust sex differences in responsiveness to psychostimulants. Indeed, females are more avid in their drug pursuit than males during all stages of self-administration [46–50]. An element that is unique to this report, however, is an evaluation of how the sexes differ on *multiple* dimensions of behavior. While the use of several criteria to assess drug susceptibility has gained popularity in recent years, in part because it resembles the diagnostic process utilized by clinicians, this work has been done predominantly in males [37,39–41], although not exclusively [36,51]. It was unknown, however, whether females are just as susceptible as males are to the multiple drug properties assessed here. Here we indicate that females are, indeed, more likely to co-express severe patterns of drug pursuit and presumably develop an addiction-like phenotype.

As is evident from these findings, sex differences are not strictly bimodal. As has been discussed in detail there are at least four types of sex differences [29,49,52,53]. Sex differences that are bimodally distributed (e.g., females ovulate, males produce spermatozoa) are *qualitative* differences. There are also *quantitative* differences (e.g., both females and males self-administer cocaine, but females tend to take more drug [30]). In some instances, the endpoint looks the same, but the neural mechanisms mediating the response are different in males and females (i.e., *convergent* differences [54]). Finally, there may also be *population* differences where different phenotypes are differentially represented in males vs. females. For example, more females than males choose cocaine over a sucrose pellet [28,36,51,55]. In terms of the effect of PS, one of the effects reported here is that PS seems to increase *the population of females* that exhibited more compulsive-like drug taking behavior.

An important nuance to our experimental design was restricted cocaine access both within and across test sessions. The length of “drug” periods was determined by the time needed to reach a predetermined criteria, preventing drug seeking during “no drug” periods from being confounded by differences in drug intake. Although there was variability in the *rate* at which cocaine was administered, correlational analyses indicated that drug seeking was not predicted by III. Consequently, variation in drug seeking was independent of differences in III.

The control of drug access *across* test sessions also served an important purpose. Because females typically acquire drug taking faster than males, for example, sex differences observed outside of the acquisition period are often confounded by differences in drug history. Stated otherwise, without strict limits on drug access, it is unclear whether observed sex differences are a consequence of (1) intrinsic, biologically-based differences that are independent of previous drug exposure or (2) sex-dependent drug intake during the preceding stage of testing, presumably mediated via drug-associated neuroadaptations. Due to this study's design, the second possibility can be eliminated as a cause of any sex difference reported here. Thus, females' greater propensity to develop compulsive-like drug habits is not reliant on a sex difference in drug intake earlier in testing.

While others have reported some animals respond for drug when animals received footshock [40,44,45], this has been reported exclusively in male rats. There is a bimodal distribution with most rats exhibiting low rates of responding and only 20% of rats exhibiting high rates of drug taking during footshock [45]. The previous reports used lever pressing, instead of nose poking, for cocaine, but we used the same dose of cocaine (0.8 mg/kg/inf) and footshock parameters as we wanted our methods to be comparable [45]. There may be a difference in the effect of footshock when all four feet are on the ground that influenced the outcome here.

We have previously reported that estradiol enhances acquisition of cocaine self-administration and motivation for cocaine [30,32,56]. If PS enhanced endogenous estradiol secretion in females, this might be a mechanism mediating the results observed. We did not see differences in body weight (negatively associated with estradiol) or estrous cycles between the female groups. In a study of sexual behavior in female rats with comparable PS there was decreased lordosis intensity, suggesting reduced estradiol, but no effect of PS on pacing of sexual behavior or mounts received during naturally occurring behavioral estrus [57]. Thus, it is more likely that PS effects on the stress response, and sex differences in the stress response, mediate the results observed [14,54,57,58].

Despite novel aspects of this study's design, patterns of drug pursuit were still consistent with those observed in similar reports. To exemplify, only a small fraction of the population ever developed a "bursting" pattern of intake. "Bursting," a marker of intense drug use across short intervals of time, distinguished the most drug-vulnerable from drug-resistant individuals in a previous report [37]. Likewise, here we found that evidence of "bursting" strongly predicted the number of addiction-like criteria satisfied by the end of testing. Consequently, although the test regimen used here was shorter than in similar experiments, it was of sufficient length for the emergence of a categorical difference between subjects that were vulnerable and resistant.

To sum, this study identifies risk factors for drug use developing compulsive-like features. Sex and PS join other preexisting differences, such as impulsivity, as modulating factors in the transition of drug use to seemingly uncontrollable levels [45,59]. Both factors have independently been implicated in aspects of drug responsiveness in other reports. An important nuance incorporated into this study, however, was including both in the same experiment to test for synergistic effects. In a previous study, it was unclear why PS

promoted sensitization to cocaine only in females and the self-administration of cocaine selectively in males [22]. The lengthened self-administration regimen used here was intended to test whether PS facilitates the drug pursuit of females with a more extensive drug and test history. In addition, we sought to test whether there is an overall sex difference in susceptibility to developing an addiction-like phenotype, as suggested by the existing literature. Indeed, after weeks of testing, females expressed a more severe profile of drug pursuit than their male littermates. Likewise, PS rats met more addiction-like criteria than No PS controls. Interestingly, the enhancing effect of PS was stronger in females than in males. Thus, PS increases the already-heightened vulnerability of females to pursue drugs, perhaps by altering individual traits that increase the ability of drugs of abuse to drive neuroplastic changes in the brain. Given the correspondence between behavioral traits measured here and the core symptoms of drug addiction, PS may represent a risk factor for enhanced drug use reaching uncontrollable levels, especially in women.

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Highlights

- Maternal restraint stress during the last week of gestation (prenatal stress; PS) results in more PS females than PS males developing an addiction-like phenotype.
- Independent of PS, females exhibited more addiction-like characteristics than did males.
- PS rats of both sexes exhibited more addiction-like characteristics than no PS rats.
- Prenatal stress may be a risk factor for addiction in both sexes, but affects females to a greater extent.

Figure 1. Experimental timeline for self-administration sessions

A. Training began on a fixed ratio (FR) 1 schedule with a criteria of 5 infusions on day 1 and increased over 9 sessions as described in text. Then the Early Trait Scores were obtained by tests of breaking point (BP) and FR 5 sessions. This was followed by a maintenance period and then the sessions for Late Trait Scores as described in the text.

B. The FR 5 sessions consisted of 3 signaled drug sessions where rats could earn a maximum of 9 infusions, with No drug period in between drug sessions when the chamber lights were out and no infusions were earned, but responses were recorded.

Figure 2. The inter-infusion interval (III; mean $\pm$ SEM) for all male and female rats for each of the first 9 self-administration sessions. Overall, the III was lower in females vs. males, independent of stress history (* $p < 0.05$).

Figure 3.

The mean (+ SEM) inter-infusion interval (III) of male and female rats with (PS) and without (No PS) a history of PS during session 34 of self-administration testing. Females self-administered cocaine at a faster rate than males (denoted by *), independent of PS. No overall or sex-dependent effect of PS was observed.

Figure 4.

Drug seeking in the absence of reinforcement, as measured by active nose pokes during periods of signaled drug inaccessibility ("no drug" active pokes). a) The distribution of scores for males and females with (PS) and without (No PS) a history of PS. Identical scores within each group are stacked along the horizontal axis. The overall population mean and 1 standard deviation above the mean (+ 1 SD) are indicated by horizontal lines on the far left side. Visual inspection of the data points suggested that PS females were overrepresented in the subpopulation of rats showing the most profound drug-seeking activity. b) We confirmed this by using 1 standard deviation above the mean as a cutoff value. As indicated by the *, a significantly higher percentage of PS females reached or exceeded the cutoff (denoted by the dashed line) when compared to all other rats in the population, irrespective of group membership.

Figure 5.

Motivation for cocaine, as determined by breaking point (BP) scores on a progressive ratio (PR) schedule. a) The distribution of BP scores for males and females with (PS) and without (No PS) a history of PS. Identical scores within each group are stacked along the horizontal axis. The overall median from the entire population is indicated by the horizontal line on the far left side, as a visual bench mark of the distribution of BP scores. Visual inspection of group distributions suggested that the PS Female group was shifted upward, relative to the other groups. b) Indeed, a greater percentage of PS females scored at or above the overall median value than that which was observed in each of the other groups (significant difference denoted by *).

Figure 6.

The percentage of each group that was positive for 0, 1, 2, and 3 addiction-like traits. As described in the Methods, individuals were considered positive for an addiction-like trait if they scored in the upper 35% of the distribution of the entire population. Females were positive for more traits than males, and rats with a history of PS (PS) met more addiction-like criteria than No PS controls. The effect of PS was stronger in females than it was in males. PS decreased the percentage of females satisfying 0 and increased the percentage of females with 3 addiction-like traits.

Figure 7.

The number of addiction-like traits satisfied by rats that did ($n = 8$) and did not ($n = 66$) develop a “bursting” pattern of cocaine intake. A “bursting” episode consisted of 6 or more infusions self-administered within a 5-minute time span, as originally reported by Belin et al (2009)[37]. Rats showing evidence of “bursting” displayed their rapid pattern of drug intake at least once per session across sessions 31–34. These individuals were positive for significantly more addiction-like traits than those that never showed a “bursting” pattern.

Table 1

Litter Characteristics.

	Stress	No Stress	P value
Litters in this study	13	12	
% with any naturally-occurring deaths	23.1	25	p = 0.91
Average number of naturally-occurring deaths/litter	0.4 ± 0.3	0.3 ± 0.3	p = 0.77
Natural male/female ratio	1.1 ± 0.2	1.1 ± 0.2	p = 0.89
Culled male/female ratio	0.9 ± 0.1	0.9 ± 0.1	p = 0.61
Natural litter size	13.9 ± 0.6	13 ± 0.9	p = 0.42
Culled litter size	9.9 ± 0.1	9.8 ± 0.1	p = 0.93

A comparison of litters derived from repeatedly-stressed (Stress; n = 13) and non-stressed (No Stress; n = 12) mothers. The mean ± SEM is shown for each group category, for the naturally-occurring deaths in the perinatal period, the percentage of litters with at least one death is shown (assessed by a chi-square test). All other measures were independently evaluated with ANOVAs. As indicated by the p values listed in the far right column, repeated maternal stress had no significant effect on any characteristic measured. Only 1–2 rats/sex/liter were used in the experiments.