Published in final edited form as:

Psychopharmacology (Berl). 2008 July ; 199(1): 119–130. doi:10.1007/s00213-008-1140-9.

Incentive salience of cocaine across the postpartum period of the female rat

Katharine M. Seip, Mariana Pereira, Michael P. Wansaw, Jenny I. Reiss, Eugenia I. Dziopa, and Joan I. Morrell

Center for Molecular and Behavioral Neuroscience, Rutgers University, 197 University Avenue, Newark, NJ 07102, USA

Abstract

Rationale—Objectives—Our prior conditioned place preference (CPP) work demonstrates that late (day16) postpartum female rats consistently prefer cocaine- over pup-associated chambers, whereas far fewer early postpartum (day8) females prefer the cocaine-associated chamber. The present study examines early and late postpartum females' preference for a cocaine-associated chamber when contrasted with a chamber associated with saline (rather than pups).

Materials and methods—Postpartum females were tested for conditioned preference for chambers associated with cocaine (10 mg/kg subcutaneous (SC) or 0.5, 5, 10, or 20 mg/kg intraperitoneal (IP) injections) versus saline; preferences of virgin female and male rats for select cocaine stimuli (10mg/kg SC or IP) were also tested. Locomotion was recorded during CPP conditioning and testing.

Results—Early and late postpartum females expressed strikingly similar preference for the cocaine-associated chamber across all administration routes and doses. IP cocaine produced an orderly, inverted U-shaped dose-preference curve, with preference peaking at the 5 mg/kg dose (83% of females). While many postpartum females preferred 10mg/kg cocaine administered either SC or IP, both virgin females and males expressed strong aversion to SC cocaine and, while virgin females strongly preferred IP cocaine, males remained relatively indifferent. Across 10mg/kg IP cocaine-conditioning sessions, locomotor sensitization occurred exclusively in cocaine- but not saline-preferring postpartum females. Locomotor rate was lower in preferred versus nonpreferred chambers at CPP test.

Conclusions—Early and late postpartum females may be equally and uniquely susceptible to sampling and/or abuse of modestly salient doses of cocaine (10mg/kg SC; 5mg/kg IP) compared to virgin females and/or males.

Keywords

Cocaine; Rat; Female; Sex differences; Lactation; Male; Motivation; Locomotor activity; Conditioning; Place preference

Introduction

Previous dual-choice conditioned place preference (CPP) studies revealed that most late postpartum female rats consistently prefer cocaine- over pup-associated chambers, whereas few early postpartum females prefer the cocaine-associated chamber, even when cocaine's incentive salience is optimized (Mattson et al. 2001; Seip and Morrell 2007). It remains

unknown, however, whether this postpartum difference is driven by a fundamentally different response to pups, cocaine, or both stimuli in relative contrast to each other. In the present study, we closely examine postpartum preference for cocaine by presenting early and late postpartum females with a simplified CPP choice between a chamber associated with cocaine and one associated with saline, a relatively neutral stimulus with little incentive salience.

CPP is a widely used Pavlovian conditioning procedure used to assess the motivation or incentive value attributed to various natural or pharmacological stimuli, like cocaine (Mucha et al. 1982; Tzschentke 1998, 2007; Bardo and Bevins 2000). In CPP, the incentive value of a stimulus is represented by a subject's conditioned response to seek out the stimulus in an environment where the stimulus was previously presented but is not currently available (Schechter and Calcagnetti 1998; Tzschentke 2007). Thus, CPP can provide important insight into the initial phases of the postpartum female's motivated response toward cocaine while in a drug-free state.

To manipulate cocaine's incentive salience, we varied two stimulus parameters, administration route and dose, that have been demonstrated to affect conditioned preference for a drug-associated CPP chamber (Nomikos and Spyraiki 1988; Mayer and Parker 1993; Durazzo et al. 1994; Tzschentke 1998; Brabant et al. 2005). Initial cocaine stimuli were identical to those used in our prior dual-choice CPP studies contrasting CPP for cocaine versus pups (Mattson et al. 2001; Seip and Morrell 2007) and were then systematically extended across a range of doses (SC 10mg/kg; IP 0.5, 5, 10, or 20mg/kg). To ensure that conditioning occurred during the most reinforcing aspect of cocaine's temporal profile (Mayer and Parker 1993; Ettenberg et al. 1999; Seip et al. 2005), the length of conditioning sessions with SC and IP cocaine coincided with rising concentrations of cocaine in serum and brain (Lau et al. 1991; Festa et al. 2004; Wansaw et al. 2005; Niyomchai et al. 2006). Conditioning session lengths thus lasted 2h for SC cocaine and 30min for IP cocaine. Select postpartum preferences were compared to those of virgin female and male rats to establish key points of comparison with well-documented CPP literature.

To provide an additional dependent measure of postpartum females' responsivity to cocaine, locomotion was recorded during CPP conditioning and test sessions. Our characterization of cocaine-induced locomotion and the induction phase of motoric sensitization in the postpartum female during conditioning sessions with IP cocaine builds upon existing dose-response locomotor analyses in males and cycling female rats (Sell et al. 2000; Hu and Becker 2003; Todtenkopf and Carlezon 2006). Locomotion during CPP testing characterizes subjects' behavioral responsivity to cocaine- and saline-associated chambers during their expression of conditioned chamber preference, offering unique insight into individual differences in motivation (Seip and Morrell 2007) and extending prior CPP work using other abused drugs (Parker 1992).

By comparing postpartum females' incentive motivation and motoric responses to various cocaine stimuli, we can explore how the postpartum female's unique endocrinological, physiological, and behavioral status may modulate cocaine CPP. While cocaine CPP is influenced by sex (Russo et al. 2003b; Carroll et al. 2004; Jackson et al. 2006), estrus cycle stage (Roberts et al. 1989; Hecht et al. 1999), and exogenous administration of ovarian hormones (Russo et al. 2003a, 2008), little research exists on cocaine CPP across the postpartum period, a complex phase characterized by subtle fluctuations in endogenous ovarian hormones and neuroactive peptides and influenced uniquely by biologically relevant stimuli (i.e., pups). The present study constitutes the first systematic examination of cocaine's incentive value across this unique period in the natural lifecycle of the female rat.

Materials and methods

Subjects

Virgin male ($n = 30$) and female ($n = 161$) Sprague-Dawley rats (90–120 days old; Charles River Laboratories, Wilmington, MA, USA) were raised in an animal colony maintained at the Laboratory Animal Facility at Rutgers University (Newark, NJ, USA), which is accredited by the American Association for Accreditation of Laboratory Animal Care. All procedures are in compliance with the “Principles of Laboratory Animal Care” and follow the “Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research” (National Research Council 2003). Details of animal husbandry, housing, and care are described previously (Mattson et al. 2001; Seip and Morrell 2007). All subjects remained healthy across the experiment. Postmortem examination of the peritoneal cavity, dermis–subderma, and (in postpartum females) mammary glands confirmed no cocaine-induced pathologies (Scott et al. 1997).

Females in postpartum groups ($n = 137$) remained with their pups for 24 h after parturition, without interruption or manipulation. Each litter was culled to eight pups on the morning that CPP began. Litters of early and late postpartum females that received cocaine injections all gained weight (Fig. 1) and weighed the same as litters of age-matched control females that did not receive cocaine (pooled age-matched experimental and control litter weights: early, $F(4, 248) = 1,851.9$, $P < 0.0001$; late, $F(4, 224) = 1,704.2$, $P < 0.0001$; both Tukey: $P < 0.05$). Behavioral observations in females’ homecages confirmed that pup retrieval, maternal nest building, and nursing proceeded normally in all postpartum females. Postmortem visual inspection of mammary glands verified that no pathological effects of cocaine developed over the course of the experiment. Brief separations of females from pups during CPP conditioning and test sessions mimicked time periods naturally spent away from the nest by mother rats (Grota and Ader 1974; Pereira et al. 2008) and did not affect litter growth or development (Wansaw et al. 2008). Thus, neither our cocaine administration parameters nor our brief female–pup deprivations negatively affected females or their litters, in accord with our prior work (Vernotica and Morrell 1998; Mattson et al. 2001; Seip and Morrell 2007).

Postpartum females were conditioned and tested either in early (days 4–8) or late (days 12–16) postpartum. Virgin females ($n = 24$) were conditioned and tested without identifying estrous stage, as vaginal lavage can elicit CPP and attenuate females’ motoric response to cocaine (Walker et al. 2002; Nazarian et al. 2004). The typical 4-day estrous cycle coincides with the length of our 4-day conditioning phase, so all cycle stages were represented equally.

Groups—Subjects either received SC (males $n_{10\text{mg}} = 14$, virgin females $n_{10\text{mg}} = 8$, early postpartum $n_{10\text{mg}} = 18$, late postpartum $n_{10\text{mg}} = 15$) or IP injections (males $n_{10\text{mg}} = 16$, virgin females $n_{10\text{mg}} = 16$, early postpartum $n_{0.5\text{mg}} = 8$, $n_{5\text{mg}} = 12$, $n_{10\text{mg}} = 23$, $n_{20\text{mg}} = 12$; late postpartum $n_{0.5\text{mg}} = 6$, $n_{5\text{mg}} = 12$, $n_{10\text{mg}} = 22$, $n_{20\text{mg}} = 10$).

Conditioned place preference procedure

Apparatus—The custom-designed conditioned place preference apparatus (Med Associates Inc., St. Albans, VT, USA, with MED-PC® Version IV Research Control and Data Acquisition System) consisted of three lidded clear Plexiglas chambers of equal size (27.5 cm W × 21 cm L × 20.5 cm H) that were each decorated with unique contextual cues (details in Seip and Morrell 2007).

Chamber preference criterion—Our prior CPP work reveals that individual rats typically spend most of their time in one of the three apparatus chambers, after learning to associate each chamber with a unique unconditioned stimulus. Individual categorization of each subject’s

chamber preference reveals important information about how individuals comprising a population respond to each stimulus-associated chamber. Standard measures of chamber times, averaged across subjects with different chamber preferences, cannot accurately represent these individual differences. We have therefore developed a quantitative preference criterion to objectively identify each individual subject's chamber preference (for additional details see Mattson et al. 2001; Seip and Morrell 2007). To meet the criterion for chamber preference, subjects must spend at least 30min in one chamber and 25% more time in that chamber than any other chamber. Subjects that do not meet preference criterion are categorized as having no preference. We also present the mean times spent in each chamber, averaged across all subjects, to allow direct comparisons with well-established CPP literature (Tzschentke 1998; Bardo and Bevins 2000).

Preconditioning baseline session—To identify any preexisting chamber preferences, subjects were exposed to the apparatus for a single baseline session before conditioning (day 1 or 9 for early and late postpartum females, respectively). Each subject was placed into the center chamber and allowed free access to all three chambers for 60min. Neither preconditioning preferences nor times differed across any groups of subjects ($n = 191$), so all data were pooled for the following preconditioning analyses. Prior to conditioning, most subjects (45%) lacked chamber preference and were randomly assigned to receive either stimulus (cocaine or saline) in one or the other side chambers. Of subjects preferring a side chamber, cocaine was typically assigned to their least-preferred chamber so that few subjects (16%) preferred the chamber where they would receive cocaine and more subjects (27%) preferred the chamber where they would receive saline. Subjects spent more time in the chamber where they would receive saline than in any other chamber ($F(2, 191) = 10.81, P < 0.0001$; Tukey: $P < 0.05$).

Conditioning phase—Subjects received a separate conditioning session with each stimulus, cocaine or saline, once a day for four consecutive days. Immediately prior to each session, subjects were injected with saline or cocaine, either SC into the dorsal flank or IP, and confined to the appropriate conditioning chamber. Conditioning sessions lasted either 2h (SC) or 30min (IP) and were identical for cocaine and saline sessions. Subjects were returned to their home cages after each conditioning session. Conditioning sessions started at 10:00 for saline and 15:00 for cocaine, ensuring that circulating concentrations of cocaine returned to baseline levels before subsequent conditioning sessions (Lau et al. 1991; Wansaw et al. 2005). Cocaine hydrochloride (National Institute of Drug Abuse, Research Triangle Park, NC, USA) was freshly dissolved in daily doses of 0.5, 5, 10, or 20mg/kg cocaine (calculated as a salt) in a 0.9% saline solution. Postpartum females received an IP dose of 0.5–20mg/kg or SC dose of 10mg/kg; virgin females and males received IP or SC doses of 10mg/kg.

Postconditioning test session—Subjects were tested for CPP for 60min on the day after the final conditioning session (for postpartum females, day 8 or 16). No cocaine or saline injections occurred. Conditioning was confirmed if, using within-subjects comparisons, subjects were differently distributed across chamber preference categories or spent different amounts of time in each chamber during the preconditioning and postconditioning sessions.

Analyses and statistics

All analyses were performed as in Seip and Morrell (2007). The significance level was $P < 0.05$. A Levine's test for equality of variance and Kolmogorov–Smirnov test preceded all parametric tests; nonparametric tests were used as needed.

Conditioned place preference—Preference data are presented as the percentage of individual subjects categorized into one of four preference categories: preference for the

cocaine-associated, center, or saline-associated chamber, or no preference. Chamber times (in minutes) were averaged across all subjects in each preference category and are presented as mean percentages of total session time, with standard errors of the mean. *Within-group comparisons.* Preconditioning and postconditioning preferences were compared within subjects using a chi-square goodness-of-fit test for specified proportions, then a one-tailed test for significance of a proportion. Preconditioning and postconditioning times were compared using two-way analyses of variance (ANOVAs; chamber, session as repeated) or one-way ANOVAs (chamber as repeated) with Tukey's honestly significant difference post hoc tests. Cocaine- and saline-associated chamber times pooled across dosage groups were compared using a Wilcoxon signed rank test. *Between-group comparisons.* Preferences were compared using Fisher's exact test or one-tailed tests for significance of difference between two proportions. Times were compared using one-way ANOVAs (chamber as repeated) with Tukey's post hoc tests. Male and virgin female data were compared to pooled postpartum data, which did not differ.

Locomotion—Locomotion was recorded during CPP for IP cocaine in postpartum females. New infrared beam breaks in each side chamber were summed during each conditioning session and divided by 30min to produce a locomotor score (rate) for that session. Beam breaks in each chamber during the postconditioning session were summed and divided by total chamber time; scores were similar across females conditioned with different IP doses and were pooled. During each conditioning and test session, scores did not differ across postpartum and were pooled.

Locomotor scores were separated by postpartum females' postconditioning preference for the cocaine- or saline-associated chamber (cocaine-preferring females $n = 59$; saline-preferring females $n = 16$). In the 10-mg/kg group, saline-conditioning scores failed to record on day1 in seven cocaine-preferring females and day3 in a saline-preferring female. Scores in each chamber during conditioning were compared within groups using two-way ANOVAs (day and chamber as repeated). Postconditioning scores were compared between groups using Mann-Whitney U tests and within groups using Wilcoxon signed rank tests.

Results

Postpartum females

Subcutaneous cocaine

Confirmation of conditioning: In both early and late postpartum females, chamber preferences changed significantly between the preconditioning and postconditioning session, indicating successful conditioning (early: $\chi^2(3, N = 18) = 10.59$; late: $\chi^2(3, N = 15) = 10.95$; both $P < 0.05$). Mean chamber times did not change after conditioning in either postpartum group.

Postconditioning preferences: Following conditioning, chamber preferences were strikingly similar in early and late postpartum females. Most late- (53%) and early postpartum (39%) females preferred the chamber associated with 10mg/kg SC cocaine; postpartum groups did not differ (Fig. 2a). Postpartum females spent similar amounts of time in each chamber (Fig. 2b).

Intraperitoneal cocaine

Confirmation of conditioning: Both before and after conditioning, chamber preferences and times did not differ between early and late postpartum females; data were subsequently pooled. In all postpartum females, chamber preferences and times changed significantly between the preconditioning and postconditioning session, confirming conditioning in all dosage groups (preferences 0.5mg/kg: $\chi^2(3, N = 14) = 9.75$; 5mg/kg: $\chi^2(3, N = 24) = 393.67$; 10mg/kg: $\chi^2(3,$

$N = 45$) = 31.09; 20mg/kg: $\chi^2(3, N = 22) = 437.64$; all $P < 0.0001$ except $P < 0.05$ for 0.5mg/kg; chamber time $\times$ session interactions 0.5mg/kg: $F(2, 26) = 4.00$; 5mg/kg: $F(2, 46) = 45.29$; 10mg/kg: $F(2, 88) = 14.50$; 20mg/kg: $F(2, 42) = 13.10$; all $P < 0.05$). After conditioning, more postpartum females preferred the cocaine-associated chamber (0.5mg/kg: $z = -2.19$; 5mg/kg: $z = -10.39$; 10mg/kg: $z = -3.68$; 20mg/kg: $z = -4.25$; all $P < 0.0001$ except $P < 0.05$ for 0.5mg/kg) and, in all dose groups but 0.5mg/kg, females spent more time in the cocaine-associated chamber (Tukey: all $P < 0.01$).

Postconditioning preferences: Regardless of dose, more postpartum females preferred the cocaine-associated chamber than the saline-associated chamber ($z = -6.45$, $P < 0.0001$; Fig. 3a). More postpartum females (83%) preferred the chamber associated with 5mg/kg cocaine than any other dose (Fisher's exact: $P < 0.05$; 0.5mg/kg: $z = -2.56$; 10mg/kg: $z = -3.14$; 20mg/kg: $z = -2.69$; all $P < 0.01$). Females also spent more time in the cocaine-associated chamber than the saline-associated chamber (Wilcoxon signed rank = 1,718, $P < 0.0001$; Fig. 3b), spent substantially more time in the cocaine-associated chamber paired with 5mg/kg than with 0.5mg/kg, and spent less time in the saline-associated chamber in the 5-mg/kg group than the 0.5- or 20-mg/kg groups (chamber $\times$ group interaction: $F(6, 202) = 2.91$, $P < 0.01$; Tukey: all $P < 0.05$; Fig. 3b).

Selective preference retesting: Some postpartum females were retested once for cocaine CPP. Most (80%) cocaine-preferring females retained their initial chamber preference when retested either after 3 ($n_{20\text{mg/kg}} = 24$; $z = 1.66$, $P = 0.05$) or 7 days ($n_{5\text{mg/kg}} = 5$; $P > 0.05$).

Subcutaneous versus intraperitoneal cocaine

More early postpartum females preferred chambers associated with IP cocaine (52%) than SC cocaine (39%) at 10mg/kg ($z = -10.98$, $P < 0.0001$), whereas late postpartum females equally preferred chambers associated with IP (59%) and SC (53%) cocaine. Postpartum females spent more time in the cocaine-associated chamber and less time in the saline-associated chamber when cocaine was injected IP versus SC (main route effect: early, $F(1, 39) = 147.0$; late, $F(1, 35) = 56.0$; both $P < 0.00001$; Tukey: $P < 0.001$).

Locomotor responses to intraperitoneal cocaine

Conditioning—Across conditioning sessions with 0.5 or 5mg/kg cocaine, locomotor scores were similar regardless of whether females were injected with cocaine or saline and regardless of eventual chamber preference (Fig. 4a,b). Across conditioning sessions with 10 or 20mg/kg cocaine, locomotor scores were higher following cocaine injections than saline injections in cocaine-preferring females (10mg/kg: chamber $\times$ day interaction $F(3, 54) = 5.88$, $P < 0.01$; Tukey: all $P < 0.001$; 20mg/kg: main chamber effect $F(1, 9) = 40.08$, $P < 0.001$; Tukey: all $P < 0.05$) but higher in saline-preferring females only with 20mg/kg cocaine (main chamber effect, $F(1, 5) = 37.97$, $P < 0.01$; Tukey: $P < 0.05$; Fig. 4c,d). Following injections of 10mg/kg cocaine, scores of cocaine-preferring females increased substantially across the four conditioning sessions (Tukey: all $P < 0.001$), while scores of saline-preferring females remained constant (Fig. 4c).

Postconditioning test—Locomotor scores of postpartum females administered with various IP cocaine doses during cocaine-conditioning were similar in each stimulus-associated chamber and thus pooled. Cocaine-preferring females' scores were substantially lower in the cocaine- than saline-associated chamber ($T = 20.0$, $P < 0.0001$), whereas the converse was true of saline-preferring females' scores ($T = 10.0$, $P < 0.01$; Fig. 5). In the cocaine-associated chamber, cocaine-preferring females' scores were lower than those of saline-preferring females ($U = 159.0$, $P < 0.0001$), whereas the converse was true in the saline-associated chamber ($U = 68.0$, $P < 0.0001$).

Males and virgin females

Subcutaneous cocaine

Confirmation of conditioning: After conditioning, preferences changed significantly in both male and virgin female rats (males: $\chi^2(3, N = 14) = 111.92$; females: $\chi^2(3, N = 8) = 1,106.80$; both $P < 0.001$). Chamber times only changed in males (chamber $\times$ session interaction, $F(2, 26) = 5.20$, $P < 0.05$).

Postconditioning preferences: No males or virgin females preferred the cocaine-associated chamber at test (Fig. 6a). More males (79%) than females (38%) preferred the saline-associated chamber ($z = 1.93$, $P = 0.05$). Males also spent less time in the cocaine-associated chamber and more time in the saline-associated chamber than virgin females (main group effect, $F(1, 20) = 7.0$; $P < 0.05$, Tukey: all $P < 0.001$) (Fig. 6c). Males spent more time in the saline- than cocaine-associated chambers [chamber $\times$ session interaction, $F(2, 26) = 5.20$, $P < 0.05$; Tukey: $P < 0.001$].

Comparison to postpartum females: More postpartum females preferred the chamber associated with 10 mg/kg SC cocaine than did males or virgin females (males: $z = -3.02$; females: $z = -2.37$; both $P < 0.01$; Fig. 6a). Fewer postpartum females than males preferred the saline-associated chamber ($z = 19.44$, $P < 0.0001$), and postpartum females spent more time in the cocaine-associated chamber and less time in the saline-associated chamber than males (chamber $\times$ group interaction: $F(2, 90) = 7.78$; $P < 0.001$; Tukey: $P < 0.05$; Fig. 6c). Chamber times differed between virgin and postpartum females, but no interaction emerged (main group effect: $F(1, 39) = 31.92$; $P < 0.0001$).

Intraperitoneal cocaine

Confirmation of conditioning: Chamber preferences and times differed after conditioning in male and virgin females (males: $\chi^2(3, N = 16) = 1,560.41$, $P < 0.0001$; main session effect, $F(1, 15) = 7.52$, $P < 0.05$; females: $\chi^2(3, N = 16) = 273.14$, $P < 0.0001$; chamber time $\times$ session interaction, $F(2, 30) = 9.00$, $P < 0.01$). More virgin females preferred the cocaine-associated chamber ($z = -2.62$, $P < 0.01$) and spent more time in that chamber after conditioning (Tukey: $P < 0.05$).

Postconditioning preferences: More virgin females (56%) than males (19%) preferred the cocaine-associated chamber ($z = -2.19$, $P < 0.05$; Fig. 6b). Virgin females spent more time in the cocaine- than saline-associated chamber and spent more time than males in the cocaine-associated chamber (chamber $\times$ group interaction, $F(2, 60) = 3.00$, $P < 0.05$; Tukey: all $P < 0.001$; Fig. 6d).

Comparison to postpartum dams: More postpartum females than males preferred the chamber associated with 10 mg/kg IP cocaine (Fisher's exact: $P < 0.05$; $z = -2.55$, $P < 0.01$; Fig. 6b). Postpartum females also spent more time in the cocaine-associated chamber and less time in the center chamber than males ($F(2, 118) = 5.86$, $P < 0.01$; Tukey: $P < 0.05$; Fig. 6d). Virgin and postpartum females expressed remarkably similar chamber preferences and times (Fig. 6b,d).

Subcutaneous versus intraperitoneal cocaine

In both males and virgin females, postconditioning chamber preferences and times differed as a result of cocaine's administration route (SC versus IP; Fisher's exact: males, $P < 0.001$; females, $P < 0.05$; chamber time $\times$ route interaction: males, $F(2, 56) = 13.11$; females, $F(2, 44) = 8.56$; both $P < 0.001$). Both males and virgin females spent more time in the chamber associated with IP cocaine than with SC cocaine (Tukey: both $P < 0.001$).

Discussion

Early and late postpartum females expressed strikingly similar patterns of cocaine-associated chamber preference. Postpartum preference for cocaine was substantial, with many postpartum females preferring cocaine whether administered SC or IP (10 mg/kg) and across a wide range of IP doses (0.5–20 mg/kg). More postpartum females preferred SC cocaine than did males or virgin females; postpartum females' robust preference for IP cocaine was similar to that of virgins but superseded that of males at 10 mg/kg. Postpartum preference persisted for at least a week. Together, our results reveal an unexpected and unique vulnerability to cocaine's incentive value in the postpartum female that remains remarkably stable across the postpartum period.

The present study also reveals a notable inverted U-shaped cocaine dose–CPP response curve in postpartum females. Nearly all postpartum females (83%) expressed robust chamber preference for 5 mg/kg IP cocaine, but only approximately half of the postpartum females preferred the cocaine-associated chamber associated with higher doses (10–20 mg/kg) and the lowest (0.5 mg/kg) dose tested. Although CPP has been considered to be relatively insensitive to dose manipulations (Bevins 2005; Bevins and Cunningham 2006), the present study supports previous reports of consistent relationships between varied drug doses and CPP or CPP effect size (Bardo et al. 1995; Roma et al. 2006; Davis et al. 2007; Rezayof et al. 2007).

That postpartum females expressed strikingly similar preferences for cocaine suggests that the distinct hormonal profiles characterizing early and late postpartum, including dynamically fluctuating concentrations and unique combinations of gonadal and lactogenic hormones, do not impact cocaine CPP. Early postpartum is characterized by very low concentrations of estrogen and high concentrations of progesterone and prolactin; concentrations gradually shift back to the normal cycling state during late postpartum (Grota and Eik-Nes 1967; Smith and Neill 1977; Taya and Greenwald 1982). Although low estrogen concentrations are associated with reduced cocaine self-administration (Roberts et al. 1989; Hecht et al. 1999) and high progesterone concentrations with attenuated cocaine CPP (Russo et al. 2003a, 2008), cocaine CPP remains nearly identical in early and late postpartum, suggesting that cocaine CPP may be relatively insensitive to subtle hormonal changes occurring across the postpartum period. Collectively, however, we believe that the unique endogenous combinations and concentrations of gonadal and lactogenic hormones present across postpartum may establish a prolonged period that subtly alters and increases the incentive value of cocaine.

Postpartum females are substantially more likely than virgin females or males to attribute incentive value to cocaine. While many postpartum females strongly preferred SC cocaine, both males and virgin females expressed dramatic place aversion to this cocaine stimulus. These findings accord with prior work indicating that male rats prefer cocaine-associated chambers only at relatively low SC doses (0.32–3.2 mg/kg; Mayer and Parker 1993; Durazzo et al. 1994) and, along with virgin females, find higher SC doses aversive (5–32 mg/kg; van Haaren and Hughes 1990; Busse et al. 2005). That a striking proportion of our postpartum females preferred SC cocaine at 10 mg/kg may point to heightened responsivity to the reinforcing properties of cocaine or blunted response to the aversive qualities of cocaine that is unique to the postpartum state.

It is important to note that, while IP cocaine was preferred equally by both postpartum and virgin females, our strict preference criteria identified very few males that strongly preferred IP cocaine, representing a select point at which cocaine's incentive salience is dissociable by gender. Prior work confirms that males display place aversion or very modest preference for 10 mg/kg IP cocaine but strongly prefer higher IP doses (20 mg/kg; Nomikos and Spyraiki 1988; Mayer and Parker 1993; Russo et al. 2003b), whereas postpartum and virgin females

prefer IP cocaine at lower doses (5–10 mg/kg; Russo et al. 2003a, b; present study). This sharp gender contrast may be partially attributable to global endocrinological, physiological, and/or pharmacokinetic differences between males and females, previously proposed to underlie cycling females' heightened vulnerability to cocaine's motoric and motivational effects (Becker 1999; Kuhn et al. 2001; Chin et al. 2002; Hu and Becker 2003; Russo et al. 2003a, b; Carroll et al. 2004; Festa et al. 2004; Festa and Quinones-Jenab 2004). Notably, cocaine pharmacokinetics remain strikingly consistent across postpartum and cycling states following cocaine administration parameters similar to those used here (Vernotica and Morrell 1998; Wansaw et al. 2005; see Dwivedi et al. 1993 for recently parturient females).

One remaining factor contributing uniquely to the incentive-motivational state of the postpartum female, however, is the continuous presence of offspring (pups). Pups are a critical and salient biological stimulus present exclusively in the postpartum females' environment. The postpartum female is uniquely and highly responsive to pups: she vigorously performs maternal caregiving behaviors toward her pups across the postpartum period and attributes considerable incentive salience to pups during early postpartum (Wilsoncroft 1969; Lee et al. 2000; Mattson et al. 2001). Pup-directed maternal behaviors substantially increase dopaminergic activity in the nucleus accumbens (Hansen et al. 1993; Champagne et al. 2004), a mesolimbic region closely tied to motivational processing, goal-directed behaviors (Berridge and Robinson 1998; Ikemoto and Panksepp 1999; Salamone and Correa 2002), and, recently, cocaine-associated chamber preference (Mattson and Morrell 2005). As dynamic endogenous changes in the female's endocrine state also modulate mesolimbic responsivity (Bazzett and Becker 1994; Bakowska and Morrell 1995), both pup-induced and endocrine-associated changes in dopaminergic tone across postpartum may contribute to cocaine's unique incentive salience in the postpartum female rat.

The present findings contribute meaningfully to an emerging body of evidence suggesting that pups may actually reduce females' preference for cocaine exclusively during early postpartum. When postpartum females were offered a choice between chambers associated with age-matched pups or with one of two cocaine stimuli examined in the present study (10 mg/kg SC, Mattson et al. 2001; 10 mg/kg IP, Seip and Morrell 2007), substantially fewer early postpartum preferred cocaine than when the same cocaine stimuli was contrasted with saline (present study); late postpartum preference for cocaine remained unchanged. We posit that the high incentive value of young pups, naturally present in the early postpartum female's environment, actively competes with cocaine's incentive value during the postpartum period, a critical time in which offspring are extremely dependent on motivated caregiving by the female.

Locomotion across the CPP procedure

The present analyses importantly extend our characterization of cocaine-induced locomotion in the postpartum female (Vernotica and Morrell 1998; Wansaw et al. 2005) during the CPP procedure, providing important points of comparison with the well-documented behavioral effects of cocaine in virgin female and male rats (Sell et al. 2000; Chin et al. 2002; Festa and Quinones-Jenab 2004; Festa et al. 2004). Following 10 mg/kg IP cocaine injections, locomotor sensitization emerged rapidly in cocaine-preferring but not saline-preferring postpartum females after only four exposures, replicating the induction phase of sensitization reported by others (Post and Rose 1976) and supporting proposed correlations between cocaine's motoric and motivational effects (Wise and Bozarth 1987). This locomotor sensitization emerged using short (30 min) conditioning sessions, indicating that longer (60–120 min) exposures are not necessary to produce sensitization to 10 mg/kg IP cocaine (Todtenkopf and Carlezon 2006). Locomotor sensitization did not emerge in cocaine-preferring females given 20 mg/kg IP, possibly due to the number of subjects (i.e., power) or relatively large variability.

Interestingly, a clear dissociation between cocaine's motoric and motivational (i.e., CPP) effects emerged exclusively at low IP doses. While 5.0 mg/kg IP cocaine produced little locomotor activation, it elicited the strongest preference in the majority of postpartum females. This dissociation may be partially attributable to dopaminergic activity. D2-like dopamine receptors in the nucleus accumbens and caudate-putamen are thought to be involved in cocaine-induced locomotion but not cocaine CPP (Baker et al. 1996), but the reverse is true for D1-like receptors (Baker et al. 1998). We speculate that changes in D2-like dopamine receptor activity associated with the maternal state may limit cocaine-induced locomotion without affecting CPP.

During the postconditioning test session, locomotor scores of postpartum females were dramatically lower in their preferred chamber, as in prior work (Parker 1992; Seip and Morrell 2007) and regardless of dose. Females displaying large cocaine-induced increases in locomotion (10–20 mg/kg) did not display conditioned locomotion during the test, contrary to others' findings (Martin-Iverson et al 1997). These scores provide a novel means of characterizing conditioned place preference for natural (Seip and Morrell 2007) and pharmacological stimuli (Parker 1992) across a range of doses and may help predict preference for stimuli such as drugs of abuse.

We posit that these findings reveal a unique postpartum vulnerability to cocaine's incentive value that remains remarkably stable across the entire postpartum period. Notably, more postpartum females attribute incentive value to various cocaine stimuli than do their virgin female and male counterparts, suggesting that the maternal state is not sufficient to offer natural protection against competing drug stimuli available in a female's environment. We posit that the unique hormonal, physiological, and behavioral factors characterizing the maternal state may contribute to the postpartum females' heightened motivation to seek out and interact with drugs with abuse potential. While the subtle hormonal fluctuations occurring across postpartum may preclude a notable difference in cocaine CPP, our present data, together with that of others, suggest that specific combinations of estrogen and progesterone may be one critical determinant of cocaine's incentive value. Identifying additional dynamic factors that contribute to postpartum females' heightened attribution of incentive salience to various cocaine stimuli, e.g., dopaminergic tone and offspring presence (Pereira et al. 2008), gain added urgency in order to protect this critical period in which the female's behavior directly helps or harms her offspring.

Acknowledgements

This research was supported by National Institutes of Health DA014025 and March of Dimes #12FY02-05-103 and #12FY05-06-134 awarded to J.I.M. The authors thank the Laboratory Animal Facility staff at Rutgers University, Newark, NJ, USA for animal breeding and care. All experiments described herein comply with the current laws of the country in which they were performed. The authors have no conflicts of interest, financial or otherwise, pertaining to any aspect of the work reported in this manuscript.

References

- Baker DA, Khroyan TV, O'Dell LE, Fuchs RA, Neisewander JL. Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. *J Pharm Exp Ther* 1996;279:392–401.
- Baker DA, Fuchs RA, Specio SE, Khroyan TV, Neisewander JL. Effects of intra-accumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. *Synapse* 1998;30:181–193. [PubMed: 9723788]
- Bakowska JC, Morrell JI. Quantitative autoradiographic analysis of D1 and D2 dopamine receptors in rat brain in early and late pregnancy. *Brain Res* 1995;703:191–200. [PubMed: 8719632]

- Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology* 2000;153:31–43. [PubMed: 11255927]
- Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci Biobehav Rev* 1995;19:39–51. [PubMed: 7770196]
- Bazzett TJ, Becker JB. Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. *Brain Res* 1994;637:163–172. [PubMed: 8180794]
- Becker JB. Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol Biochem Behav* 1999;64:803–812. [PubMed: 10593204]
- Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev* 1998;28:309–369. [PubMed: 9858756]
- Bevins RA. The reference–dose place conditioning procedure yields a graded dose–effect function. *Int J Comp Psychol* 2005;18:101–111.
- Bevins, RA.; Cunningham, CL. Place conditioning: a methodological analysis. In: Anderson, M., editor. *Tasks and techniques: a sampling of methodologies for the investigation of animal learning, behavior, and cognition*. Nova Science; Hauppauge: 2006. p. 99–110.
- Brabant C, Quertemont E, Tirelli E. Influence of the dose and the number of drug-context pairings on the magnitude and the long-lasting retention of cocaine-induced conditioned place preference in C57BL/6J mice. *Psychopharmacology* 2005;180:33–40. [PubMed: 15682299]
- Busse GD, Freeman KB, Riley AL. The interaction of sex and route of drug administration in cocaine-induced conditioned taste aversions. *Pharmacol Biochem Behav* 2005;81:814–820. [PubMed: 16005058]
- Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. *Trends Pharmacol Sci* 2004;25:273–279. [PubMed: 15120494]
- Champagne FA, Chretien P, Stevenson CW, Zhang TY, Gratton A, Meaney MJ. Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *J Neurosci* 2004;24:4113–4123. [PubMed: 15115806]
- Chin J, Sternin O, Wu HB, Burrell S, Lu D, Jenab S, Perrotti LI, Quinones-Jenab V. Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. *Brain Res* 2002;945:123–130. [PubMed: 12113959]
- Davis CM, Roma PG, Dominguez JM, Riley AL. Morphine-induced place conditioning in Fischer and Lewis rats: acquisition and dose–response in a fully biased procedure. *Pharmacol Biochem Behav* 2007;86:516–523. [PubMed: 17306352]
- Durazzo TC, Gauvin DV, Goulden KL, Briscoe RJ, Holloway FA. Cocaine-induced conditioned place approach in rats: the role of dose and route of administration. *Pharmacol Biochem Behav* 1994;49:1001–1005. [PubMed: 7886067]
- Dwivedi C, Engineer FN, Vaughan SL. Alterations in biodistribution of cocaine may explain differential toxicity in pregnant and postpartum rats. *Toxicol Appl Pharmacol* 1993;118:131–134. [PubMed: 8430420]
- Ettenberg A, Raven MA, Danluck DA, Necessary BD. Evidence for opponent-process actions of intravenous cocaine. *Pharmacol Biochem Behav* 1999;64:507–512. [PubMed: 10548263]
- Festa ED, Quinones-Jenab V. Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. *Horm Behav* 2004;46:509–519. [PubMed: 15555492]
- Festa ED, Russo SJ, Gazi FM, Niyomchai T, Kemen LM, Lin SN, Foltz R, Jenab S, Quinones-Jenab V. Sex differences in cocaine-induced behavioral responses, pharmacokinetics, and monoamine levels. *Neuropharmacology* 2004;46:672–687. [PubMed: 14996545]
- Grota LJ, Eik-Nes KB. Plasma progesterone concentrations during pregnancy and lactation in the rat. *J Reprod Fertil* 1967;13:83–91. [PubMed: 6066784]
- Grota LJ, Ader R. Behavior of lactating rats in a dual-chambered maternity cage. *Horm Behav* 1974;5:275–282. [PubMed: 4477150]
- Hansen S, Bergvall AH, Nyiredi S. Interaction with pups enhances dopamine release in the ventral striatum of maternal rats: a microdialysis study. *Pharmacol Biochem Behav* 1993;45:673–676. [PubMed: 7687357]

- Hecht GS, Spear NE, Spear LP. Changes in progressive ratio responding for intravenous cocaine throughout the reproductive process in female rats. *Dev Psychobiol* 1999;35:136–145. [PubMed: 10461127]
- Hu M, Becker JB. Effects of sex and estrogen on behavioral sensitization to cocaine in rats. *J Neurosci* 2003;23:693–699. [PubMed: 12533629]
- Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Res Rev* 1999;31:6–41. [PubMed: 10611493]
- Jackson LR, Robinson TE, Becker JB. Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology* 2006;31:129–138. [PubMed: 15920500]
- Kuhn CM, Walker QD, Kaplan KA, Li ST. Sex, steroids, and stimulant sensitivity. *Ann N Y Acad Sci* 2001;937:188–201. [PubMed: 11458537]
- Lau CE, Imam A, Ma F, Falk JL. Acute effects of cocaine on spontaneous and discriminative motor functions: relation to route of administration and pharmacokinetics. *J Pharmacol Exp Ther* 1991;257:444–456. [PubMed: 2020002]
- Lee A, Clancy S, Fleming AS. Mother rats bar-press for pups: effects of lesions of the MPOA and limbic sites on maternal behavior and operant responding for pup-reinforcement. *Behav Brain Res* 2000;108:215–231. [PubMed: 10701665]
- Martin-Iverson MT, Reimer AR, Sharma S. Unbiased cocaine conditioned place preference (CPP) obscures conditioned locomotion, and nimodipine blockade of cocaine CPP is due to conditioned place aversions. *Psychopharmacology* 1997;130:327–333. [PubMed: 9160848]
- Mattson BJ, Morrell JI. Preference for cocaine- versus pup-associated cues differentially activates neurons expressing either Fos or cocaine- and amphetamine-regulated transcript in lactating, maternal rodents. *Neuroscience* 2005;135:315–328. [PubMed: 16112474]
- Mattson BJ, Williams S, Rosenblatt JS, Morrell JI. Comparison of two positive reinforcing stimuli: pups and cocaine throughout the postpartum period. *Behav Neurosci* 2001;115:683–694. [PubMed: 11439457]
- Mayer LA, Parker LA. Rewarding and aversive properties of IP and SC cocaine: assessment by place and taste conditioning. *Psychopharmacology* 1993;112:189–194. [PubMed: 7871018]
- Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. *Brain Res* 1982;243:91–105. [PubMed: 6288174]
- National Research Council. Guidelines for the care and use of mammals in neuroscience and behavioral research. Washington DC: National Academies Press; 2003.
- Nazarian A, Russo SJ, Festa ED, Kraish M, Quinones-Jenab V. The role of D1 and D2 receptors in the cocaine conditioned place preference of male and female rats. *Brain Res Bull* 2004;63:295–299.
- Niyomchai T, Akhavan A, Festa ED, Lin SN, Lamm L, Foltz R, Quiñones-Jenab V. Estrogen and progesterone affect cocaine pharmacokinetics in female rats. *Brain Res Bull* 2006;68:310–314. [PubMed: 16377436]
- Nomikos GG, Spyraiki C. Cocaine-induced place conditioning: importance of route of administration and other procedural variables. *Psychopharmacology* 1988;94:119–125. [PubMed: 3126520]
- Parker LA. Place conditioning in a three- or four-choice apparatus: role of stimulus novelty in drug-induced place conditioning. *Behav Neurosci* 1992;106:294–306. [PubMed: 1317184]
- Pereira, M.; Seip, KM.; Morrell, JI. Maternal motivation and its neural substrate across the postpartum period. In: Bridges, B., editor. *The neurobiology of the parental mind*. Amsterdam: Elsevier; 2008. (in press)
- Post RM, Rose H. Increasing effects of repetitive cocaine administration in the rat. *Nature* 1976;260:731–732. [PubMed: 944382]
- Rezayof A, Golhasani-Keshtan F, Haeri-Rohani A, Zarrindast MR. Morphine-induced place preference: involvement of the central amygdala NMDA receptors. *Brain Res* 2007;1133:34–41. [PubMed: 17184750]
- Roberts DC, Bennett SA, Vickers GJ. The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology* 1989;98:408–411. [PubMed: 2501818]
- Roma PG, Flint WW, Higley JD, Riley AL. Assessment of the aversive and rewarding effects of alcohol in Fischer and Lewis rats. *Psychopharmacology* 2006;189:187–199. [PubMed: 17013639]

- Russo SJ, Festa ED, Fabian SJ, Gazi FM, Kraish M, Jenab S, Quinones-Jenab V. Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. *Neuroscience* 2003a;120:523–533. [PubMed: 12890521]
- Russo SJ, Jenab S, Fabian SJ, Festa ED, Kemen LM, Quinones-Jenab V. Sex differences in the conditioned rewarding effects of cocaine. *Brain Res* 2003b;970:214–220. [PubMed: 12706263]
- Russo SJ, Sun WL, Minerly AC, Weierstall K, Nazarian A, Festa ED, Niyomchai T, Akhavan A, Luine V, Jenab S, Quinones-Jenab V. Progesterone attenuates cocaine-induced conditioned place preference in female rats. *Brain Res* 2008;1189:229–235. [PubMed: 18067879]
- Salamone JD, Correa M. Motivational view of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav Brain Res* 2002;137:2–25.
- Schechter MD, Calcagnetti DJ. Continued trends in the conditioned place preference literature from 1992 to 1996, inclusive, with a cross-indexed bibliography. *Neurosci Biobehav Rev* 1998;22:827–846. [PubMed: 9809314]
- Scott DW, Morrell JI, Vernotica EM. Focal necrotizing panniculitis and vascular necrosis in rats given subcutaneous injections of cocaine hydrochloride. *J Cutan Pathol* 1997;24:25–29. [PubMed: 9027629]
- Seip KM, Morrell JI. Increasing the incentive salience of cocaine challenges preference for pup- over cocaine-associated stimuli during early postpartum: place preference and locomotor analyses in the lactating female rat. *Psychopharmacology* 2007;194:309–319. [PubMed: 17589831]
- Seip KM, Reiss JI, Dziopa EI, Wansaw MP, Morrell JI. Further place preference studies on the rewarding effects of cocaine in the postpartum female rat (abstract). *Soc Neurosci* 2005;35:4198.
- Sell SL, Scalzitti JM, Thomas ML, Cunningham KA. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. *J Pharmacol Exp Ther* 2000;293:879–886. [PubMed: 10869388]
- Smith MS, Neill JD. Inhibition of gonadotropin secretion during lactation in the rat: relative contribution of suckling and ovarian steroids. *Biol Reprod* 1977;17:255–261. [PubMed: 889995]
- Taya K, Greenwald GS. Peripheral blood and ovarian levels of sex steroids in the lactating rat. *Endocrinol Jpn* 1982;29:453–459. [PubMed: 7151743]
- Todtenkopf MS, Carlezon WA Jr. Contribution of drug doses and conditioning periods to psychomotor stimulant sensitization. *Psychopharmacology* 2006;185:451–458. [PubMed: 16550389]
- Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* 1998;56:613–672. [PubMed: 9871940]
- Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol* 2007;12:227–462. [PubMed: 17678505]
- van Haaren F, Hughes CE. Cocaine-induced conditioned taste aversions in male and female Wistar rats. *Pharm Biochem Behav* 1990;37:693–686.
- Vernotica EM, Morrell JI. Plasma cocaine levels and locomotor activity after systemic injection in virgin and in lactating maternal female rats. *Physiol Behav* 1998;64:399–407. [PubMed: 9748111]
- Walker QD, Nelson CJ, Smith D, Kuhn C. Vaginal lavage attenuates cocaine-stimulated activity and establishes place preference in rats. *Pharm Biochem Behav* 2002;73:743–752.
- Wansaw MP, Lin SN, Morrell JI. Plasma cocaine levels, metabolites, and locomotor activity after subcutaneous cocaine injection are stable across the postpartum period in rats. *Pharmacol Biochem Behav* 2005;82:55–66. [PubMed: 16115667]
- Wansaw, MP.; Pereira, M.; Morrell, JI. Hormones Behav. 2008. Characterization of maternal motivation in the lactating rat: contrasts between early and late postpartum responses. (in press)
- Wilsoncroft WE. Babies by bar-press: maternal behavior in the rat. *Behav Res Meth Instrum* 1969;1:229–230.
- Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev* 1987;94:469–492. [PubMed: 3317472]

Fig. 1.

Percentage of average weight gained by each eight-pup litter of early (*circles*) and late postpartum (*triangles*) females. Mean weights (in grams) and standard error of the mean recorded on the morning of each day are listed for litters of experimental females (*black shapes*) and age-matched litters of control females, which received no injections but were deprived of pups for similar amounts of time (*grey shapes*). Weights of experimental and control litters did not differ on any day, but all weights increased significantly on each day in each group (*asterisks*). All $P < 0.05$

Fig. 2.

Preferences and times for chambers associated with *subcutaneous cocaine* in the postpartum female rat. The percentage of early (*light grey*) and late postpartum (*dark grey*) females categorized into each of four chamber preference categories (**a**) and mean percentage of total session time that females spent in each apparatus chamber (**b**) during the postconditioning session, after conditioning with subcutaneous injections of 10 mg/kg cocaine and saline. No differences existed across postpartum

Fig. 3.

Preference and times for chambers associated with *intraperitoneal cocaine* in the postpartum female rat. The percentage of early (*light grey*) and late postpartum (*dark grey*) females categorized into each of four chamber preference categories (**a**) and mean percentage of time that females spent in each apparatus chamber (**b**) during the postconditioning session, after conditioning with intraperitoneal injections of cocaine and saline. During conditioning, IP cocaine was administered at one of four doses, either 0.5, 5, 10, or 20 mg/kg; doses are listed on *bars* representing cocaine-associated preference, with graphical order of doses remaining consistent across all chambers and preference categories. No differences existed across postpartum at any dose. *Asterisks* denote significant difference between doses; *Number signs* denote significant difference between chambers; all $P < 0.05$

Fig. 4.

Locomotor response to four doses of intraperitoneal cocaine across conditioning sessions in the postpartum female rat. Each dose is displayed separately; see top left corner of each subpanel for dose. Mean locomotor scores during each conditioning session within each conditioning chamber, calculated separately for cocaine-preferring postpartum females (*black shapes*; $n_{0.5 \text{ mg/kg}}=4$; $n_{5 \text{ mg/kg}}=20$; $n_{10 \text{ mg/kg}}=25$; $n_{20 \text{ mg/kg}}=10$) and saline-preferring postpartum females (*white shapes*; $n_{0.5 \text{ mg/kg}}=5$; $n_{5 \text{ mg/kg}}=1$; $n_{10 \text{ mg/kg}}=4$; $n_{20 \text{ mg/kg}}=6$). *Circles* represent cocaine-conditioning sessions (*COC*) and *triangles* represent saline-conditioning sessions (*SAL*). *Asterisks* denote significant differences between cocaine- and saline-conditioning sessions within the same preference group, while *numbers* denote significantly higher scores than those on day 1 and 1–2, respectively; all $P < 0.05$

Fig. 5.

Locomotor response to intraperitoneal cocaine during the postconditioning (test) session. Scores did not differ across postpartum group or cocaine dose and were pooled. Mean locomotor scores in each stimulus-associated chamber during the 1-h postconditioning test session were calculated separately for cocaine-preferring postpartum females (*black*; $n=59$) and saline-preferring postpartum females (*white*; $n=16$). *Number signs* denote significant differences between groups and *asterisks* for within groups; all $P<0.05$

Fig. 6.

Preference and times for chambers associated with *subcutaneous* or *intraperitoneal* cocaine in postpartum female, virgin female, and male rats. No differences existed across postpartum, so data from all postpartum females were pooled for these comparisons. The percentage of males (*striped*), virgin females (*white*), and postpartum females (*black*) categorized into each of four chamber preference categories (**a, c**) and mean percentage of time that males, virgin females, and postpartum females spent in each apparatus chamber (**b, d**) during the postconditioning session, after conditioning with either subcutaneous (**a, b**) or intraperitoneal (**c, d**) injections of 10 mg/kg cocaine and saline. Significant differences are marked with asterisks for between groups; number signs for within groups; all $P < 0.05$