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EFFECTS OF MENSTRUAL CYCLE PHASE ON COCAINE SELF-ADMINISTRATION IN RHESUS MACAQUES

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Abstract

Epidemiological findings suggest that men and women vary in their pattern of cocaine use resulting in differences in cocaine dependence and relapse rates. Preclinical laboratory studies have demonstrated that female rodents are indeed more sensitive to cocaine's reinforcing effects than males, with estrous cycle stage as a key determinant of this effect. The current study sought to extend these findings to normally cycling female rhesus macaques, a species that shares a nearly identical menstrual cycle to humans. Dose-dependent intravenous cocaine self-administration (0.0125, 0.0250, and 0.0500 mg/kg/infusion) using a progressive-ratio schedule of reinforcement was determined across the menstrual cycle. The menstrual cycle was divided into 5 discrete phases: menses, follicular, periovulatory, luteal, and late luteal phases: verified by the onset of menses and plasma levels of estradiol and progesterone. Dependent variables including number of infusions self-administered per session, progressive ratio breakpoint, and cocaine intake were analyzed according to cocaine dose and menstrual cycle phase. Analysis of plasma hormone levels verified phase-dependent fluctuations of estradiol and progesterone, with estrogen levels peaking during the periovulatory phase, and progesterone peaking during the luteal phase. Progressive ratio breakpoint, infusions self-administered, and cocaine intake did not consistently vary based on menstrual cycle phase. These findings demonstrate that under the current experimental parameters, the reinforcing effects of cocaine did not vary across the menstrual cycle in a systematic fashion in normally cycling rhesus macaques.

Keywords

self-administration; cocaine; estrogen; progesterone; non-human primate; female; menstrual cycle

INTRODUCTION

Though epidemiological findings report that cocaine use continues to be more prevalent among men than women (Cotto et al., 2010), the rates of new initiates to cocaine use among the adolescent population have been increasing among females, while remaining constant among males (SAMHSA, 2008). In conjunction with these statistics, women exhibit a more rapid progression from first use to cocaine-related substance use disorders (Carroll et al., 2002; Cotto et al., 2010) and are less successful when seeking treatment for these disorders

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relative to men due to low rates of treatment retention (Siqueland et al., 2002) or high rates of relapse to cocaine use (Hyman et al., 2007). Over the last decade, complementary preclinical data in laboratory animals have illustrated sex-dependent behavioral effects of cocaine. Many of these studies have demonstrated that laboratory animal models used to assess cocaine's abuse liability can be implemented to further understand the neuroendocrine basis for sex-dependent differences among the human cocaine-using population.

Cocaine self-administration studies in laboratory animals have probed this question by directly comparing behavior between males and females. Overall, these findings indicate that female rats demonstrate greater sensitivity to cocaine's reinforcing effects relative to males with shorter acquisition times (Lynch and Carroll, 1999; Carroll et al., 2002), higher rates of cocaine self-administration (Roberts et al., 1989; Lynch and Taylor, 2004, 2005), and greater resistance to extinction (Lynch and Carroll, 2000; Kerstetter et al., 2008). Studies examining the role of gonadal hormones on cocaine-maintained behaviors found that females demonstrate the greatest sensitivity to cocaine's reinforcing effects (Roberts et al., 1989; Hecht et al., 1999; Feltenstein et al., 2009) and resistance to extinguish responding previously paired with cocaine (Kippin et al., 2005; Kerstetter et al., 2008) during estrus, when circulating estradiol levels are at their highest and progesterone levels are at their lowest. Specifically, plasma progesterone levels appear to predict the behavioral effects of cocaine such that responding was inversely correlated with progesterone plasma levels, with lower progesterone levels predicting greater responding (Feltenstein and See, 2007). Other studies have shown that exogenous progesterone, and its metabolite, allopregnanolone, attenuate acquisition of cocaine self-administration, escalation of cocaine self-administration, and reinstatement of cocaine-seeking (Lynch et al., 2001; Larson et al., 2005, 2007; Jackson et al., 2006; Anker et al., 2007; Hu and Becker, 2008; Feltenstein et al., 2009).

Controlled human laboratory studies have also investigated the extent to which cocaine's effects vary between males and females. Findings from early human studies investigating sex-dependent differences in the subjective effects of cocaine have been mixed (Kosten et al., 1996; Haney et al., 1998; Evans et al., 1999), challenging the generalizability of cocaine's sex-dependent differences observed in rodent models. However, these studies varied with respect to the route of cocaine administered and they did not control for menstrual cycle phase. Several studies have directly investigated the extent to which menstrual cycle phase contributes to sex differences in the subjective response to cocaine (Lukas et al., 1996; Mendelson et al., 1999; Sofuoglu et al., 1999; Evans et al., 2002; Evans and Foltin, 2006a; Collins et al., 2007), but again, the results have been inconsistent, particularly among studies that administered intranasal cocaine (Lukas et al., 1996; Collins et al., 2007). In contrast, studies with smoked cocaine have revealed a clearer contribution of menstrual cycle phase to cocaine's subjective effects. The subjective effects of smoked cocaine are similar between men and women tested during the follicular phase, when estradiol levels are high and progesterone levels are low (Sofuoglu et al., 1999; Evans and Foltin, 2006a), whereas during the luteal phase (when progesterone levels are high) the subjective response to smoked cocaine is decreased relative to females tested during the follicular phase (Sofuoglu et al., 1999; Evans et al., 2002; Evans and Foltin, 2006a; but see Reed et al., 2011) or to men (Sofuoglu et al., 1999; Evans and Foltin, 2006a). Further, administration of exogenous progesterone decreased subjective responses to smoked cocaine in females during the follicular phase (Sofuoglu et al., 2002; Evans and Foltin, 2006a), but not in men (Evans and Foltin, 2006a). Exogenous progesterone also decreased the subjective ratings of 'high' produced by intravenous cocaine in a sample of men ($n = 6$) and women ($n = 4$) tested during the follicular phase (Sofuoglu et al., 2004). Despite these differences in subjective response to cocaine, and in contrast to the preclinical literature in rodents, no

studies in humans have observed differences in cocaine self-administration as a function of sex (Sofuoglu et al., 1999, 2004; Lynch et al., 2008), menstrual cycle phase (Sofuoglu et al., 1999; Reed et al., 2011), or progesterone administration (Sofuoglu et al., 2004; Reed et al., 2011). Thus, it remains unclear whether differences in the subjective response to cocaine between men and women, possibly due to naturally occurring fluctuations of gonadal hormone levels, contribute to differences in the reinforcing effects of cocaine. There are inherent limitations of human-subjects research, such as constraints on study length, restriction on the number and range of cocaine doses that can be tested, and inability to control for variability between participants that present obstacles for systematically investigating the role of gonadal hormones on cocaine's behavioral effects in humans. To overcome these limitations, the current study investigated the effects of menstrual cycle phase on cocaine self-administration in non-human primates, a species that can perhaps provide the most generalizable findings to predict factors that contribute to human drug abuse and dependence (Weerts et al., 2007).

Given the similarity in menstrual cycle in terms of duration and hormonal fluctuations between female macaques and humans (Appt, 2004; Shimizu, 2005), macaques are an ideal species to use for attempting to elucidate the role of fluctuations of gonadal across the menstrual cycle on cocaine reinforcement. Despite these similarities between humans and non-human primates, only 3 studies, all from the same laboratory, have been conducted to evaluate the reinforcing effects of intravenous cocaine in non-human primates as a function of sex, menstrual cycle phase, and administration of exogenous gonadal hormones (Mello et al., 2007, 2008, 2011). In one study (Mello et al., 2007), 4 gonadally intact, cycling female and 2 gonadally intact male cynomolgus monkeys (*Macaca fascicularis*) self-administered intravenous cocaine using a progressive ratio schedule of reinforcement. In that study, menstrual cycle phase did not consistently alter responding in female monkeys, although females reached a higher progressive ratio breakpoint than males for all doses tested, demonstrating a clear sex difference. Mello and colleagues also showed that exogenous estradiol failed to consistently affect cocaine self-administration in female rhesus monkeys (2 gonadally intact and 2 ovariectomized; Mello et al., 2008), whereas exogenous progesterone decreased intravenous cocaine self-administration of 0.01 mg/kg/injection in female rhesus monkeys (4 gonadally intact and 1 ovariectomized; Mello et al., 2011). These findings are important since they bridge the results in rodents with the results in humans. Therefore, the present study had two objectives. Since the majority of non-human primate research related to the reinforcing effects of cocaine have been conducted in rhesus monkeys (Mello and Negus, 1996), the first objective was to extend the results obtained in female cynomolgus monkeys (Mello et al., 2007) to rhesus monkeys, by determining whether cocaine's reinforcing effects varied as a function of menstrual cycle phase using a progressive ratio schedule of cocaine self-administration. The second objective was to relate these findings to studies conducted in human female cocaine abusers (Evans et al., 2002; Evans and Foltin, 2006a; Reed et al., 2011).

METHODS

Subjects

Five adult female rhesus monkeys (*Macaca mulatta*), weighing 6.0 to 10.0 kg, were housed unrestrained as described below for the duration of the study in a room that was maintained on a 12 hour light/dark cycle, with lights on at 7 AM. Each day, monkeys received fruit, a daily vitamin, and monkey chow to maintain stable body weight; approximately 7–9 chow (105–135 grams; High protein monkey diet #5047, 3.37 kcal/g; LabDiets®, PMI Feeds, Inc., St. Louis, MO). In addition, monkeys periodically received various food treats such as raisins, cookies, candy and fruit-flavored juices. Monkeys were housed in customized, squeeze-capable, rack-mounted, non-human primate cages (Hazleton Systems, Inc,

Aberdeen, MD) in the AAALAC-approved animal care facility of The New York State Psychiatric Institute. Each monkey had access to 2 identically sized chambers (61.5 cm wide × 66.5 cm deep × 88 cm high) connected by a 40 cm × 40 cm opening. Water was freely available from spouts located on the back panels of both chambers. Four of the five monkeys had participated in previous studies assessing the pharmacokinetics of cocaine across the menstrual cycle (Evans and Foltin, 2004; Evans and Foltin, 2006b). Investigators and veterinarians routinely monitored the health of the monkeys. Cages were positioned to allow all monkeys to have visual, auditory and olfactory social contact with other monkeys, the animal caretakers and other staff. In addition to the operant procedures, when self-administration sessions were not in progress, monkeys had other various forms of environmental enrichment including: music, cartoon videos (e.g., Scooby Doo, Nemo), a puzzle box, a mirror, and other tactile and chew toys. Lastly, monkeys had multiple positive interactions each day with the primary caregivers, as well as with the Investigators, who all are knowledgeable about environmental enrichment, primate behavior and the evaluation of primate behavior. All study procedures and aspects of animal maintenance complied with the US National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the New York State Psychiatric Institute Animal Care and Use Committee.

Surgery

For long-term intravenous drug administration, monkeys were surgically implanted with a chronic indwelling catheter (Access Technologies, Skokie, IL) that terminated in a subcutaneous vascular access port (VAP) (Wojnicki et al., 1994). An outside veterinary team with extensive expertise in VAP surgeries performed all of the VAP surgeries. Briefly, intravenous propofol was administered before an intertracheal tube was inserted. The monkey was connected to isoflurane gas, and the anesthesia level was monitored and maintained within physiological limits throughout the surgery, and an angiocath was placed in the saphenous vein, allowing an intravenous fluid line to be established. Under aseptic surgical conditions, a subcutaneous VAP connected to a silicone rubber-rounded-tip (intinsil tip) catheter was implanted in the right atrium through a femoral or jugular vein and was anchored to the vein with sutures. The VAP was anchored to the muscle and fascia in the pocket between the scapulae, and the port was assessed for patency with 0.5 ml of heparin 10,000 IU/ml. Upon recovery from anesthesia, the monkey was returned to its home cage and received buprenorphine (0.03 mg/kg, i.m.) twice within the first 24 hours after surgery. Cefatab (20 mg/kg) was also administered orally for 7 days, and the catheter was flushed every 3 days with 0.5 ml of heparin (200 IU/ml). Monkeys were given a two-week recovery period after surgery before resuming any experimental procedures. For maintenance, the catheter and port were flushed each self-administration session and a solution of heparin and gentamycin with sterile saline was inserted into the port to maintain patency, which was routinely assessed. If any signs of infection occurred, blood samples were taken and a request for a hematology profile and culture and sensitivity testing for the site of infection and appropriate antibiotics were administered. If an infection was suspected, experimental sessions were suspended.

Cocaine Self-Administration Sessions

The reinforcing effectiveness of 3 cocaine doses was tested (0.0125, 0.0250 and 0.0500 mg/kg/injection), and the order of doses systematically varied across monkeys. For each monkey, self-administration of each cocaine dose was studied for 3 to 5 menstrual cycles, with occasional saline sessions. Sessions occurred in the mornings, approximately 4 days per week. Monkeys were taken from their home cages and guided into customized wheel-mounted primate chairs (Primate Products Inc., Redwood City, CA) located in the same room as the home-cages. Primate chairs were placed in front of the cocaine session panels

mounted on the wall, facing the monkeys. Six session lights (CM 1820, 24 v, Chicago Miniature, Buffalo Grove, IL) with white lenses were evenly spaced around the outside edges of each panel. Two Lindsley levers (BRS-LVE, Beltsville, MD), with a light over each, were mounted at the bottom of each panel. For cocaine delivery, the VAP was accessed using a Huber needle with customized sterile extension tubing attached to a drug pump and a saline pump placed on an extension on the back of each chair. Schedule contingencies were controlled by customized software (Eureka Software, Cary, NC) running on two Macintosh 610 computers (Cupertino, CA) located in an adjacent area. Monkeys were first trained to respond on a fixed-ratio schedule for a cocaine dose of 0.10 mg/kg/infusion. Once stable responding was observed (less than 20% variability in total number of injections earned for 3 consecutive sessions), training on a progressive ratio schedule of reinforcement for 0.0125 mg/kg/infusion cocaine commenced. The size of the initial ratio (100 or 180 responses) was increased until stable responding was observed. For this schedule, a step size multiplier of 1.40 was used, such that the number of lever pulls required increased by 140% (e.g., 100, 140, 196, ... 2066) after each cocaine infusion (Hodos, 1961) and progressive ratio breakpoint was defined as the final ratio of responses completed for a cocaine injection. The start of the session was signaled by the illumination of a session light and each infusion was accompanied by a blinking light and tone. Cocaine infusions were delivered in a volume of 0.50 ml over 10 sec and followed by a 1.0 ml saline flush. Sessions ended 2.5 hours after the beginning of the session or when 10 infusions were earned. At the end of each session, the catheter and port were flushed and a solution of heparin and gentamycin with sterile saline was inserted into the port to maintain patency. Then the Huber needle was removed from the VAP and monkeys were returned to their home cages.

Determination of Hormone Levels and Menstrual Cycle Phase

Approximately one day per week, 2.0 ml whole blood was drawn from the saphenous vein while monkeys were seated in the primate chair to obtain plasma estradiol and progesterone levels. Within 30 minutes of collection, samples were centrifuged to obtain plasma (approximately 0.5 ml) and frozen until analysis. Estradiol and progesterone plasma levels were determined by Dr. Michel Ferin at the College of Physicians and Surgeons of Columbia University, Department of Obstetrics and Gynecology (New York, NY) using a commercial solid-phase, chemiluminescent enzyme immunoassay (Immulite, Diagnostic Products Co, DPC, Los Angeles, CA) validated for monkeys. Using this assay, progesterone levels of 1.5 ng/ml during the periovulatory or luteal phase indicated that the monkey had a normal ovulatory cycle. The sensitivity of the estradiol assay was 20 pg/ml and the intra- and inter-assay coefficients of variation were 4.3 and 10.5, respectively. The sensitivity of the progesterone assay was 0.2 ng/ml and the intra- and inter-assay coefficients of variation were 6.6 and 7.9, respectively. Onset of menses was determined primarily by observation of menstrual flow.

Drugs

Cocaine hydrochloride was obtained from the National Institute on Drug Abuse and was dissolved in sterile saline for injection U.S.P. All cocaine solutions were filtered using a millipore filter before injection. Cocaine doses were administered intravenously in a volume of 0.50 ml over 10 sec. The reinforcing effectiveness of 0.0125, 0.0250 and 0.0500 mg/kg/infusion were assessed based on previous studies demonstrating that these doses produce dose-dependent responding in rhesus monkeys (Negus et al., 1995, 1996; Rowlett, 2000). Because cocaine has been shown to disrupt the hypothalamic-pituitary-gonadal axis in rhesus monkeys (Mello et al., 2004), precautions were made to limit cocaine intake to avoid disruption of the menstrual cycle; relatively low doses of cocaine were used (Mello et al.,

1997; Potter et al., 1998), and self-administration sessions were limited to 4 days a week, with a maximum of 10 infusions each session.

Data Analysis

Plasma hormone levels—Plasma estradiol and progesterone levels and the onset of menses guided menstrual cycle phase determinations. Menses and follicular phases were determined based upon rising estradiol and low progesterone plasma levels, with menses usually defined as days 1–5 from onset of menstruation, and the follicular phase as days 6–10 after the onset of menstruation based on a typical 28-day menstrual cycle. The periovulatory phase was marked by peak estradiol and low progesterone plasma levels, usually 11–14 days from onset of menstruation. The luteal and late luteal phases were defined by high progesterone and low estradiol plasma levels, with the luteal phase typically occurring 6–14 days before the onset of menses, and the late luteal phase occurring 5 days before the onset of menses. The specific days used to define each menstrual cycle phase were adjusted according to plasma hormone levels and menstrual cycle length (e.g., if elevated progesterone levels were observed earlier/later in the cycle and the cycle length was shorter/longer than 28 days). Plasma hormones levels were analyzed separately according to menstrual cycle phase (menses, follicular, periovulatory, luteal, and late luteal) using a one factor repeated-measures analyses of variance (ANOVA). Cycles were considered to be anovulatory if there was no evidence to confirm ovulation based on the estradiol and progesterone levels obtained for any given cycle. Plasma hormone levels and self-administration data from anovulatory cycles were omitted from all analyses.

Cocaine self-administration—For each dose of cocaine tested, infusions earned, progressive ratio breakpoint (defined as the final ratio of responses completed for a cocaine injection), and cocaine intake was analyzed separately using a one factor repeated-measures analyses of variance (ANOVA) with menstrual cycle phase (menses, follicular, periovulatory, luteal, and late luteal) as the factor. Menstrual cycle phase was defined according to plasma estradiol and progesterone levels and onset of menses as described above. Three of the five monkeys self-administered all three cocaine doses during ovulatory cycles, whereas two of the five monkeys self-administered two of the three cocaine doses tested during ovulatory cycles (0.025 and 0.050 mg/kg/infusion). One of these monkeys (7CX) was tested for 7 cycles before being euthanized due to the development of uterine fibroids. Therefore analysis for 0.0125 mg/kg/infusion included data from three monkeys and analysis for 0.025 and 0.050 included data from all five monkeys.

All statistical analyses were calculated with Prism 4.0a for the Macintosh (GraphPad Software, Inc, 2003). Results were considered statistically significant when *p* values were equal to or less than 0.05. When significant main effects were detected, Bonferroni's multiple comparison tests were performed to further determine phase-dependent differences in hormone levels and cocaine self-administration (breakpoint, injections earned, and cocaine intake).

RESULTS

Menstrual cyclicity and plasma hormone levels

Table 1 depicts the range and average duration of each monkey's menstrual cycles and the number of ovulatory cycles over which cocaine self-administration was tested. Across the group, cocaine self-administration took place over 16.8 ± 3.1 ovulatory cycles with an average cycle length of 29.4 ± 0.4 days. Anovulatory cycles were observed in 4 of the 5 monkeys tested based upon plasma hormone levels and cycle length (66U, RQ205, and 44U2 exhibited 1 anovulatory cycle, and 7CX exhibited 5 anovulatory cycles). Anovulatory

cycles occurred when the highest cocaine dose was being tested for 3 monkeys (RQ205, 44U2, and 7CX) and when the low dose was tested for 1 monkey (66U).

Figure 1 shows that plasma estradiol and progesterone levels varied as a function of menstrual cycle phase (estradiol, $p = 0.0001$; progesterone, $p = 0.0001$). Bonferroni's multiple comparison tests revealed plasma estradiol levels to be the highest during the periovulatory phase compared to all other phases ($p = 0.001$), whereas plasma progesterone levels were highest during the luteal phase relative to all other phases ($p = 0.001$). Progesterone and estradiol hormone levels of individual monkeys reflected the group effect.

Cocaine Self-Administration

Infusions—Figure 2 illustrates infusions earned for the group excluding 66U and for individual monkeys as a function of cocaine dose and menstrual cycle phase. Infusions earned did not consistently vary as a function of menstrual cycle phase ($p = 0.05$). Four of five monkeys self-administered more active cocaine doses relative to saline (6ED, 4U2, 7CX and RQ205), but only one of those monkeys (RQ205) demonstrated consistent increases in infusions earned with increasing cocaine doses. Self-administration data for all three cocaine doses were obtained from only three of the five monkeys, with one of the three (66U) failing to self-administer more for active cocaine relative to saline, preventing statistical analysis of cocaine's dose-dependent effects. No consistent changes were observed with respect to the number of cocaine injections as a function of menstrual cycle phase across individual monkeys.

Breakpoint—Similar to infusions earned, progressive ratio breakpoint values did not consistently vary as a function of menstrual cycle phase (Table 2, $p = 0.05$). Breakpoint values were higher for the two higher cocaine doses relative to saline and the low cocaine dose for the group (excluding 66U). For individual monkeys, progressive ratio breakpoint values followed the same pattern observed for infusions earned. No pattern among the individual monkeys emerged for breakpoint values as a function of menstrual cycle phase.

Cocaine Intake—Figure 3 depicts cocaine intake as a function of cocaine dose and menstrual cycle phase for the group excluding 66U and individual monkeys. Cocaine intake did not vary as a function of menstrual cycle phase ($p = 0.05$) though consumption increased when higher cocaine doses were available for self-administration. Data from individual monkeys consistently demonstrated no effect of menstrual cycle phase on cocaine intake across monkeys.

DISCUSSION

The current study was designed to assess the impact of menstrual cycle phase on the reinforcing effectiveness of cocaine in healthy, regularly cycling female rhesus monkeys. Though there is considerable evidence derived from rodent self-administration studies to show that both phase and exogenous sex-hormone administration affects cocaine's reinforcing effects, few studies have investigated these effects in non-human primates, a species that shares a similar menstrual cycle with humans. Verifying menstrual cycle phase with plasma hormone levels and monitoring the onset of menses, fluctuations across the menstrual cycle in cocaine self-administration on a progressive ratio schedule across a range of doses was assessed. Under the current experimental parameters, progressive ratio breakpoint, cocaine infusions earned, and cocaine intake did not vary according to menstrual cycle phase.

The present findings are similar to a previous report in female cynomolgus monkeys demonstrating no changes in progressive ratio breakpoint values across the menstrual cycle

for a wide range of cocaine doses across a range, with the exception that the breakpoint for the lowest dose tested (0.0032 mg/kg/infusion) was greater during the follicular phase than during the late luteal phase (Mello et al., 2007). Several differences between the study by Mello and colleagues (2007) and the current study demonstrate the generalizability of this effect: differences in the progressive ratio schedule of reinforcement, the number and range of cocaine doses tested, the number of drug infusions available for self-administration per session, and the drug and training histories of the monkeys. In the previous study by Mello and colleagues (2007), effects of menstrual cycle phase on cocaine's reinforcing effects were assessed over a broader range of cocaine doses than currently tested (0.0032–0.032 mg/kg/infusion vs. 0.0125–0.0500 mg/kg/infusion). Though both studies used a progressive ratio schedule, the contingency used in the current study was more behaviorally demanding; the current study utilized a progressive ratio schedule that started at 100 or 180 responses for the first infusion and increased by a multiplier of 1.4 with each successive infusion, whereas the schedule used by Mello and colleagues (2007) started at 20 responses for the first infusion and increased by 0.05 log units with each successive infusion. The results from the earlier study also differed from the current one in that the breakpoint values during the follicular phase were significantly higher than those obtained during the luteal phase for a dose of cocaine that was more than a half-log unit lower than those tested in the current study (0.0032 mg/kg/infusion) (Mello et al., 2007), suggesting menstrual cycle phase-dependent changes in cocaine's reinforcing effects may only be detected at low doses under low ratio requirements. A subsequent study demonstrated that administration of progesterone (0.2 and 0.3 mg/kg) increased progesterone plasma levels beyond physiological levels (75–125 ng/ml) and shifted the cocaine self-administration dose-effect function down and to the right, significantly decreasing the number of injections self-administered for the cocaine dose that maintained the highest rates of behavior (0.01 mg/kg/infusion cocaine) (Mello et al., 2011). While estradiol administration (0.0001–0.01 mg/kg) increased 17 β -estradiol plasma levels by more than 10-fold, estradiol had no consistent effect on cocaine-self-administration (Mello et al., 2008). Together, these studies in female non-human primates suggest that the natural fluctuation of progesterone and estradiol levels across the menstrual cycle minimally influence cocaine self-administration. These findings are consistent with human studies reporting that smoked cocaine self-administration does not vary as a function of menstrual cycle phase (Sofuoglu et al., 1999; Reed et al., 2011). However, cocaine's positive subjective effect ratings are lower during the luteal phase relative to the follicular phase (Sofuoglu et al., 1999, 2002; Evans et al., 2002; Evans and Foltin, 2006a) and during the follicular phase when exogenous progesterone is administered (Sofuoglu et al., 2002, 2004; Evans and Foltin, 2006a). Based upon the current findings and these previous reports, it is evident that consistent changes in cocaine's effects as a function of menstrual cycle phase may be modest and can only be identified under limited experimental parameters and select dependent measures.

The current limitations of the study were primarily related to precautions in relation to cocaine's effects on the hypothalamic-pituitary-gonadal (HPG) axis. By limiting cocaine exposure, a ceiling effect may have occurred where the maximum number of infusions available were earned; this appeared to be the case for only one animal. It is unknown if breakpoints, infusions earned, and cocaine intake would have been higher if intake not been limited or if the dose range tested had been wider, but previous studies by Mello and colleagues (Mello et al., 2008, 2011) have shown that rhesus monkeys will respond for approximately 50 doses of 0.01 mg/kg/infusion cocaine when working on a FR30 schedule of reinforcement. Furthermore, by limiting the number of sessions per week to 4 in the current study, cocaine-maintained behavior may not have been as stable as being exposed to the operant and reinforcer on a daily basis as was implemented by Mello and colleagues (Mello et al., 2008, 2011). Stimulus control of cocaine self-administration was negatively affected by the infrequent sessions as reflected in the lack of consistent dose-dependent

increases in responding for cocaine and the high rates of responding for saline for 2 monkeys (66U and RQ205). Although limiting cocaine self-administration served as a precaution to prevent cocaine's effects on the HPG axis, 3 of 5 monkeys exhibited an isolated anovulatory cycle and one monkey exhibited occasional anovulatory cycles, all of which occurred when active cocaine doses were self-administered. These anovulatory cycles, in part, also prevented the collection of sufficient data for complete dose-response curves. The lack of behavioral sensitivity, in combination with the effects of cocaine on ovulation, demonstrate the difficulty in assessing fluctuating hormone levels on cocaine's effects in non-human primates. Another limitation is that hormone levels were only measured weekly; greater accuracy with respect to the determination of menstrual cycle phases and the time of ovulation would have occurred if samples had been collected more frequently.

Though the findings from this study suggest that normally fluctuating estradiol and progesterone levels fail to affect cocaine self-administration, it would be informative to continue to assess lower cocaine doses under lower ratio requirements, increase the number of injections available each session, and increase the frequency of self-administration sessions. These modifications would have less impact on the HPG axis, increase stimulus control, and potentially provide a greater spectrum of behavior affording a more sensitive baseline with which subtle differences in cocaine self-administration may be detected across the menstrual cycle.

Though there is growing evidence that gonadal hormones can potentially alter cocaine's behavioral effects, these findings are sometimes subtle and not always replicated. However, the present study in rhesus monkeys extends the previous findings in cynomolgus monkeys (Mello et al., 2007), that there are minimal changes in cocaine self-administration across the menstrual cycle, despite clear fluctuations in gonadal hormones. Similarly, though progesterone (both endogenous and exogenous) was shown to alter smoked cocaine's effects in women (Sofuoglu et al., 1999, 2002; Evans et al., 2002; Evans and Foltin, 2006a), these findings were not replicated in a later study (Reed et al., 2011). Furthermore, although oral progesterone decreased the subjective effects of intravenous (Sofuoglu et al., 2004) and smoked (Evans and Foltin, 2006a) cocaine, progesterone did not affect intravenous (Sofuoglu et al., 2004) and smoked (Reed et al., 2011) cocaine self-administration. These discrepancies demonstrate that the potential effects gonadal hormones have on cocaine may be masked by experimental procedures that are not designed to detect subtle changes. Therefore, behavioral contingencies and experimental parameters should be manipulated in effort to increase assay sensitivity.

CONCLUSION

The results from the current study in rhesus monkeys indicate that normal fluctuations of estradiol and progesterone associated with the menstrual cycle do not alter cocaine's reinforcing effects. Although cocaine self-administration did not change as a function of menstrual cycle phase in the current study, strengthening stimulus control and manipulating experimental variables to increase experimental sensitivity may further enhance our understanding of the subtle effects of gonadal hormones on cocaine reinforcement. Taken together with the existing literature in both humans and laboratory rodents, non-human primates appear to be a valid model for understanding the role of gonadal hormones and the menstrual cycle with respect to the reinforcing effects of cocaine.

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References

- Anker JJ, Larson EB, Gliddon LA, Carroll ME. Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. *Exp Clin Psychopharmacol.* 2007; 15:472–480. [PubMed: 17924781]
- Appt SE. Usefulness of the monkey model to investigate the role soy in postmenopausal women's health. *ILAR J.* 2004; 45:200–211. [PubMed: 15111739]
- Carroll ME, Morgan AD, Lynch WJ, Campbell UC, Dess NK. Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. *Psychopharmacology.* 2002; 161:304–313. [PubMed: 12021834]
- Collins SL, Evans SM, Foltin RW, Haney M. Intranasal cocaine in humans: effects of sex and menstrual cycle. *Pharmacol Biochem Behav.* 2007; 86:117–124. [PubMed: 17257663]
- Cotto JH, Davis E, Dowling GJ, Elcano JC, Staton AB, Weiss SR. Gender effects on drug use, abuse, and dependence: a special analysis of results from the National Survey on Drug Use and Health. *Gend Med.* 2010; 7:402–413. [PubMed: 21056867]
- Evans SM, Foltin RW. Pharmacokinetics of intravenous cocaine across the menstrual cycle in rhesus monkeys. *Neuropsychopharmacology.* 2004; 29:1889–1900. [PubMed: 15138443]
- Evans SM, Foltin RW. Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharmacology.* 2006a; 31:659–674. [PubMed: 16160708]
- Evans SM, Foltin RW. Pharmacokinetics of repeated doses of intravenous cocaine across the menstrual cycle in rhesus monkeys. *Pharmacol Biochem Behav.* 2006b; 83:56–66. [PubMed: 16426669]
- Evan SM, Foltin RW. Does the response to cocaine differ as a function of sex and hormonal status in human and non-human primates? *Horm Behav.* 2010; 58:13–21. [PubMed: 19733571]
- Evans SM, Haney M, Fischman MW, Foltin RW. Limited sex differences in response to “binge” smoked cocaine use in humans. *Neuropsychopharmacology.* 1999; 21:445–454. [PubMed: 10457542]
- Evans SM, Haney M, Foltin RW. The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology.* 2002; 159:397–406. [PubMed: 11823892]
- Feltenstein MW, Byrd EA, Henderson AR, See RE. Attenuation of cocaine-seeking by progesterone treatment in female rats. *Psychoneuroendocrinology.* 2009; 34:343–352. [PubMed: 18977603]
- Feltenstein MW, See RE. Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. *Drug Alcohol Depend.* 2007; 89:183–189. [PubMed: 17240083]
- Haney M, Foltin RW, Fischman MW. Effects of pergolide on intravenous cocaine self-administration in men and women. *Psychopharmacology.* 1998; 137:15–24. [PubMed: 9631952]
- 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]
- Hodos W. Progressive ratio as a measure of reward strength. *Science.* 1961; 134:943–944. [PubMed: 13714876]
- Hu M, Becker JB. Acquisition of cocaine self-administration in ovariectomized female rats: effect of estradiol dose or chronic estradiol administration. *Drug Alcohol Depend.* 2008; 94:56–62. [PubMed: 18054446]

- Hyman SM, Paliwal P, Sinha R. Childhood maltreatment, perceived stress, and stress-related coping in recently abstinent cocaine dependent adults. *Psychol Addict Behav*. 2007; 21:233–238.
- 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]
- Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE. Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. *Psychopharmacology*. 2008; 198:63–75. [PubMed: 18265959]
- Kippin TE, Fuchs RA, Mehta RH, Case JM, Parker MP, Bimonte-Nelson HA, See RE. Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. *Psychopharmacology*. 2005; 182:245–252. [PubMed: 16001116]
- Kosten TR, Kosten TA, McDougle CJ, Hameedi FA, McCance EF, Rosen MI, Oliveto AH, Price LH. Gender differences in response to intranasal cocaine administration to humans. *Biol Psychiatry*. 1996; 39:147–148. [PubMed: 8717615]
- Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME. Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. *Exp Clin Psychopharmacol*. 2007; 15:461–471. [PubMed: 17924780]
- Larson EB, Roth ME, Anker JJ, Carroll ME. Effect of short- vs. long-term estrogen on reinstatement of cocaine-seeking behavior in female rats. *Pharmacol Biochem Behav*. 2005; 82:98–108. [PubMed: 16111740]
- Lukas SE, Sholar M, Lundahl LH, Lamas X, Kouri E, Wines JD, Kragie L, Mendelson JH. Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. *Psychopharmacology*. 1996; 125:346–354. [PubMed: 8826539]
- Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology*. 1999; 144:77–82. [PubMed: 10379627]
- Lynch WJ, Carroll ME. Reinstatement of cocaine self-administration in rats: sex differences. *Psychopharmacology*. 2000; 148:196–200. [PubMed: 10663435]
- Lynch WJ, Kalayasiri R, Sughondhabirorn A, Pittman B, Coric V, Morgan PT, Malison RT. Subjective responses and cardiovascular effects of self-administered cocaine in cocaine-abusing men and women. *Addict Biol*. 2008; 13:403–410. [PubMed: 18782384]
- Lynch WJ, Roth ME, Mickelberg JL, Carroll ME. Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. *Pharmacol Biochem Behav*. 2001; 68:641–646. [PubMed: 11526960]
- Lynch WJ, Taylor JR. Sex differences in the behavioral effects of 24-h/day access to cocaine under a discrete trial procedure. *Neuropsychopharmacology*. 2004; 29:943–951. [PubMed: 14872204]
- Lynch WJ, Taylor JR. Decreased motivation following cocaine self-administration under extended access conditions: effects of sex and ovarian hormones. *Neuropsychopharmacology*. 2005; 30:927–935. [PubMed: 15647749]
- Mello NK, Knudson IM, Kelly M, Fivel PA, Mendelson JH. Effects of progesterone and testosterone on cocaine self-administration and cocaine discrimination by female rhesus monkeys. *Neuropsychopharmacology*. 2011; 36:2187–2199. [PubMed: 21796112]
- Mello NK, Knudson IM, Mendelson JH. Sex and menstrual cycle effects on progressive ratio measures of cocaine self-administration in cynomolgus monkeys. *Neuropsychopharmacology*. 2007; 32:1956–1966. [PubMed: 17251908]
- Mello NK, Mendelson JH, Kelly M, Diaz-Migoyo N, Sholar JW. The effects of chronic cocaine self-administration on the menstrual cycle in rhesus monkeys. *J Pharmacol Exp Ther*. 1997; 281:70–83. [PubMed: 9103482]
- Mello NK, Mendelson JH, Negus SS, Kelly M, Knudson I, Roth ME. The effects of cocaine on gonadal steroid hormones and LH in male and female rhesus monkeys. *Neuropsychopharmacology*. 2004; 29:2024–2034. [PubMed: 15199372]
- Mello NK, Negus SS. Preclinical evaluation of pharmacotherapies for treatment of cocaine and opioid abuse using drug self-administration procedures. *Neuropsychopharmacology*. 1996; 14:375–424. [PubMed: 8726752]

- Mello NK, Negus SS, Knudson IM, Kelly M, Mendelson JH. Effects of estradiol on cocaine self-administration and cocaine discrimination by female rhesus monkeys. *Neuropsychopharmacology*. 2008; 33:783–795. [PubMed: 17507915]
- Mendelson JH, Mello NK, Sholar MB, Siegel AJ, Kaufman MJ, Levin JM, Renshaw PF, Cohen BM. Cocaine pharmacokinetics in men and in women during the follicular and luteal phases of the menstrual cycle. *Neuropsychopharmacology*. 1999; 21:294–303. [PubMed: 10432477]
- Negus SS. Choice between heroin and food in nondependent and heroin-dependent rhesus monkeys: effects of naloxone, buprenorphine, and methadone. *J Pharmacol Exp Ther*. 2006; 317:711–723. [PubMed: 16456085]
- Negus SS, Mello NK, Lamas X, Mendelson JH. Acute and chronic effects of flupenthixol on the discriminative stimulus and reinforcing effects of cocaine in rhesus monkeys. *J Pharmacol Exp Ther*. 1996; 278:879–890. [PubMed: 8768743]
- Negus SS, Mello NK, Lukas SE, Mendelson JH. Diurnal patterns of cocaine and heroin self-administration in rhesus monkeys responding under a schedule of multiple daily sessions. *Behav Pharmacol*. 1995; 6:763–775. [PubMed: 11224379]
- Potter DA, Moreno A, Luther MF, Eddy CA, Siler-Khodr TM, King TS, Schenken RS. Effects of follicular-phase cocaine administration on menstrual and ovarian cyclicity in rhesus monkeys. *Am J Obstet Gynecol*. 1998; 178:118–125. [PubMed: 9465814]
- Reed SC, Evans SM, Bedi G, Rubin E, Foltin RW. The effects of oral micronized progesterone on smoked cocaine self-administration in women. *Horm Behav*. 2011; 59:227–235. [PubMed: 21192940]
- 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]
- Rowlett JK. A labor-supply analysis of cocaine self-administration under progressive-ratio schedules: antecedents, methodologies, and perspectives. *Psychopharmacology*. 2000; 153:1–16. [PubMed: 11255919]
- Shimizu K. Studies on reproductive endocrinology in non-human primates: application of non-invasive methods. *J Reprod Dev*. 2005; 51:1–13. [PubMed: 15750292]
- Siqueland L, Crits-Christoph P, Gallop R, Barber JP, Griffin ML, Thase ME, Daley D, Frank A, Gastfriend DR, Blaine J, Connolly MB, Gladis M. Retention in psychosocial treatment of cocaine dependence: predictors and impact on outcome. *Am J Addict*. 2002; 11:24–40. [PubMed: 11876581]
- Sofuoglu M, Babb DA, Hatsukami DK. Effects of progesterone treatment on smoked cocaine response in women. *Pharmacol Biochem Behav*. 2002; 72:431–435. [PubMed: 11900816]
- Sofuoglu M, Dudish-Poulsen S, Nelson D, Pentel PR, Hatsukami DK. Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Exp Clin Psychopharmacol*. 1999; 7:274–283. [PubMed: 10472516]
- Sofuoglu M, Mitchell E, Kosten TR. Effects of progesterone treatment on cocaine responses in male and female cocaine users. *Pharmacol Biochem Behav*. 2004; 78:699–705. [PubMed: 15301924]
- Substance Abuse and Mental Health Services Administration. Treatment Episode Data Set (TEDS). Office of Applied Studies; Rockville, MD: 2008.
- Weerts EM, Fantegrossi WE, Goodwin AK. The value of nonhuman primates in drug abuse research. *Exp Clin Psychopharmacol*. 2007; 15:309–327. [PubMed: 17696678]
- Wojnicki FH, Bacher JD, Glowa JR. Use of subcutaneous vascular access ports in rhesus monkeys. *Lab Anim Sci*. 1994; 44:491–494. [PubMed: 7844959]

HIGHLIGHTS

- Female rhesus macaques and humans have similar menstrual cycles
- Cocaine self-administration did not vary systematically across the menstrual cycle
- Future studies should manipulate behavioral contingencies to detect subtle changes

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Figure 1.

Mean ($\pm$ standard error of the mean, SEM) plasma estradiol and progesterone as a function of menstrual cycle phase [menses (M), follicular (F), periovulatory (PO), luteal (L), and late luteal (LL)] for the group (top left panel) and each individual monkey. Differences in estradiol levels between the periovulatory phase and other phases are indicated by an *, $p < 0.001$. Differences in progesterone levels between the luteal phase and other phases are indicated by a #, $p < 0.001$.

Figure 2.

Average ($\pm$ SEM) number of infusions earned as a function of cocaine dose and menstrual cycle phase for the group (excluding 66U; top left panel) and each individual monkey. Saline values were obtained from sessions across menstrual cycles, independent of menstrual cycle phase. Portions of these data were published in Evans and Foltin (2010).

Figure 3.

Average ($\pm$ SEM) cocaine intake (mg/kg) as a function of cocaine dose and menstrual cycle phase for the group (excluding 66U; top left panel) and each individual monkey. Saline values were obtained from sessions across menstrual cycles, independent of menstrual cycle phase.

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Table 1
Number, range, and length of ovulatory cycles for individual monkeys and the group.

	Monkey ID					Group
	66U	RQ206	6ED	4U2	7CX	
Ovulatory Cycles (#)	24	16	23	14	7	16.8 ± 3.1 *
Range (Days)	23 – 34	26 – 39	26 – 41	16 – 37	28 – 32	16 – 41
Length (Days; Mean ± SEM)	28.9 ± 0.5	28.9 ± 0.8	31.6 ± 0.8	27.1 ± 1.5	29.4 ± 0.6	29.4 ± 0.4 *

* Data represent mean ± SEM

Table 2

Mean **progressive ratio breakpoint** values for individual monkeys and the group (excluding 66U) as a function of menstrual cycle phase ^{*}.

Dose (mg/kg/infusion)	Phase [§]	Monkey ID				Group
		66U	RQ206	6ED	4U2	7CX
0.0125	M	1017.5 ± 48.5	803.0 ± 173.2	365.6 ± 153.0	-	-
	F	1140.9 ± 39.9	369.3 ± 153.3	1027.8 ± 118.9	-	-
	PO	1464.7 ± 121.4	179.2 ± 63.2	1313.8 ± 74.1	-	-
	L	1253.5 ± 35.0	492.6 ± 139.6	1268.1 ± 86.0	-	-
	LL	1404.4 ± 118.1	450.3 ± 80.7	1168.3 ± 207.0	-	-
0.0250	M	1279.3 ± 114.2	1286.0 ± 144.6	1478.0 ± 102.5	492.2 ± 60.8	1460.3 ± 336.0
	F	1185.8 ± 127.3	1350.0 ± 224.7	1419.5 ± 25.9	593.4 ± 49.6	1662.1 ± 162.4
	PO	1199.3 ± 182.4	1421.6 ± 172.2	1321.1 ± 186.4	781.7 ± 149.6	1672.7 ± 0.0
	L	1143.9 ± 121.6	1405.9 ± 170.2	1357.4 ± 116.7	564.2 ± 91.6	1552.2 ± 110.7
	LL	1181.6 ± 181.7	1335.0 ± 250.6	1289.9 ± 149.5	664.2 ± 56.8	1637.1 ± 337.8
0.0500	M	1033.8 ± 159.2	2439.0 ± 447.4	1145.9 ± 155.5	761.2 ± 55.3	1166.8 ± 161.7
	F	1363.1 ± 254.1	2271.5 ± 372.9	1169.1 ± 118.4	803.8 ± 82.4	1306.0 ± 257.4
	PO	1368.8 ± 304.6	2149.9 ± 521.2	1158.6 ± 219.0	782.1 ± 77.3	1124.3 ± 70.3
	L	1260.7 ± 145.0	2038.3 ± 318.3	1329.0 ± 153.8	732.3 ± 48.6	1380.1 ± 227.7
	LL	1145.1 ± 109.6	2154.7 ± 414.7	1551.9 ± 237.2	725.6 ± 86.9	1308.0 ± 189.4

^{*} Data represent mean ± SEM **progressive ratio breakpoint** value as a function of cocaine dose and menstrual cycle phase.

[§] M, menses; F, follicular, PO, periovulatory; L, luteal; LL, late luteal.