

# PROGESTERONE DOES NOT AFFECT COCAINE-INDUCED CONDITIONED PLACE PREFERENCE OR LOCOMOTOR ACTIVITY IN MALE RATS

**Introduction:** The present study aimed to determine if, as occurs in female rats, progesterone attenuates cocaine-induced reward and psychomotor responses in male rats.

**Methods:** The role of progesterone in the acquisition and/or expression of cocaine-induced conditioned place preference (CPP) and locomotor responses of intact male rats was studied. For chronic progesterone treatment, rats received Silastic capsules with either progesterone (100%) or vehicle 1 week prior to conditioning. For acute progesterone treatment, rats received subcutaneous injections of progesterone (500 µg) or vehicle (sesame oil) 4 hours before intraperitoneal injections of saline or cocaine administration (20 mg/kg) on conditioning days (acquisition phase-formation of reward associations) or before testing (expression phase-recall of reward associations).

**Results:** Both progesterone-treatment paradigms produced equivalent progesterone serum levels. Progesterone administered chronically or acutely during the acquisition and expression phases of cocaine conditioning did not block cocaine-induced CPP. Nor did progesterone affect ambulatory or rearing behaviors after cocaine administration.

**Conclusion:** These results suggest that, unlike the findings with female rats (in which similar treatment paradigms inhibited the formation and recall of cocaine-induced CPP), progesterone plays a limited role in the cocaine-induced reward or psychomotor responses of male rats. (*Ethn Dis.* 2010;20:[Suppl 1]:S1-73-S1-77)

**Key Words:** Reward, Sex Differences, Conditioned Place Preference, Progesterone

---

From Department of Psychology, Hunter College, New York, NY and Doctoral Subprogram in Biopsychology and Behavioral Neuroscience, Graduate School, City University of New York, New York, NY.

Address correspondence and reprint requests to Vanya Quinones-Jenab, PhD; Department of Psychology; Hunter College; 695 Park Avenue; New York, New York, 10065; 212-772-4649; 212-772-4619 (fax); vanya.quinones@hunter.cuny.edu

Scott J. Russo, PhD; Wei Lun Sun, PhD;  
Ana Christina E. Minerley, PhD; Karen Weierstall, PhD;  
Arbi Nazarian, PhD; Eugene D. Festa, PhD;  
Tipyamol Niyomchai, PhD; Alaleh Akhavan, BS; Shirzad Jenab, PhD;  
Vanya Quinones-Jenab, PhD

## INTRODUCTION

Both clinical and preclinical studies conducted in females have shown that progesterone attenuates some cocaine-induced behavioral responses. Specifically, women who use cocaine have attenuated subjective responses and less desire to smoke cocaine during the luteal phase (when serum levels of progesterone are the highest) than during the follicular phase of the menstrual cycle.<sup>1,2</sup> Progesterone administration attenuates cocaine's subjective effects in women during the follicular phase.<sup>3,4</sup> Similarly, in female rats, progesterone administration inhibits cocaine-induced conditioned place preference (CPP) and reduces the acquisition, escalation, and reinstatement of cocaine self-administration.<sup>5,6,7</sup> Feltstein and See demonstrated an inverse relationship between cocaine-seeking behaviors and plasma progesterone levels in female rats, thus suggesting that progesterone reduces craving of cocaine.<sup>8</sup> Progesterone also dose-dependently attenuates cocaine-induced psychomotor responses in female rats.<sup>9</sup> Few studies have addressed whether progesterone affects reward and psychomotor responses to cocaine in males. This study aimed to determine if progesterone inhibits the development (acquisition) and/or recall (expression) of learned association between environmental cues and cocaine-induced reward effects in male rats.

## METHODS

### Animals

Intact male Fischer rats (8 weeks old) purchased from Charles River

(Raleigh, NC) were housed in single-animal cages with free access to food and water and maintained on a 12-hour light/dark cycle. Rats were randomly assigned to cocaine or saline administration and then further subdivided into two pretreatment conditions: vehicle or progesterone, with a final  $n=10$  per experimental group. Separate sets of animals were run for each experimental manipulation, each of which consisted of at least three cohorts. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

### Drug Treatments

Rats received 20 mg/kg (diluted in saline [0.9%]) of cocaine or saline via intraperitoneal injections. This dose has previously been shown to represent the optimal dose needed to produce cocaine-induced CPP and locomotor responses in male rats.<sup>10,11</sup>

### Progesterone Administration

The progesterone administration paradigms were as previously described and were consistent with doses reported in the literature.<sup>10,11</sup> Briefly, for acute progesterone administration, rats received subcutaneous injections of either vehicle (sesame oil) or 500 µg of progesterone 4 hours before behavioral testing. For chronic progesterone administration, rats were anaesthetized with isoflourane and received an implant with Silastic capsules (Dow Corning) containing either dry cholesterol (3 mm, 100%-cholesterol, 40 mg cholesterol) or pro-

gestosterone (3 mm, 100%-progesterone, 40 mg of progesterone) inserted into the nape of the animal's neck. After 1 week of administration of progesterone via Silastic implantation, progesterone serum levels were approximately 10 ng/mL of serum. One week after surgery, rats were conditioned for cocaine-CPP or tested for locomotor activities.

### Progesterone Serum Level Determinations

A separate cohort of rats was run to determine serum levels of progesterone. For chronic treatment, rats were sacrificed 1 week after the capsule implant. For acute treatment, rats were sacrificed 4 hours after progesterone administration. Animals were sacrificed by decapitation after a brief (20-second) exposure to CO<sub>2</sub>. Trunk blood was collected and centrifuged at 3,000 rpm for 20 minutes at 4°C. Serum was stored at -80°C until use. To determine serum levels, samples—run in triplicates—were analyzed with a Count-A-Count for progesterone (Diagnostic Product Corporation, CA). An *n* of 8 per group was used for these analyses.

### Cocaine-CPP Procedure

Place preference cages purchased from Med Associates (Georgia, VT) consisted of a rectangular box with three distinct chambers—one neutral chamber between two conditioning chambers. Conditioning was conducted as described previously.<sup>10,11</sup> For pre-conditioning, rats were placed into a neutral-gray chamber area (for a 5-minute acclimation period) and then allowed to freely explore all three chambers for 15 minutes. Rats were then randomly assigned to cocaine/saline conditioning or saline/saline control groups and further sub-divided into vehicle or progesterone pretreatment groups. The next phase consisted of 4 days (2 cocaine/saline pairings) of conditioning; rats received cocaine on days 1 and 3 and saline on days 2 and 4 and were placed in alternate chambers.

Control rats received saline on all 4 days of conditioning. After each drug treatment, rats were immediately placed into the schedule CPP-chamber for 20 minutes. Chambers were counter balanced to avoid bias. On the testing day, rats were placed into the neutral gray chamber (5-minute acclimation period) and then allowed 15 minutes with free access to all three chambers in a drug-free state. Time-spent in each chamber was recorded by a computerized photobeam system run within the MED PC software.

To determine the effects of chronic progesterone administration on cocaine-induced CPP, rats received Silastic capsules 1 week prior to being conditioned for cocaine; thus, the hormone was present throughout the entire CPP paradigm. To determine the effects of acute progesterone administration on the expression of cocaine-induced CPP, on the testing day of the paradigm rats received vehicle or progesterone via subcutaneous injections 4 hours before placement in the CPP chamber. To determine the effects of progesterone on the acquisition of cocaine-induced CPP, rats received either vehicle or progesterone via subcutaneous injections 4 hours prior to cocaine or saline on conditioning days (*n*=10 per group).

### Locomotor Activity

Locomotor measurements were performed in each rat's home cage according to previously described methods.<sup>5</sup> Ambulatory (defined as breaking two beams of lights) and rearing (defined as vertical movements) activities were monitored after cocaine or saline treatment for 1 hour with a Photobeam Activity System from San Diego Instruments.

### STATISTICS

For CPP analysis, dependent measure *t*-test analyses were used to determine statistically significant differences

between time spent in each chamber. To assess the effect of cocaine and its interaction with progesterone on locomotor activities, cocaine CPP scores, or progesterone serum levels, ANOVAs were used (drug [saline, cocaine] x hormone [vehicle, progesterone]). When analysis identified significant interactions, a Newman-Keuls post hoc test was used to assess the differences among treatment groups. Significance was at the .05 level for all comparisons.

### RESULTS

Although progesterone serum levels were induced by hormone treatment ( $F=31.13$ ;  $P<.05$ ), overall progesterone levels were higher than in vehicle-treated controls. However, no differences were observed between the two manners of progesterone treatment ( $F=1.31$ ;  $P>.30$ ; Table 1). Throughout the study, vehicle-treated rats receiving saline treatment in both conditioning compartments (saline-saline pairing) did not exhibit significant differences in the time spent in each chamber (Table 2), confirming the unbiased nature of the procedure. Progesterone-treated rats receiving saline-saline control did not exhibit significant differences in the time spent in each chamber (see Table 2), demonstrating that progesterone has no basal effect.

As shown in Figure 1A, acute-vehicle-treated male rats acquired place preferences for the cocaine-paired chamber (acute-vehicle administration during the acquisition phase:  $t=2.45$ ;  $P<.05$ ; acute-vehicle administration during the expression phase:  $t=4.72$ ,  $P<.05$ ). Acute administration of progesterone during conditioning (acquisition phase) or testing (expression phase) of the CPP paradigm did not alter the development or expression of cocaine-induced CPP (acute-progesterone administration during the acquisition phase:  $t=3.21$ ,  $P<.05$ ; acute-progester-

**Table 1. Serum levels of progesterone after acute and chronic progesterone administration in male rats**

Length of hormone treatment	Hormone treatment	Drug treatment	Progesterone levels (ng/mL)
Acute	vehicle	saline	4.0 ± 2.5
	vehicle	cocaine	6.1 ± 2.0
	progesterone	saline	28.4 ± 3.5*
	progesterone	cocaine	30.5 ± 3.6*
Chronic	vehicle	saline	2.2 ± .5
	vehicle	cocaine	2.5 ± .4
	progesterone	saline	25.0 ± 3.5*
	progesterone	cocaine	27.5 ± 4.8*

Data are expressed as mean ± SEM.

\* Represents a hormone main effect. n=8 rats per group.

one administration during the expression phase:  $t=2.72$ ,  $P<.05$ ; see Figure 1). No differences were observed in the intensity of cocaine conditioning (CPP-scores) within the acute-treatment paradigm ( $F=.07$ ,  $P>.05$ ; Figure 1B).

As shown in Figure 1C, when progesterone was present throughout the cocaine-induced CPP paradigm (chronic-treatment paradigm), both vehicle- and progesterone-treated rats developed a significant CPP for cocaine (vehicle-treated:  $t=5.93$ ,  $P<.05$ ; progesterone-treated:  $t=2.91$ ,  $P<.05$ ). No differences were observed in the intensity of cocaine conditioning (CPP-scores) within chronic-progesterone-treatment paradigms ( $F=1.33$ ,  $P>.3$ ; Figure 1C).

Overall, cocaine increased rearing and ambulatory activities (main drug effect; rearing:  $F=38.18$ ,  $P<.000001$ ; ambulatory:  $F=28.56$ ,  $P<.000001$ ; Figure 2). However, acute progesterone administration did not affect ambulatory

or rearing responses to acute cocaine administration (Figure 2).

## DISCUSSION

In female rats progesterone administration has consistently been shown to attenuate cocaine-induced reward CPP,<sup>7,10</sup> but in this study of male rats, progesterone did not alter the formation or recall of cocaine-reward associations. This finding is consistent with human studies in which progesterone did not alter cocaine's subjective effects in men.<sup>3</sup>

The observed sex differences in progesterone's inhibitory effects on the acquisition and expression of cocaine CPP between our previous study in females and this investigation in males were not due to differences in serum levels of progesterone.<sup>7</sup> Equivalent serum levels after acute progesterone administration were found in female rats in the Russo et al study and in male

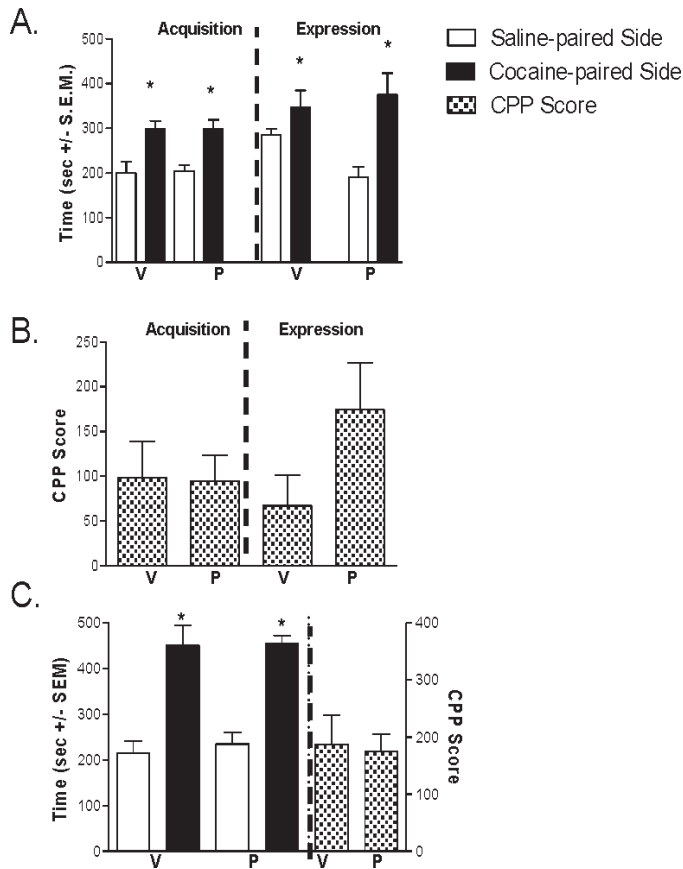
rats in this study.<sup>7</sup> Moreover, because cocaine-induced locomotor and rearing activities also were equivalent between our present study and our earlier one using female rats,<sup>7</sup> we postulate that sex differences in progesterone's ability to inhibit cocaine-induced CPP responses are not related to sex differences in cocaine-induced motor effects. It is yet to be determined whether sex disparities in progesterone's effects on the formation of regulation of cocaine-CPP are mediated through intrinsic differences between the sexes in progesterone-mediated responses. Still, the lack of progesterone effects on cocaine's CPP responses in males indirectly supports the hypothesis that sex differences in how progesterone alters cocaine-induced responses may be partly responsible for known differences in how the sexes develop and recall reward effects of this psychostimulant. In most studies, however, males and females are given equivalent progesterone doses, regardless of intrinsic differences in progesterone plasma levels or progesterone receptor distribution. The possibility that males may need higher progesterone concentrations than females to inhibit cocaine's rewarding/subjective effects still needs to be tested.

Romieu et al demonstrated that in male mice progesterone blocked cocaine-induced CPP.<sup>12</sup> Discrepancies between the Romieu study<sup>12</sup> and the reported observations herein could be related to species differences and/or doses and length of progesterone replacement. Anker et al showed that although both progesterone and allopregnanolone (ALLOP) decreased cocaine-primed reinstatement in females, ALLOP had no effect on cocaine-primed reinstatement in males.<sup>5</sup> However, the progesterone metabolite, pregnanolone, attenuated discriminative stimulus effects of cocaine in male rats.<sup>13</sup> A similar dichotomy in locomotor activity was observed. Although progesterone administration did not alter locomotor or rearing responses to

**Table 2. Time-spent in the cocaine unpaired and paired chambers on the test day of saline/saline control groups**

Replacement Paradigm	Hormone	Unpaired	Paired
Chronic	vehicle	278.5 ± 30.0	200.1 ± 41.0
	progesterone	258.6 ± 25.9	245.5 ± 6.7
Acute-Acquisition	vehicle	310.1 ± 56.6	215.1 ± 21.0
	progesterone	235.0 ± 39.0	274.5 ± 36.5
Acute-Expression	vehicle	215.1 ± 22.6	200.1 ± 28.0
	progesterone	248.6 ± 25.0	254.5 ± 17.9

All data are expressed as the mean (seconds)±SEM.



**Fig 1.** (A, B) The effects of acute progesterone administration during the acquisition and expression of the CPP paradigm in male rats. Data are represented as the (A) mean ( $\pm$ SEM) time spent in each chamber (white bar=saline side and black bar=cocaine side) or (B) CPP score (checkered bars). (C) The effects of chronic progesterone administration on the development of cocaine-induced CPP in male rats. The left side of the figure shows the mean ( $\pm$ SEM) time spent in each chamber; the right side shows the CPP score. \* Represents statistically significant difference between cocaine- and saline-paired sides. [P=progesterone administration; V=vehicle administration].  $n=10$  rats per group

cocaine, a recent study by Wu et al showed that inhibition of progesterone receptor activation via administration of RU486 (a progesterone receptor antagonist) attenuated locomotor responses to cocaine in male rats, thus suggesting a possible effect of progesterone on cocaine-induced responses.<sup>14</sup> In female rats, progesterone's effects on cocaine-induced psychomotor responses were dose and time dependent.<sup>9</sup> Thus, it is feasible that under different experimental conditions, progesterone might inhibit cocaine-psychostimulation in males. This possibility needs testing.

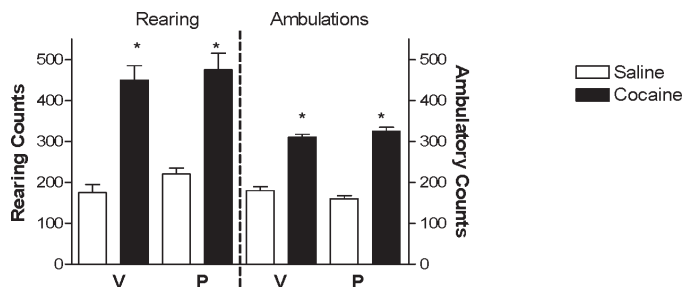
Several studies have demonstrated that progesterone is involved at a certain level with other cocaine-induced behavioral responses and neurochemical adaptations in male rats (eg, progesterone attenuated sleep deprivation associated with cocaine-enhanced genital reflexes in male rats<sup>17</sup>) Furthermore, Wu et al demonstrated the activation of progesterone receptors in males' nucleus accumbens after acute cocaine administration.<sup>15</sup> Cocaine also increased progesterone serum and brain levels in males.<sup>15,16</sup> In this study, however, although progesterone serum levels were higher in male rats after cocaine admin-

istration than in their respective controls, this induction failed to reach statistical significance. Wu et al demonstrated that progesterone serum levels were transiently induced in male rats 10 and 15 minutes after 15 and 30 mg/kg (respectively) of cocaine administration.<sup>14</sup> Thus, discrepancies between the findings of Wu et al and our observations may be due to differences in the time course of tissue collection and/or cocaine doses.<sup>14</sup> However, the current literature consistently has shown that in male rats, progesterone may mediate some cocaine-induced behavioral responses, and the activation of progesterone-mediated responses is in part involved in regulating cocaine-induced responses. Although the mechanisms of action of progesterone's effects are yet to be established, as recently reviewed in Quinones-Jenab and Jenab,<sup>17</sup> progesterone and/or its metabolites may affect cocaine responses either through its own receptor-mediated mechanisms or through other responses such as activation of GABA, sigma-1, or dopamine receptors.

### IMPLICATIONS FOR IMPROVING HEALTH DISPARITIES

Studies of cocaine abuse have shown that the user's sex affects treatment outcomes and relapse rates; women report shorter abstinence periods between cocaine uses than do men. To date, all of the published studies have shown that in females progesterone attenuates reward, initiation and relapse of cocaine self-administration, and positive subjective effects, and so it is feasible to predict that progesterone may be a potential treatment to regulate cocaine abuse in women.

Similar to our results, progesterone has also been shown to be ineffective in reducing drug-related responses in men.<sup>18</sup> However, further studies are needed in males to determine if differ-



**Fig 2. Ambulatory and rearing behavioral responses in male rats after acute progesterone and cocaine administration. Intact rats received progesterone (500 µg, s.c., 4 hr before cocaine) or vehicle and cocaine or saline. Behavior was recorded for 30 minutes after cocaine/saline administration. \* Represents statistically significant difference from respective saline-treated male rats. [P=progesterone administration; V=vehicle administration]. n=10 rats per group**

ent concentrations and/or length of treatment may be needed to attenuate cocaine’s reward. The data presented in this article have important clinical applications because they indicate that the observed sexual incongruities in overall cocaine use and rates of relapse may in part be explained by progesterone’s effects on cocaine reward associations.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Patricia Stephens for her editorial comments. This work was supported by SCORE 506-GM60654 and DA 12136.

**REFERENCES**

1. Evans SM, Haney M, Foltin RW. The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology (Berl)*. 2002;159(4):397–406.

2. Sofuoglu M, Babb D, Hatsukami DK. Effects of progesterone treatment on smoked cocaine response in women. *Pharmacol Biochem Behav*. 2002;72(1–2):431–435.
3. Evans SM, Foltin RW. Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharmacol*. 2006;31(3):659–674.
4. Sofuoglu M, Babb DA, Hatsukami DK. Progesterone treatment during the early follicular phase of the menstrual cycle: effects on smoking behavior in women. *Pharmacol Biochem Behav*. 2001;69(1–2):299–304.
5. Anker JJ, Larson EB, Gliddon L, Carroll ME. Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. *Exp Clin Psychopharmacol*. 2007;15(5):472–480.
6. Larson EB, Anker JJ, Gliddon L, Fons K, 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(15):461–471.
7. Russo SJ, Sun WL, Minerly ACE, et al. Progesterone attenuates cocaine-induced conditioned place preference in female rats. *Brain Res*. 2008;1189:229–235.

8. Feltenstein M, See RE. Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. *Drug Alcohol Depend*. 2007;89(2–3):183–189.
9. Niyomchai T, Russo SJ, Festa ED, Akhavan A, Jenab S, Quinones-Jenab V. Progesterone inhibits behavioral responses and estrogen increases corticosterone levels after acute cocaine administration. *Pharmacol Biochem Behav*. 2005;80(4):603–610.
10. Russo SJ, Festa ED, Fabian SJ, et al. Gonadal hormones differentially modulate cocaine-induced place preference in male and female rats. *Neuroscience*. 2003;120(2):523–533.
11. Russo SJ, Jenab S, Fabian SJ, Festa ED, Kemen LM, Quinones-Jenab V. Sex differences in the conditioned rewarding effects of cocaine. *Brain Res*. 2003;970(1–2):214–220.
12. Romieu P, Martin-Fardon R, Bowen WD, Maurice T. Sigma 1 receptor-related neuroactive steroids modulate cocaine-induced reward. *J Neurosci*. 2003;23(9):3572–6.
13. Quinton MS, Gerak LR, Moerschbaecher JM, Winsauer PJ. Effects of pregnanolone in rats discriminating cocaine. *Pharmacol Biochem Behav*. 2006;85(2):385–392.
14. Wu HBK, Niyomchai T, Minerly ACE, et al. Effects of RU 486 and tamoxifen on cocaine-induced behavioral and endocrine activations in male and female Fischer rats. *Ethn Dis*. 2008;18(2 Suppl 2):S2-81–S2-86.
15. Wu HBK, Fabian SJ, Jenab S, Quinones-Jenab V. Progesterone receptor activation after acute cocaine administration. *Brain Res*. 2006;1126(1):188–192.
16. Quinones-Jenab V, Minerly ACE, Niyomchai T, Akhavan A, Jenab S, Frye CA. Progesterone and allopregnanolone are induced by cocaine in serum and brain tissues of male and female rats. *Pharmacol Biochem Behav*. 2008;89(3):292–297.
17. Quinones-Jenab V, Jenab V. Progesterone attenuates cocaine-induced responses. *Horm Behav*. 2009; in press.
18. Evans SM, Foltin RW. Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharm*. 2006;31(3):659–74.