

# TESTOSTERONE PLAYS A LIMITED ROLE IN COCAINE-INDUCED CONDITIONED PLACE PREFERENCE AND LOCOMOTOR ACTIVITY IN MALE RATS

Growing evidence suggests that sex differences in cocaine reward responses are regulated by endogenous gonadal hormones. However, few studies have addressed the role of testosterone on cocaine reward and psychomotor activation. This study aimed to determine whether testosterone influences the development of psychomotor and reward responses to cocaine. Castrated 8-week-old male Fisher rats received placebo or testosterone via Silastic capsules (1–3 capsules of 100% testosterone) or subcutaneous injections (400, 800, or 1200 µg/kg) concurrent with cocaine administration. Although chronic testosterone administration did not alter cocaine-induced conditioned place preference (CPP), concurrent administration of testosterone and cocaine affected the development of cocaine CPP dose-dependently; 400 µg/kg blocked the expression of cocaine-induced CPP. Testosterone did not affect cocaine-induced locomotor activity. Furthermore, testosterone-saline-treated controls did not develop CPP, suggesting that at these doses, testosterone does not produce rewarding or motor responses. These data suggest that testosterone may play a limited role in cocaine-induced reward associations and locomotor responses and thus has a limited effect in the previously reported sexually dimorphic responses to cocaine. (*Ethn Dis.* 2008;18[Suppl 2]:S2-200–S2-204)

**Key Words:** Gender Differences, Reward, Castrated, Gonadal Hormones, Testosterone, Cocaine

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## INTRODUCTION

Sex differences in cocaine-induced behavioral and subjective responses have been reported, wherein females typically exhibit more pronounced locomotor responses and development of cocaine-induced conditioned place preference (CPP) than do males.<sup>1</sup> The prevailing theory suggests that gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine.<sup>1,2</sup> However, although the current literature postulates that testosterone contributes to the sexually dimorphic responses to cocaine, a direct link between testosterone and cocaine has yet to be established.

To date, few studies have addressed the role of testosterone in male responses to cocaine. In castrated males, testosterone has been found to delay and reduce cocaine-induced stereotypical behavior.<sup>3–5</sup> Testosterone also reduces cocaine-induced brain activity in the nucleus accumbens (a reward-associated area in the brain),<sup>6</sup> suggesting that testosterone is able to modify the reward effects of cocaine by reducing activation of reward circuitry in the brain.<sup>6</sup> Further, testosterone serum levels increase after cocaine administration, suggesting that an interaction exists between testosterone and cocaine effects.<sup>7</sup>

CPP has been used to determine the rewarding effects of drugs by establishing associations between the rewarding and incentive motivational effects of drugs and environmental stimuli.<sup>8</sup> Using this experimental technique, we aimed to determine if testosterone dose-dependently affects locomotor activity and the formation of reward associations with cocaine.

## METHODS

### Animals

Eight-week-old castrated male Fischer rats (Charles River Laboratories, Kingston, NY) were singly housed in animal cages with free access to food and water and were maintained on a 12-hour light/dark cycle (lights on at 9:00 AM). Animals were handled daily after their arrival. Animals were randomly assigned to either saline or cocaine treatment and further subdivided into hormone-replacement groups. For both the chronic and acute testosterone replacements, experiments were conducted in at least three cohorts with 8–10 animals per group. All National Institutes of Health and Institutional Animal Care and Use Committee guidelines for the care and use of laboratory animals were strictly followed.

### Testosterone Replacement Paradigms

All chemicals, unless otherwise stated, were purchased from Sigma Chemical Co. (Saint Louis, Mo). Testosterone (Innovative Research of America, Sarasota, Fla or Sigma Chemical Co., Saint Louis, Mo) was administered via a chronic or an acute replacement paradigm. For chronic testosterone replacement, after sedation with isofluorane, rats were implanted with either one 15-mm or one to three 30-mm Silastic capsules [.058 in. ID × .077 in. OD; Dow Corning (Midland, Mich)] filled with 100% testosterone. Control rats were implanted with empty capsules. Rats were behaviorally tested 1 week after hormone replacement. For acute testosterone administration, testosterone

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(0, 400, 800, or 1200 µg/kg) or vehicle (100% dimethyl sulfoxide) was administered via subcutaneous injections at the nape of the neck. These doses and the manner of administration were previously shown to produce physiological and pharmacokinetic testosterone serum levels.<sup>1</sup> Cocaine was prepared daily in .9% saline solution and administered via intraperitoneal injection at a concentration of 20 mg/kg. This dose was chosen because it reliably produces cocaine-induced CPP<sup>9-11</sup> and locomotor activity<sup>1,9</sup> in males tested with similar paradigms.

### CPP Apparatus and Procedure

The CPP apparatus used was a Single Station Package Place Preference Apparatus from Med Associates (Georgia, Vt) with three chambers (a white chamber, a black chamber, and a central gray neutral chamber). The central chamber was 12 cm long and had a smooth polyvinylchloride floor. The black chamber was 28 cm long and had a stainless steel grid rod floor; the white chamber, also 28 cm long, had a stainless steel mesh floor. The three chambers were separated by computer-automated guillotine doors. Pairings of cocaine or saline with each chamber were counter-balanced. Spontaneous locomotor activity was monitored by a Photobeam Activity System within each conditioning chamber.

A four-day conditioning paradigm was used as previously described.<sup>9,10</sup> For preconditioning, rats were placed into the neutral gray area for a five-minute acclimation period and then allowed free access to all three chambers for 15 minutes. The conditioning phase consisted of a four-day paradigm (two cocaine/saline pairings). Thirty minutes after the lights were turned on, rats received intraperitoneal injections of saline or cocaine in the conditioning chambers. On conditioning days 1 and 3, rats in the acute testosterone replacement group received a coadministration of cocaine and testosterone, whereas rats

in the chronic testosterone replacement group received only cocaine. The rats were then immediately confined to one chamber for 30 minutes. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 30 minutes. Control rats received saline on all four days, but on days 1 and 3 received either testosterone and saline or vehicle and saline. On the testing day, rats were placed into the neutral gray chamber for a five-minute acclimation period and then allowed 15 minutes of free access to all three chambers in a drug-free state. Time spent in each chamber and total locomotor activity (sum of all horizontal counts) was recorded by using a computerized photo-beam system run with MED-PC software.

### Testosterone Radioimmunoassay

After the final behavioral test, rats were sacrificed by rapid decapitation (after a 20-second exposure to CO<sub>2</sub>). Trunk blood was collected, centrifuged (at 3000 rpm for 30 minutes at 4°C), and serum was stored at -80°C until used. Testosterone serum levels were determined with a Coat-A-Count radioimmunoassay kit from Diagnostic Product Corporation (Los Angeles, Calif). The intra-assay coefficient of variance averaged <10%. Results were determined by using a log-logit analysis within GRAPHPAD PRISM (Graph-Pad Software, Calif). Testosterone serum levels are expressed as nanograms per milliliter.

### Data Analysis

For CPP data studies, behavioral data are presented as the mean time plus or minus standard error of the mean (SEM) in seconds spent in each side of the place preference apparatus. Total locomotor counts are represented as the mean sum of photobeam breaks ± SEM. For CPP data, dependent measures *t* test (two-tailed) within each group were used to determine statistically significant differences between the

time spent in each chamber. The CPP score was calculated to determine the magnitude of the place preference by subtracting the amount of time spent on the saline-paired side of the chamber from the amount of time spent on the cocaine-paired side. Differences in CPP scores, serum levels across the range of testosterone doses, and total locomotor activity were assessed by means of one-way analysis of variance. When appropriate, Fisher least significant difference post hoc tests were conducted. In all cases *P*<.05 was considered significant.

## RESULTS

### Chronic Testosterone Administration

After chronic testosterone administration, testosterone serum levels were found to increase dose-dependently ( $F(3, 79)=28.34$ ,  $P<.001$ ; Figure 1A): Silastic capsules administering 30, 60, and 90 of testosterone produced significantly higher testosterone serum levels than empty capsules (control rats), and both 60 mm and 90 mm increased serum levels to a greater degree than did 30 mm ( $P<.05$  for all comparisons).

Control rats (saline/saline-treated) did not differ in the time spent on each chamber, confirming the unbiased nature of the procedure (data not shown). Overall, cocaine increased the time spent in the drug-paired side as compared with time spent in the saline-paired side ( $F(1, 60)=36.609$ ,  $P<.01$ ). Furthermore, chronic testosterone administration did not produce CPP or altered locomotor activity in any of the testosterone-saline-treated rats (data not shown).

In cocaine-treated groups, regardless of the testosterone dose, rats spent more time in the cocaine-paired side of the apparatus than in the saline-paired side (0 mm:  $t(12)=4.402$ ,  $P<.05$ ; 15 mm:  $t(6)=2.924$ ,  $P<.05$ ; 30 mm:

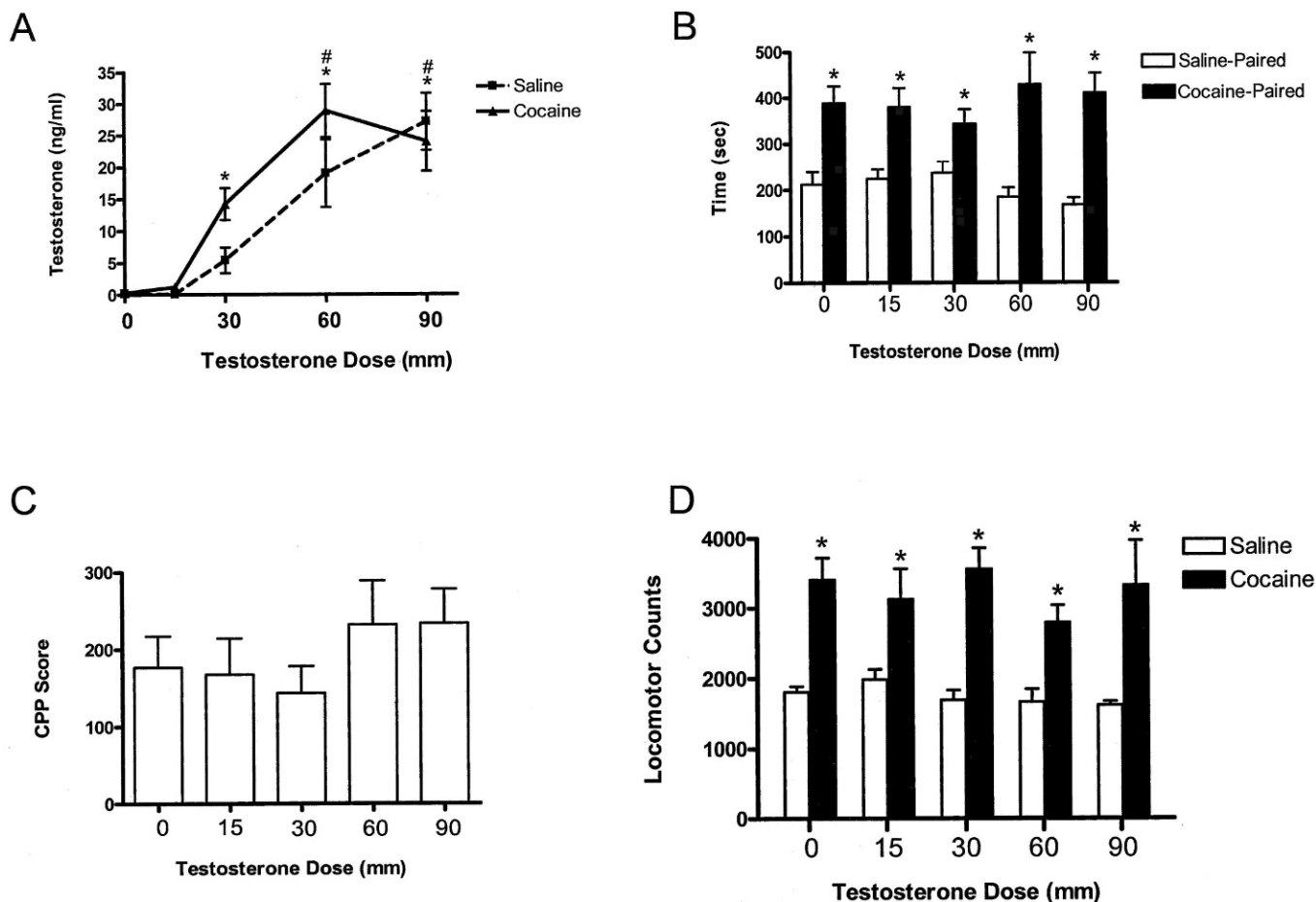


Fig 1. Effects of chronic testosterone administration on serum levels, cocaine-induced conditioned place preference (CPP), and locomotor activity in castrated male rats. (A) Testosterone serum levels: Data represent mean plus or minus standard error of the mean (SEM) serum levels, measured in ng/mL, in cocaine-treated (solid line) and saline-treated (dotted line) rats ( $n=7$  per group). (B) Cocaine-induced CPP: Data represent the time, measured in seconds, spent in saline-paired (white bars) and cocaine-paired (black bars) compartments of the CPP chambers on the test day. \*Indicates significant differences between saline- and cocaine-paired compartments within each testosterone dose. (C) Cocaine CPP scores: Data represent the differences in time, measured in seconds, spent in the saline-paired and cocaine-paired compartments of the CPP chambers on the test day for each testosterone dose used. (D) Locomotor responses: Data represent the mean total locomotor counts  $\pm$  SEM among saline-treated (white bars) and cocaine-treated (solid bars) groups. \*Indicates differences between saline and cocaine treatments within each testosterone dose.

$t(13)=2.047, P<.05$ ; 60 mm:  $t(4)=2.792, P<.05$ ; 90 mm:  $t(6)=4.930, P<.05$ ; Figure 1B). However, chronic testosterone replacement did not alter the intensity of cocaine-induced CPP; no significant differences in the CPP scores were observed ( $F(4, 47)=.8658, P>.05$ ; Figure 1C). As shown in Figure 1D, although overall cocaine increased locomotor activity ( $F(1, 60)=36.6, P<.05$ ), no significant interaction between hormone administration and cocaine was observed.

### Acute Testosterone Administration

In the acute-testosterone-treated rats, 48 hours after the last testosterone dose, no significant changes in testosterone serum levels were observed (Figure 2A). Similar to the chronic replacement experiment, control rats (saline/saline-treated) did not differ in the time spent on either side of the chamber (data not shown). Overall, cocaine increased the time spent in the drug-paired side as compared to the

saline-paired side ( $F(1, 60)=36.609, P<.01$ ). Both replacement paradigms produced equivalent CPP scores. Furthermore, acute testosterone administration did not alter CPP or locomotor activity in any of the testosterone/saline-treated rats (data not shown).

As shown in Figure 2B, acute testosterone administration dose-dependently altered cocaine-induced CPP; only 800 and 1200  $\mu\text{g}/\text{kg}$  produced cocaine-induced CPP (0  $\mu\text{g}/\text{kg}$  of testosterone:  $t(7)=2.923, P<.05$ ; 400  $\mu\text{g}/$

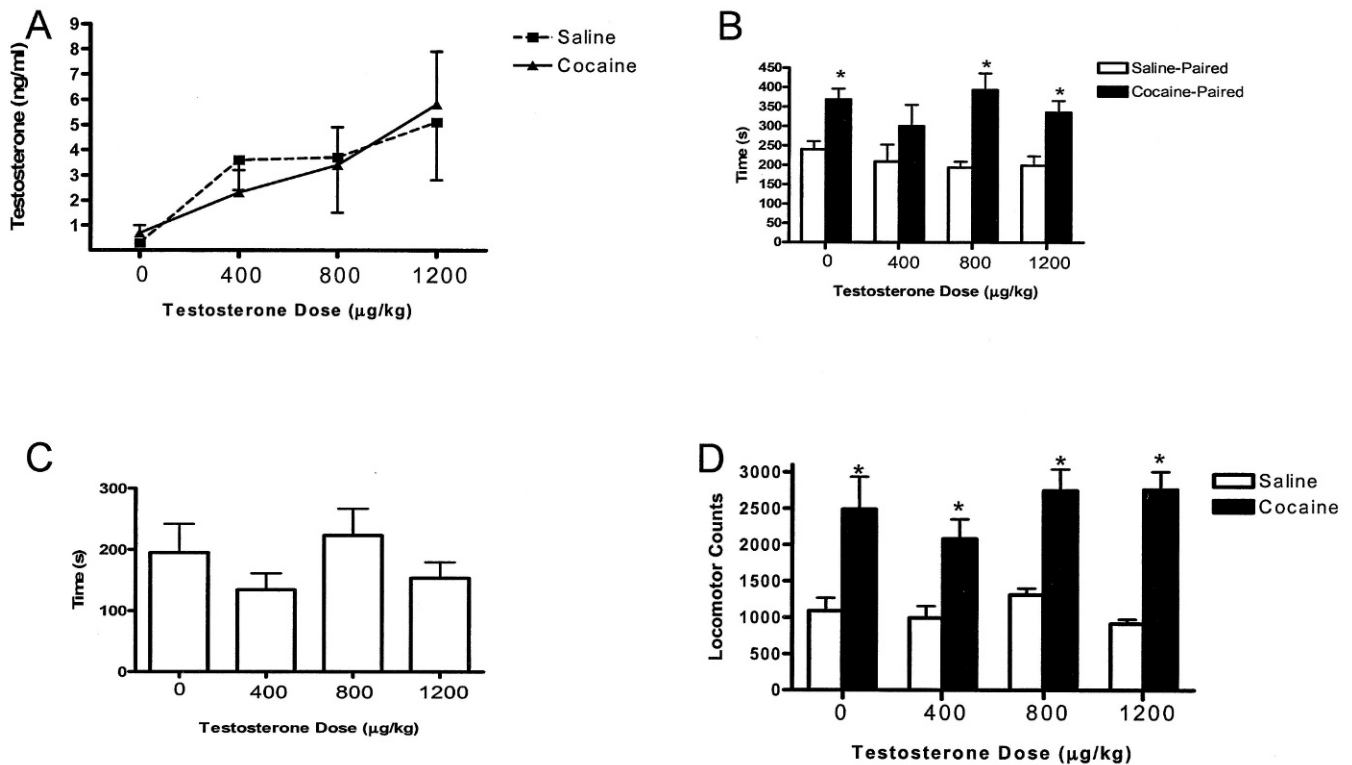


Fig 2. Effects of acute testosterone administration on serum levels, cocaine-induced conditioned place preference (CPP), and locomotor activity in castrated male rats. (A) Testosterone serum levels: Data represent mean plus or minus the standard error of the mean (SEM) serum levels, measured in ng/mL, in cocaine-treated (solid line) and saline-treated (dotted line) rats. ( $n=7$  per group). (B) Cocaine-induced CPP: Data represent the time, measured in seconds, spent in saline-paired (white bars) and cocaine-paired (black bars) compartments of the CPP chambers on the test day. \*Indicates significant differences between saline- and cocaine-paired compartments within testosterone dose. (C) Cocaine CPP Scores: Data represent the differences in time, measured in seconds, spent in the saline- and cocaine-paired compartments of the CPP chambers on the test day for each testosterone dose used. (D) Locomotor responses: Data represent the mean total locomotor counts  $\pm$  SEM among saline-treated (white bars) and cocaine-treated (solid bars) rats. \*Indicates differences between saline and cocaine treatments within each testosterone dose.

kg:  $t(7)=1.133$ ,  $P>.05$ ; 800  $\mu\text{g}/\text{kg}$ :  $t(6)=4.467$ ,  $P<.05$ ; 1200  $\mu\text{g}/\text{kg}$ :  $t(6)=5.512$ ,  $P<.05$ ). However, no significant differences in CPP scores were observed [ $(F(3, 27)=1.126$ ,  $P>.05$ ); Figure 2C]. Overall, cocaine increased locomotor activity ( $F(1, 51)=56.4$ ,  $P<.05$ ; Figure 2D), but no significant interaction between acute testosterone replacement doses and cocaine was observed.

## DISCUSSION

Chronic testosterone replacement did not affect the intensity of cocaine-

induced CPP or locomotor activity, which suggests that chronic testosterone replacement after gonadectomy does not influence the formation of cocaine-rewarding effects. Although acute testosterone administration inhibited the development of cocaine-induced CPP, this effect was limited to one dose. Taken together, our results suggest a limited interaction between testosterone and cocaine on cocaine-rewarding effects and psychomotor responses. Dramatic sex differences in term of cocaine rewarding and psychoactive behavioral responses have been reported, but these differences may mostly come from the contributions of other

gonadal hormones, ie, estrogen and progesterone, and not testosterone.

We observed some discrepancy between the manner of testosterone administration and the behavioral outcome. Whereas testosterone replacement via Silastic capsules provides relatively steady levels of testosterone, subcutaneous injections produce transient increases of serum levels of the hormone. These replacement methods may induce differential mechanisms of actions (genomic vs nongenomic mechanisms).<sup>1,12,13</sup> Therefore, the fact that acute testosterone (400  $\mu\text{g}/\text{kg}$ ) replacement inhibited cocaine-induced CPP may in part suggest that testosterone's

effects on cocaine-induced CPP behavioral responses occur via nongenomic (rapid effects) mechanisms.

Chronic administration of testosterone in castrated rats has also been shown not to be a critical determinant of cocaine-reinforcing effects in male rats.<sup>14</sup> Moreover, chronic administration of testosterone has no effect on the reward of intracranial self-stimulation of the brain to amphetamine treatments.<sup>15</sup> However, acute testosterone administration has been shown to affect locomotor activity in mice<sup>5</sup> and rewarding effects in mice and rats<sup>15-20</sup> to cocaine. In those studies, however, testosterone administration was given to gonadally intact males. Thus, acute and transient surges of testosterone serum levels, above testosterone physiological levels in intact rats, may be necessary for an interaction between testosterone or other anabolic androgenic steroids and psychoactive substances.

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