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Circulating androgens enhance sensitivity to testosterone self-administration in male hamsters

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Abstract

Young adult men are more likely to abuse steroids than individuals with low testosterone, including women, boys and older men. This suggests that circulating testosterone may enhance sensitivity to exogenous androgens. This hypothesis was tested using intracerebroventricular (i.c.v.) testosterone self-administration in orchidectomized males without testosterone (Orchx, n=8) and in orchidectomized males with chronic physiologic testosterone replacement (Orchx+T, n=8). Beginning 1 week after surgery, hamsters self-administered testosterone for 4 h/day in operant chambers at three doses (0.1, 1.0 and 2.0 μg/μl), each for 8 days. Afterwards, testosterone was replaced with vehicle for 8 days to test extinction. At 1.0 and 2.0 μg/μl, Orchx+T and Orchx males self-administered similar amounts of testosterone. However, at 0.1 μg/μl testosterone, only Orchx+T males showed a significant preference for the active nose-poke (Orchx+T active: 35.1±8.4 responses/4 h [mean±S.E.M.] vs. inactive: 16.5±1.7 responses/4 h, p<0.05; Orchx active: 16.7±4.9 responses/4 h vs. inactive: 13.5±3.1 responses/4 h, p>0.05). There was little change in operant behavior during extinction in Orchx+T males. However, when vehicle replaced testosterone, Orchx males extinguished their preference for the active nose-poke hole by day 6. These results support our hypothesis that circulating androgens enhance sensitivity to testosterone self-administration.

Keywords: Testosterone; Androgens; Anabolic agents; Self-administration; Hamsters; Sex behavior, animal

Abuse of androgenic anabolic steroids (AAS) is an increasing public health concern affecting not only athletes, but also other segments of the population (Clark and Henderson, 2003; Yesalis et al., 1993). Steroid users often take steroids in quantities 10–100 times higher than medically recommended (Clark and Henderson, 2003; Leshner, 2000b). AAS abuse is associated with a variety of reversible (testicular atrophy) and irreversible side-effects (baldness, gynecomastia, early growth termination), as well as life-threatening cardiovascular disease and hepatic carcinoma (Leshner, 2000a). In spite of the risks of steroid abuse, we know relatively little about androgen reinforcement and the potential for AAS dependence. According to Brower, AAS users initiate steroid use for anabolic effects, but they develop dependence with continued use (Brower, 2002). Steroid abusers often continue taking drugs in spite of the negative effects on health and social relations. In addition, many users report withdrawal symptoms, such as mood swings, depression, fatigue, loss of appetite and the desire to take more steroids (Leshner, 2000b). Of particular concern, recent evidence suggests that the incidence of AAS abuse among adolescents in the US is on the rise. In the last decade, the Monitoring the Future study found a significant increase in AAS use among middle and high school students (Johnston et al., 2003). Moreover, according to the National Household Survey on Drug Abuse, the median age for first use of AAS is 18 years (SAMHSA/OAS, 1996). First time AAS abuse correlates with high serum testosterone levels in men. Young men have the highest incidence of steroid use compared to individuals with low endogenous androgens, including prepubertal boys, women and older men (Yesalis et al., 1993).

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In humans, it is difficult to separate the direct psychoactive effects of AAS from reinforcement due to the anabolic effects of androgens on the body and increased athletic performance. Animal studies are helpful to investigate androgen reinforcement in a context in which the anabolic effects of steroids are irrelevant. Studies in laboratory rodents have demonstrated androgen reinforcement using a variety of experimental models. Using conditioned-place-preference, male rats (Alexander et al., 1994; De Beun et al., 1992) and mice (Arnedo et al., 2000) prefer an environment where they previously received testosterone. Indeed, when delivered as small volumes in an aqueous solution, rats will also form a conditioned place-preference to testosterone injections directly into nucleus accumbens (Packard et al., 1997). In a related conditioned taste aversion test, estrogen decreased, but testosterone increased consumption of a saccharin solution (Miele et al., 1988). The increase in saccharin consumption in testosterone-treated males suggests that testosterone is reinforcing. Estradiol plus progesterone potentiates brain stimulation reward in female rats (Bless et al., 1997). While testosterone does not directly stimulate lever-pressing in male rats, it does enhance responding in the presence of amphetamine (Clark et al., 1996). In our laboratory, male rats and Syrian hamsters self-administer exogenous testosterone orally, or via intravenous or intracerebroventricular (i.c.v.) cannula (Wood, 2002; Wood et al., 2004). The foregoing studies suggest that steroids are reinforcing, independent of their anabolic effects. In particular, steroid reinforcement demonstrated by self-administration, brain stimulation reward or conditioned-place-preference is evident in experimental models that do not require exercise or social interaction. However, the physiologic conditions (age, sex, steroid levels) that modify androgen self-administration remain largely undetermined.

Serum testosterone levels in men peak during young adulthood (Baier et al., 1974) when the prevalence of AAS abuse is the highest (SAMHSA/OAS, 1996). Accordingly, we hypothesized that elevated testosterone in circulation enhances sensitivity to self-administered androgens. To test this hypothesis, the present study used i.c.v. self-administration in male hamsters. Orchidectomized males without testosterone (Orchx), or orchidectomized males with chronic physiologic testosterone replacement (Orchx+T) were tested for i.c.v. testosterone self-administration. To compare androgen sensitivity in the two groups, three concentrations of testosterone were available in increasing order (0.1, 1.0 and 2.0 μg/μl). Gonad-intact male and female hamsters self-administer testosterone at 1.0 μg/μl (Triemstra and Wood, 2004; Wood et al., 2004). If circulating androgens enhance responsiveness to testosterone self-administration, Orchx+T males will self-administer more androgen than Orchx males. As a further test of androgen reinforcement, testosterone was replaced with vehicle for 8 days, followed by 8 days of testosterone reinstatement (1.0 μg/μl). Finally, males were tested for sexual behavior at the end of the study to determine whether the amount of testosterone consumed is behaviorally relevant.

1. Materials and methods

1.1. Subjects

Sixteen sexually naïve adult male Syrian hamsters (122–172 g BW) were obtained from Charles River Laboratories (Kingston, NY). Animals were singly housed with food and water available ad libitum. Hamsters were maintained under a long-day photoperiod (14:10 LD, lights on at 7 p.m.) and were tested during the first 4 h of the dark phase when activity peaks. All experimental procedures were approved by the USC Institutional Animal Care and Use Committee and were conducted in accordance with the "NIH Guide for Care and Use of Laboratory Animals" (Publication No. 85-23, revised 1985).

1.2. Surgery

All 16 males were castrated (Orchx). Half of them (Orchx+T) received a 10-mm Silastic implant (ID: 1.98 mm, OD: 3.18 mm; Dow Corning, MI) subcutaneously (s.c.) to maintain physiologic levels of testosterone (Powers et al., 1985). All surgeries were performed under anesthesia with pentobarbital sodium (80 mg/kg). At the time of orchidectomy, Orchx and Orchx+T hamsters were implanted with a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) into the lateral ventricle (AP: +1.0 mm, ML: +1.0 mm, DV: −3.0 to 5.0 mm relative to bregma). Beginning 1 week after surgery, testosterone was infused via a 28-gauge internal cannula inserted into the guide cannula immediately before testing. At other times, the guide cannula was protected by a dummy cannula with dust cap. Female hamsters used as stimulus animals during mating tests were ovariectomized via dorsal incision and were treated chronically with estradiol-17B (Sigma, St. Louis, MO) via a 4-mm Silastic capsule implanted s.c. immediately after ovariectomy. To induce lordosis, each female received 350 μg progesterone (2.5 mg/ml in sesame oil; Sigma) s.c. 4 h before testing.

1.3. Apparatus

Each operant conditioning chamber (Med Associates, St. Albans, VT) was enclosed in a sound-attenuating cubicle with a fan for ventilation. Operant chambers were equipped with a house light, two nose-poke holes and a computer-controlled syringe pump (Med Associates) with balance arm and fluid swivel (Instech, Plymouth Meeting, PA). Testosterone solutions from a 100-μl glass syringe (Hamilton, Reno, NY) were delivered to the swivel through

Teflon tubing. The tubing connecting the swivel to the cannula was protected by a metal spring. Nose-poke holes were located 6 cm above the cage floor on the left wall of the chamber, beneath the house light. The location of the active hole (to the front or rear of the chamber) was balanced to control for side preferences. To aid in discrimination of the active hole, the house light was extinguished and a stimulus light in the active hole was illuminated during the 5-s infusion interval (0.2 μl/s). Further nose poking in the active hole during this 5-s time-out period was recorded, but not reinforced with additional drug delivery. Likewise, nose-poking in the inactive hole was recorded, but produced no response.

1.4. Procedures

Beginning 1 week after surgery, hamsters were tested five times a week in daily 4-h sessions for acquisition of testosterone self-administration, according to methods of Wood et al. (2004). Testosterone (Steraloids, Newport, RI) for i.c.v. self-administration was dissolved in an aqueous vehicle of 13% 2-hydroxypropyl-β-cyclodextrin (RBI, Natick, MA). During testosterone self-administration, operation of the active nose-poke delivered 1 μl testosterone solution. Three concentrations of testosterone (0.1, 1.0 and 2.0 μg/μl) were tested, each for 8 days, beginning with the lowest concentration (0.1 μg/μl).

Following self-administration of the highest testosterone concentration (2.0 μg/μl), testosterone was replaced with vehicle and males were tested for extinction for the next 8 days. Finally, testosterone (1.0 μg/μl) was reinstated for an additional 8 days. Immediately after the final self-administration session, all animals were observed for sexual behavior with a receptive female in a single 10-min test. The number of mounts, intromissions and ejaculations were recorded.

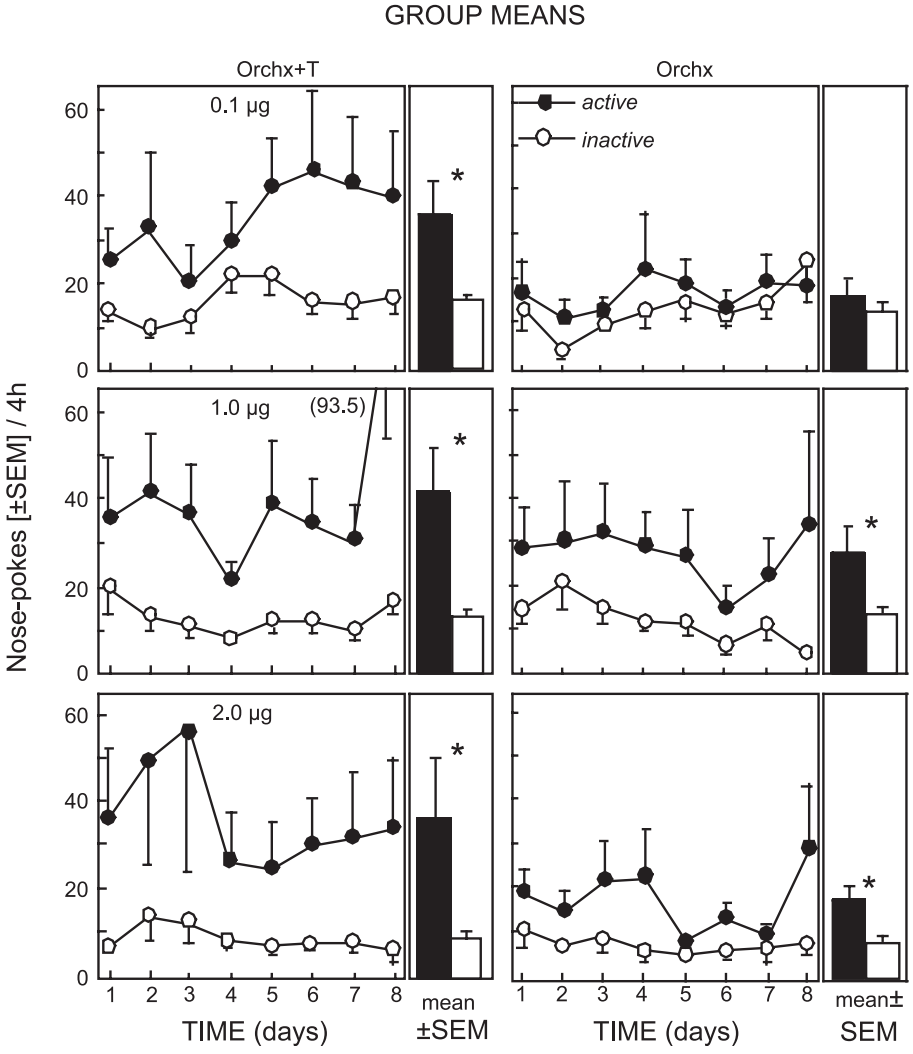

Fig. 1. Average daily operant responses (mean±S.E.M.) for intracerebroventricular testosterone (0.1, 1.0 and 2.0 μg/μl) in Orchx+T (left) and Orchx (right) male hamsters (n=8). Each concentration of testosterone was available for 8 days in increasing order. Responses in active (black) and inactive (white) nose-poke holes were monitored in daily 4-h test sessions. Bars indicate mean responses at each dose. Asterisk indicates significant difference in operation of the active versus inactive holes.

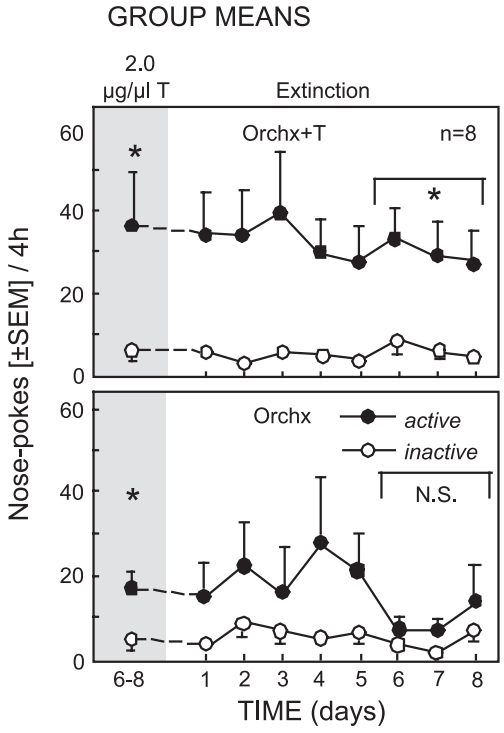

Fig. 2. Daily operant responses (mean±S.E.M.) during extinction in Orchx and Orchx+T male hamsters. Following self-administration of 2.0 µg/µl testosterone, testosterone was replaced with vehicle for 8 days (extinction). Responses in active (black) and inactive (white) nose-poke holes were monitored in daily 4-h test sessions. Asterisk indicates significant difference in operation of the active versus inactive holes.

1.5. Data analysis

Data from the active and inactive holes were collected by a PC-running Windows-compatible software (WMPC, Med Associates). Daily responses on the two nose-poke holes were averaged for each animal across each 8-day session (0.1, 1.0 and 2.0 µg/µl, extinction). Due to substantial individual variation in operant behavior, a Mann–Whiney *U*-test with Bonferroni correction for multiple comparisons was used to compare androgen intake and operant responses on the active nose-pokes between Orchx and Orchx+T males. Furthermore, the Wilcoxon signed rank test was used to compare responses on the inactive and active nose-poking among treatments and groups. Sexual behavior was analyzed by MANOVA and simple regression. In addition, the number of days to express a consistent preference for the active nose-poke hole was calculated for each animal. Consistent preference was defined as the first of 4 consecutive days for which responses in the active hole exceeded those in the inactive hole (Wood et al., 2004). A test of proportion (Ferguson, 1966) was used to compare acquisition of consistent preference between groups. Statistical analyses were completed using Statview 5.0 (SAS Institute). Data are presented as mean±S.E.M. For all comparisons, $p<0.05$ was considered statistically significant.

2. Results

2.1. Dose–response

Fig. 1 presents mean±S.E.M. responses for i.c.v. testosterone in Orchx and Orchx+T males. At the lowest concentration of testosterone (0.1 µg/µl), Orchx+T males showed a significant preference for the active nose-poke hole (35.1±8.4 responses/4 h on the active vs. 16.5±1.7 responses/4 h on the inactive hole, $p<0.05$). Moreover, six of eight Orchx+T males acquired a consistent preference for the active nose-poke after only 2.8±0.8 days of exposure. By contrast at the same 0.1 µg/µl androgen dose, only three of eight Orchx males acquired a consistent preference for the active nose-poke (1.7±0.7 days). Moreover, for all Orchx males, operant responses on the active hole (16.7±4.9/4 h) were equivalent to those on the inactive hole (13.5±3.0 responses/4 h, $p>0.05$). At higher testosterone concentrations (1.0 and 2.0 µg/µl), both Orchx and Orchx+T males preferred the active nose-poke hole over the inactive nose-poke ($p<0.05$). However, as determined by repeated measures ANOVA, there were no differences in operant responses on the active nose-poke between Orchx and Orchx+T males across the three testosterone concentrations, $p>0.05$.

2.2. Extinction

As shown in Fig. 2, substituting vehicle for testosterone failed to extinguish the behavior in Orchx+T males, but extinguished operant responding on the active hole in Orchx males. Orchx males showed a significant preference for the active nose-poke during the last 3 days of testosterone self-administration at 2.0 µg/µl (active: 18.0±5.1 responses/4 h vs. inactive: 7.6±2.5 responses/4 h, $p<0.05$). When testosterone was replaced with vehicle, operant responding slowly declined. During days 6–8 of vehicle self-administration, responses on the active and inactive nose-pokes were not significantly different (active: 10.8±4.5 responses/4 h vs. inactive: 5.8±1.4 responses/4 h, $p<0.5$). By contrast, operant responding did not extinguish in Orchx+T males when vehicle was substituted for testosterone. During the last 3 days of 2.0 µg/µl testosterone self-administration, responding on the active nose-poke averaged 35.2±13.7 responses/4 h compared with 7.3±2.3 responses/4 h on the inactive nose-poke. During days 6–8 of vehicle self-administration, there was little change in responses on the

Table 1
Mean±S.E.M. testosterone intake (µg/4 h) in Orchx and Orchx+T male hamsters ($n=8$ each) during self-administration of 0.1, 1.0 and 2.0 µg/µl testosterone

	0.1 µg/µl	1.0 µg/µl	2.0 µg/µl
Orchx	0.9±0.2*	15.6±3.2	19.1±2.8
Orchx+T	1.8±0.3	22.8±5.1	40.3±14.3

Asterisk indicates significant differences between groups, $p<0.05$.

Table 2
Percent of Orchx+T (n=5) and Orchx (n=7) male hamsters expressing copulatory behaviors (mounts, intromission, ejaculation) after i.c.v. testosterone self-administration

	Mount	Intromission	Ejaculation
Orchx (n=7)	86	43	0
Orchx+T (n=5)	100	100	100

active (29.5±9.3 responses/4 h) or inactive (8.0±3.4 responses/4 h) nose-pokes.

2.3. Testosterone intake

For all three doses of testosterone, at least half of the responses (51–57%) on the active hole were reinforced. At 0.1 μg/μl testosterone, Orchx+T males self-administered 1.8±0.3 μg testosterone/4 h, while Orchx males consumed less than half as much androgen (0.8±0 μg/4 h, p<0.05 vs. Orchx+T males). At 1.0 and 2.0 μg/μl testosterone, androgen intake was not statistically different between Orchx and Orchx+T males (Table 1).

2.4. Male sexual behavior

Table 2 illustrates the percentage of Orchx (n=7) and Orchx+T (n=5) males expressing mounts, intromissions and ejaculations when tested for copulatory behavior at the end of the study. Testosterone self-administration was comparable in the two groups during the preceding week. All Orchx+T males copulated to ejaculation during the mating test, and there was no correlation between testosterone intake and sexual behavior. However, since all males were sexually inexperienced at the time of testing, the overall expression of sexual behavior was relatively low (19.6±1.5 intromissions/10 min), compared with the behavior of sexually experienced males reported previously (26.0±2.9 intromissions/10 min; Wood, 2002). In Orchx males, six of seven mounted, three intromitted and none ejaculated. Moreover, copulatory behavior varied with androgen intake. The three Orchx males who achieved intromission self-administered 12.0±3.9 μg testosterone/4 h during the preceding week, while males who did not intromit averaged only 3.2±0.3 μg testosterone/4 h. Compared with Orchx males, Orchx+T males exhibited significantly higher sexual behavior (mounts F=63.4, intromissions F=156.2, ejaculations F=101.8, p<0.05). However, combining Orchx and Orchx+T males, testosterone intake did not correlate with sexual behavior.

3. Discussion

This study provides evidence that circulating androgens enhance sensitivity to exogenous androgen self-administration. Castrated male hamsters with chronic systemic testosterone replacement self-administered testosterone i.c.v. at all concentrations tested (0.1, 1.0 and 2.0 μg/μl). Castrates without systemic testosterone did not show a preference for the active nose-poke at 0.1 μg/μl testosterone. At higher testosterone concentrations (1.0 and 2.0 μg/μl), both Orchx and Orchx+T males self-administered i.c.v. testosterone at levels similar to intact female and male hamsters in previous studies from our laboratory (Triemstra and Wood, 2004; Wood et al., 2004). The implication is that high circulating androgens may enhance the reinforcing effects of AAS. Interestingly, when testosterone was replaced by vehicle, Orchx males rapidly extinguished operant behavior, while Orchx+T males continued to respond on the active nose-poke.

Self-administration is a well-established model to determine the potential for dependence and addiction to drugs of abuse (Madden et al., 1980; Panlilio et al., 2003; Ross et al., 1978). Previous studies from our laboratory have demonstrated intravenous and i.c.v. testosterone self-administration in rats and hamsters (Triemstra and Wood, 2004; Wood et al., 2004). In particular, testosterone self-administration into the cerebral ventricles suggests that the reinforcing effects of androgens are mediated by the brain. Operant responding is specific to testosterone because male and female hamsters in our previous studies did not self-administer the cyclodextrin vehicle (Triemstra and Wood, 2004; Wood et al., 2004). Nonetheless, it is evident that androgen reinforcement is not comparable to that of cocaine or heroin. In part, this may be due to the slow time-course of testosterone effects. While cocaine and heroin produce a "rush" in human users, former opiate addicts did not report euphoria following an injection of testosterone (Fingerhood et al., 1997). Likewise, in the Orchx and Orchx+T males as in our previous studies, testosterone self-administration across a 20-fold concentration range does not demonstrate a strong dose–response relationship (Triemstra and Wood, 2004; Wood et al., 2004).

Castration was used here to test for androgen reinforcement in the presence of chronic low circulating androgens. In humans, testosterone production peaks in the late teens and early twenties, and circulating androgens are substantially lower in women, older men and young boys (Baier et al., 1974). Nonetheless, AAS abuse is increasing in these populations (Leshner, 2000b). For comparison with Orchx male hamsters, we used Orchx+T males to ensure continued high levels of androgens in circulation. In intact males, the negative feedback of exogenous testosterone on the hypothalamic–pituitary–gonadal axis ultimately suppresses endogenous androgens (Tilbrook and Clarke, 2000). Thus, the Orchx+T model provides a more consistent contrast with Orchx males. Operant responding for testosterone in Orchx and Orchx+T males from the present study was similar to that of gonad-intact females and males, respectively, in our earlier studies (Triemstra and Wood, 2004; Wood et al., 2004). This suggests that Orchx and Orchx+T males are an appropriate model for normal physiologic states.

Males were exposed to each testosterone concentration for 8 days because this duration is sufficient for gonad intact males and females to acquire a consistent preference for 1.0 μg/μl testosterone. We interpret the lack of response to 0.1 μg/μl testosterone in castrates to reflect reduced responsiveness to low concentrations of androgen. However, castration has been shown to impair aspects of learning and memory (Sawyer et al., 1984; Van Oyen et al., 1980). Perhaps, learning deficits in castrated males account for the failure to self-administer 0.1 μg/μl testosterone. In this regard, castration does not impair performance in a discriminated lever-press avoidance test in rats (Van Oyen et al., 1981). In addition, gonadectomized rats are able to use sex odors to obtain a water reward (Carr and Caul, 1962) and are also able to discriminate morphine in a drug discrimination test (Craft et al., 1999). In hamsters, castration does not impair performance in conditioned taste avoidance (Peters et al., 2004) or a cost-benefit test of food reward (Chu and Wood, 2002). Moreover, in the present study, Orchx males showed a more rapid extinction response than Orchx+T males when testosterone was replaced by vehicle. This indicates that castrated males can learn operant responses and can quickly adjust their behavior.

Although the concentrations of androgen achieved in the brain during self-administration are not known, the amount is behaviorally relevant for mating. Steroids act in the central nervous system to facilitate a number of rewarding social behaviors, including sex (Mani et al., 1994; McEwen, 1994; Siegel, 1985; Week and Levine, 1995). Castrated male hamsters show little interest in receptive females and it takes 2–3 weeks of testosterone replacement to reinstate sexual behavior to pre-castration levels (Noble and Alsum, 1975; Payne and Bennett, 1976). In the present study, males were tested for sexual activity 9–10 weeks after castration. This duration is sufficient to eliminate copulation in castrated males (Noble and Alsum, 1975; Payne and Bennett, 1976; Siegel, 1985). When tested for mating after 9–10 weeks of testosterone self-administration, 86% of Orchx males mounted. However, the relatively low levels of sexual activity may reflect the lack of prior sexual experience, the interruption of androgen replacement during extinction, and/or the lack of peripheral androgens. Androgen levels and sex drive in male rodents have a "threshold" relationship. That is, above normal concentrations, androgens do not increase sexual activity (McGill et al., 1966). Our observations of mating behavior in Orchx+T males support the threshold model: there was no correlation between androgen intake and sexual behavior in this group.

Whether testosterone reinforcement is due to the androgenic or estrogenic metabolites of testosterone in not known. In the brain, testosterone may be aromatized into estradiol or reduced to the potent non-aromatizable androgen, dihydrotestosterone. AAS users "stack" combinations of aromatizable and/or reducible steroids (Clark and Henderson, 2003). Likewise, steroid actions in the brain may be mediated by genomic and/or non-genomic mechanisms (Frye, 2001; McEwen, 1991). In particular, rapid responses to exogenous androgens (<30 min) are more likely to reflect non-genomic mechanisms. In the present study, males made 20% of their responses on the active nose-poke during the first half hour of each 4-h session. In a similar manner, testosterone can produce a conditioned place preference in male rats with exposure to the test chamber for only 30 min after testosterone injection (Alexander et al., 1994). Likewise, testosterone can produce a conditioned place preference when injected directly into the nucleus accumbens (Frye et al., 2002; Packard et al., 1998), a brain area with few classical androgen receptors (Simerly et al., 1990). Together, these observations support the possibility of non-genomic receptors mediating androgen reinforcement. However, androgens up-regulate classical androgen receptors in the brain (Menard and Harlan, 1993). In the present study, the increased sensitivity to low testosterone concentrations in Orchx+T males may reflect increased levels of genomic receptors.

Although AAS abuse affects over a million people in the United States (Taylor, 2002), it is most common in young men, compared with adolescents, women and older men (Leshner, 2000b). In humans, many factors contribute to AAS abuse. However, the results of the present study suggest that the reinforcing effects of AAS may be increased in individuals with high circulating androgens. The implication is that young men may be at higher risk for steroid dependence.

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