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Self-administration of 3α-androstanediol increases locomotion and analgesia and decreases aggressive behavior of male hamsters

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Abstract

Androgens, such as testosterone (T), can have reinforcing effect, which may be due in part to actions of T's metabolite, 3α-androstanediol (3α-diol). To investigate rewarding effects of 3α-diol, gonadally intact adult male hamsters were given a two-bottle choice test to determine the amount of 3α-diol that would be self-administered over 4 days of exposure. After 2 days of habituation and 4 days of monitoring of consumption, hamsters were tested in an activity monitor and the open field (locomotion/exploration), paw lick (analgesia) and resident–intruder (aggression) tasks. Hamsters consumed significantly more 3α-diol than vehicle in the two-bottle choice test. Hamsters that were allowed to self-administer 3α-diol made significantly more beam breaks and total entries in the open field had increased latencies to pawlick, and engaged in significantly fewer attacks, than did hamsters with access to vehicle alone. Hamsters that self-administered 3α-diol had higher levels of 3α-diol in serum, hippocampus, prefrontal cortex, striatum and midbrain than did hamsters with access to vehicle alone. Together, these data suggest that 3α-diol may have rewarding effects.

Keywords: Non-genomic; Neurosteroid; Anabolic–androgenic androgens; Reward; Reinforcement

1. Introduction

Anabolic–androgenic steroids (AAS), synthetic derivatives of the primary androgen secreted by the testes, testosterone (T), are commonly abused substances. The epidemiology of AAS abuse is similar to other drugs of abuse with use by elite groups (body builders, athletes) being followed by widespread use among other segments of the population (non-athletes, young adults, adolescents) (Arnedo et al., 2000; Buckley et al., 1988; Faigenbaum et al., 1998; Kann et al., 1996; Yesalis et al., 1990). Adverse effects of AAS (kidney/liver damage, heart disease, hypertension, testicular atrophy, gynomastia amenorrhea) may be greatest among adolescents (NIDA, 1991) and include premature growth plate closing and early baldness (Bahrke et al., 1996a,b; Haupt and Rovere, 1984; Yesalis and Bahrke, 1995; Yesalis and Cowart, 1998). As well, AAS abuse can also lead to abuse of other drugs (Arnedo et al., 2000; Arvary and Pope, 2000; Miller et al., 2005), which adolescents may be particularly vulnerable to. Although AAS use may be due to primary reinforcing effects on appearance and/or performance, there may be secondary reinforcing hedonic effects (Arnedo et al., 2000; Bahrke et al., 1996a,b; 1990; as reviewed by Katz and Pope, 1990; Wilson, 1988; Yesalis and Cowart, 1998). Users report positive mood effects from AAS (Brower et al., 1991; Taylor, 1987). AAS can elicit similar electroencephalographic changes as do amphetamines and antidepressants (Bahrke et al., 1990). Moreover, T was used as an antidepressant (Altschule and Tilletson, 1948) and can enhance mood when administered to depressed men with low endogenous T levels (Pope et al., 2003). A question surrounding most drugs of abuse is to what extent they produce euphorogenic effects, which maintain and/or exacerbate use. However, unlike classic drugs of abuse, relatively little is known about factors underlying AAS abuse.

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In animal models, T can have rewarding effects. T increases rates of bar pressing for electrical brain stimulation (Caggiula, 1970; Campbell, 1970; Kornetsky and Esposito, 1981; Olds, 1958). Animals can become physically dependent on AAS (Foltin, 1992; Peters and Wood, 2005). In studies of conditioned place preference (CPP), T conditions a place preference (Alexander et al., 1994; DeBeun et al., 1992; Caldarone et al., 1996; Frye et al., 2001, 2002; King et al., 1999; Packard et al., 1997; 1998; Rosellini et al., 2001; Schroeder and Packard, 2000), when administered systemically, or when applied centrally to the nucleus accumbens or medial preoptic area. However, in some studies, CPP with T was seen only with very high systemic dosages (Caldarone et al., 1996). Further, hamsters will self-administer T, as well as other AAS (Ballard and Wood, 2005; Johnson and Wood, 2001; Wood, 2002; Wood et al., 2004).

T has anti-anxiety effects that are mediated in part by 3α-androstanediol (3α-diol). The 5α-reductase and 3α-hydroxysteroid dehydrogenase enzymes convert T to dihydrotestosterone (DHT) and then 3α-diol. T or 3α-diol administration systemically or to the hippocampus of gonadectomized rats increases anti-anxiety behavior and 3α-diol, but not T, levels in the hippocampus (Edinger and Frye, 2005; Frye and Edinger, 2004; Frye and Seliga, 2001). Blocking formation of 3α-diol attenuates T's anti-anxiety effects (Frye and Edinger, 2004). 3α-diol has agonist-like effects at γ-aminobutyric acid $(GABA)_A$/benzodiazepine receptor complexes (GBRs; Frye et al., 1996a; Gee, 1988). T and 3α-diol both produce CPP and blocking T's metabolism to 3α-diol prevents this (Frye et al., 2001, 2002; Rosellini et al., 2001). Whether 3α-diol would be self-administered was of interest. We hypothesized that, if 3α-diol has reinforcing effects, hamsters would consume more 3α-diol than vehicle and this would alter behavior.

2. Methods

2.1. Animals and housing

Adult (<55 days old), gonadally intact male LVG hamsters (*Mesocricetus auratus*; $N=14$) were bred from stock originally obtained from Harlan Laboratories (Indianapolis, IN) in the temperature-controlled (22±4 °C) Laboratory Animal Care Facility in the Social Science Building at The University at Albany. Hamsters were housed in a room maintained on a 10:14 h dark/light cycle (lights off between 0800 and 2200 h). Hamsters were raised group-housed (4 per cage) and transitioned to single housing 2 weeks prior to the experiment. Hamsters had food available *ad libitum* and water was available as described below.

2.2. Procedure and two-bottle choice test

2.2.1. Hamsters were tested over a 4-week period

Week 1: All hamsters were given access to two 100 ml graduated, inverted, leak-proof drinking bottles in their home cages which contained vehicle (water, 1% ethanol v/v). Each day at noon, the volume of liquid (in ml) that hamsters drank from the right and left bottles were recorded by an observer who was uninformed of the experimental hypothesis (AB). Bottles were refilled and carefully checked to ascertain that there was no leakage prior to replacement in the cage. The first 2 days of consumption were considered habituation and the subsequent 4 days were considered the experimental period after which hamsters were behaviorally tested (tasks described below).

Week 2: During the second week, one of the bottles contained vehicle and the other 3α-diol (800 μg/ml in 1% ethanol vehicle). The placement of the vehicle or 3α-diol containing bottles on the right or left side of the cages were counterbalanced to prevent any potential differences in side preference. As described above, the consumption from each of the bottles was monitored daily. The first 2 days of consumption were considered habituation and the subsequent 4 days were considered the experimental period after which hamsters were behaviorally tested.

Week 3: All hamsters had two bottles filled with vehicle for a washout period. Consumption was monitored daily but there was no behavioral testing.

Week 4: Hamsters were randomly assigned to the vehicle or 3α-diol condition. Hamsters in the vehicle condition were given two bottles filled with vehicle. Hamsters in the 3α-diol condition were administered two bottles, one filled with vehicle and one filled with 3α-diol. Consumption was monitored daily, the first 2 days were considered habituation and the subsequent 4 days were considered the experimental period, which were followed by behavioral testing and tissue collection immediately thereafter for later measurement of 3α-diol levels. NB: Consumption of 3α-diol did not appear to produce changes in body weight and/or general health of hamsters.

2.3. Behavioral testing

Hamsters were tested in the following battery of tasks, without a rest period between tasks, by an investigator that was blind to the experimental hypothesis (AB).

2.3.1. Open field

Hamsters were placed in the Digiscan activity monitor (39×39×30 cm), with 16 square grid floor, for a 5-min test period (Frye et al., 2004a,b). The number of beam breaks (as a measure of general motor activity) was mechanically recorded by the apparatus. In addition, the movement of hamsters in the open field was traced and recorded by an observer. The total and central square entries were recorded (Frye et al., 2004a,b).

2.3.2. Pawlick

Hamsters were placed on a hotplate that was 50 °C. The latency (maximum latency 30 sec) for hamsters to shake and lick their paws was recorded (Frye et al., 2000).

2.3.3. Resident intruder

In the resident–intruder task, a gonadally intact, weight- and age-matched conspecific was placed into the home cage of the experimental hamster for 3 min. The latency and/or number of offensive (attacks, bites) and defensive (submissive postures) aggressive behaviors made by the resident and intruder, respectively, were recorded (Frye et al., 2002).

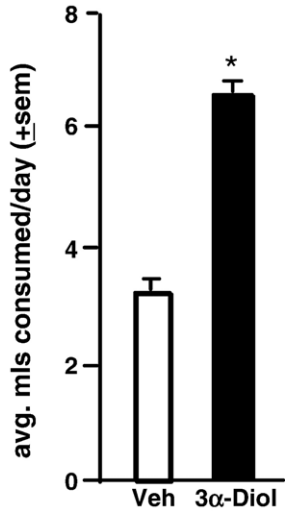

Fig. 1. The mean (±S.E.M.) volume (in ml) of vehicle (open bar) or 3α-diol (black bar) consumed per day. * indicates a significant difference from vehicle ($p<0.05$).

2.4. Tissue collection

Immediately after testing, hamsters were rapidly decapitated and trunk blood and whole brains were collected on dry ice. Following refrigerated centrifugation, serum and whole brains were stored at −70 °C for about 1 month until radioimmunoassay was performed to determine 3α-diol levels. At the time of measurement, the hippocampus, cortex, striatum and midbrain were rapidly dissected from whole brains that had been gently thawed on ice.

2.5. 3α-diol radioimmunoassay

3α-diol was measured with radioimmunoassay techniques previously described in detail (Edinger and Frye, 2004; Frye and Bayon, 1999; Frye and Edinger, 2004; Frye et al., 1996a,b). Briefly, 3α-diol was extracted from plasma and brain tissue (homogenized with a glass/Teflon homogenizer in distilled water) with diethyl ether and trace amounts of ^{3}H 3α-diol (purchased from New England Nuclear, Boston, MA). The antibody for 3α-diol (X-144, Dr. P.N. Rao, Southwest Foundation for Biomedical Research, San Antonio, TX) is highly specific to 3α-diol (Rao et al., 1977). The 1:20,000 dilution of this antibody binds ~ 96% of [^{3}H] 3α-diol (NET-806: specific activity=41.00 Ci/mmol). All standard curves were prepared in duplicate (range=50 pg–2000 pg). The standards were added to BSA assay buffer, followed by addition of the appropriate antibody and [^{3}H] steroid and incubated overnight at room temperature. Separation of bound and free was accomplished by the rapid addition of dextran-coated charcoal. Following incubation with charcoal, samples are centrifuged at 1200×*g*. The supernatant was pipetted into a glass scintillation vial with scintillation cocktail. Sample tube concentrations are calculated using the logit-log method of Rodbard and Hutt (1974), interpolation of the standards and correction for recovery. The intra- and inter-assay coefficients of variance were 0.09 and 0.10, respectively.

2.6. Statistical analyses

Paired *t*-tests were used to determine differences in daily consumption of vehicle or 3α-diol. Unpaired *t*-tests compared effects of vehicle or 3α-diol condition on behavior, plasma and central 3α-diol levels. The α level for the determination of statistical significance was $p \leq 0.05$.

3. Results

3.1. Consumption of vehicle

During weeks 1 and 3, when only vehicle was available, the quantity of vehicle consumed from the bottle on the right (3.1±0.6 ml) or left (4.5±0.8 ml) side of the cage did not differ.

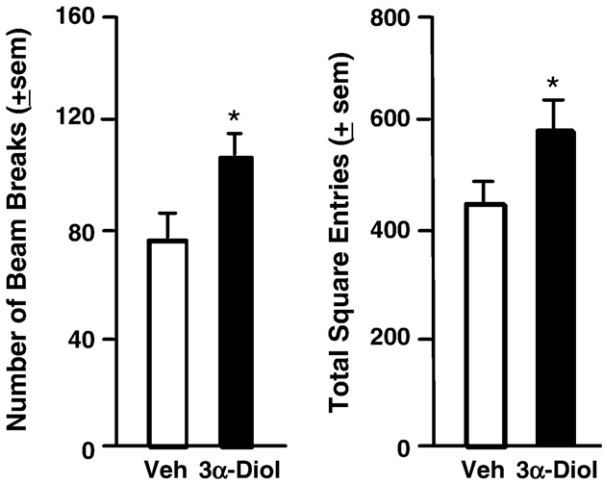

Fig. 2. The mean (±S.E.M.) number of beam breaks (left) in the activity chamber and total (right) squares entered in the open field task of hamsters with access to vehicle (open bars) or 3α-diol (black bars). * indicates a significant difference from vehicle ($p<0.05$).

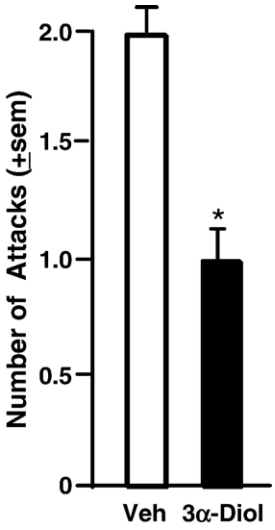

Fig. 3. The mean (±S.E.M.) number of attacks in the resident–intruder task of hamsters with access to vehicle (open bar) or 3α-diol (black bar). * indicates a significant difference from vehicle ($p<0.05$).

3.2. Consumption of 3α-diol

During weeks 2 and 4, when both 3α-diol and vehicle were available, significantly ($T(27)=3.63$, $p=0.001$) more 3α-diol than vehicle was consumed (Fig. 1). The preference for the 3α-diol solution over vehicle was 77%.

3.3. Effect of 3α-diol on activity, anxiety and analgesia

Hamsters that had access to 3α-diol were more active than hamsters that only had access to vehicle. Those with access to 3α-diol made significantly ($T(26)=2.37$, $p=0.03$) more beam breaks in the open field than did hamsters only exposed to vehicle (Fig. 2, top). Similarly, hamsters with access to 3α-diol made significantly more total ($T(26)=3.04$, $p=0.01$) entries in the open field than did hamsters only exposed to vehicle (Fig. 2, bottom).

There were no significant differences in the number of central entries for those with access to 3α-diol (12.6±1.8) versus vehicle (8.2±1.6). As well, the percentage of central entries to total entries did not differ for those with access to 3α-diol (11.4±1.4%) versus vehicle (10.3±1.4%).

Hamsters that had access to 3α-diol also had significantly ($T(26)=2.07$, $p=0.05$) longer latencies to lick their front paws (19.8±6.6 s) than did hamsters only exposed to vehicle (5.5±2.0 s).

3.4. Effect of 3α-diol on aggression

Access to 3α-diol did not alter latencies to attack (3α-diol − 110±21 s, vehicle −67±17 s) but was associated with significantly fewer attacks ($T(26)=1.97$, $p=0.05$) than hamsters that only had access to vehicle (Fig. 3). There was no difference in number of bites for hamsters with access to 3α-diol (1.0±0.5) or vehicle (1.6±0.4). Neither the latency to, nor number of, submissive postures were different for hamsters with access to 3α-diol (latency 2.1±0.4 s, number 4.6±0.4 s) or vehicle (latency 2.0±0.4 s, number 4.7±0.6 s).

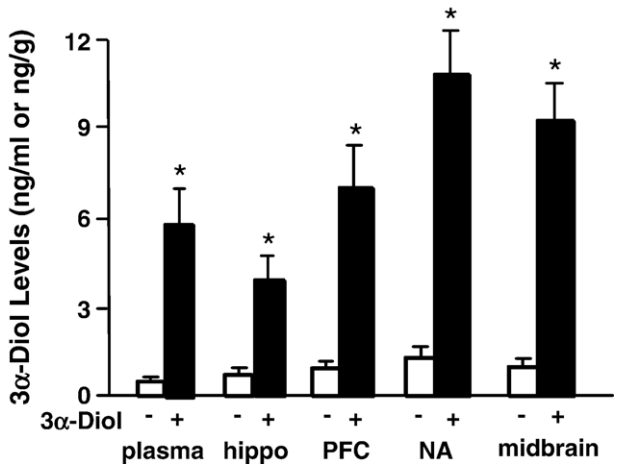

Fig. 4. The mean (±S.E.M.) plasma and central levels of 3α-diol produced by access to vehicle (open bar) or 3α-diol (black bars) * indicates a significant difference from vehicle ($p<0.05$).

3.5. Levels of 3α-diol produced

Access to 3α-diol produced higher concentrations of 3α-diol in plasma ($T(12)=5.77$, $p=0.001$), hippocampus ($T(12)=2.27$, $p=0.04$), prefrontal cortex ($T(12)=4.42$, $p=0.001$, striatum ($T(12)=3.61$, $p=0.001$) and midbrain ($T(12)=3.48$, $p=0.001$) than did access to vehicle alone (Fig. 4).

4. Discussion

The hypothesis that 3α-diol has reinforcing effects was supported. Hamsters consumed more 3α-diol than vehicle in the two-bottle choice test. Hamsters that self-administered 3α-diol demonstrated greater activity as indicated by a significant increase in the number of beam breaks in the activity monitor and a greater number of grid entries in the open-field, than vehicle-administered hamsters. Access to 3α-diol also produced analgesia: pawlick latencies were increased with access to 3α-diol versus vehicle alone. Hamsters that self-administered 3α-diol also showed less offensive aggression: they had longer latencies to, and made fewer, attacks than did hamsters with access to vehicle alone. Hamsters that self-administered 3α-diol had plasma, hippocampal, cortical, striatal and midbrain levels of 3α-diol that were significantly greater than that of hamsters that only had access to vehicle. Together, these data suggest that hamsters will self-administer 3α-diol in sufficient quantities to produce greater circulating and central levels of 3α-diol and increase activity, analgesia and decrease offensive aggression, than is seen among hamsters that only have access to vehicle.

These findings confirm and extend previous research that suggests that 3α-diol can have rewarding effects. For example, when systemic 3α-diol is administered to intact male rats immediately prior to exposure to the originally non-preferred side of the chamber in the CPP task, the preference for the originally non-preferred side is significantly increased on test day, over that produced by pairing with vehicle (Frye et al., 2001, 2002). Even more robust increases in CPP with 3α-diol are seen when 3α-diol is applied directly to the shell, but not the core of nucleus accumbens, prior to exposure to the originally non-preferred side of the chamber. Further, administration of 3α-diol systemically, or with implants or infusions to the dorsal hippocampus, of gonadectomized rats increases anti-anxiety behavior in the open field, elevated plus maze and enhances performance in the inhibitory avoidance paradigms (Edinger and Frye, 2004, 2005; Edinger et al., 2004; Frye et al., 2004a,b). As well, rats that are administered systemic, hypothalamic or hippocampal 3α-diol have longer tailflick and/or pawlick latencies than do control rats (Edinger and Frye, 2004, 2005; Frye et al., 1996b). That hamsters will preferentially consume 3α-diol to produce levels which are comparable to those observed in rats in the above studies, extend these previous findings to demonstrate that in this self-administration paradigm, 3α-diol can also have rewarding effects and increase 3α-diol in brain areas important for these functional effects.

These findings that hamsters will self-administer 3α-diol also confirm and extend prior work that demonstrates that hamsters will self-administer T, its metabolites and other AAS. When hamsters in an operant chamber can use nose-poke to gain access

to i.c.v. infusions of T for 4-h a day, self-administration of T readily occurs (DiMeo and Wood, 2004; Triemstra and Wood, 2004; Wood et al., 2004). In this model, some hamsters consume so much T that effects similar to opiate intoxication, central nervous system depression and death occur (Peters and Wood, 2005; Wood, 2004). In this approach, male hamsters also self-administer T's aromatized and 5α-reduced metabolites, estradiol and dihydrotestosterone (the precursor to 3α-diol) (DiMeo and Wood, 2006). Other AAS including drostanolone>nandrolone>oxymetholone>stanozolol are also self-administered by hamsters in this paradigm (Ballard and Wood, 2005). Thus, the present results that hamsters self-administer oral 3α-diol suggest that, like other AAS, 3α-diol can have rewarding effects.

In addition to rewarding effects, androgens may have other effects and/or mechanisms that are similar to other drugs of abuse. First, in the present experiment, 3α-diol had clear-cut stimulant effects. 3α-diol, like amphetamine and cocaine, all stimulate locomotor activity (Sahakian et al., 1975), induce CPP (Bardo et al., 1995) and are orally self-administered (Ufer et al., 1999). Amphetamine and cocaine (Carroll and Lac, 1997; Koob et al., 1994) are self-administered intravenously, which enables different concentration-dependent effects to be examined. Given that dosage and a number of conditioning variables that can influence drug effects are not as readily controlled in oral-self-administration studies (Stewart et al., 1984; Todtenkopf and Carlezon, 2006), it will be important to examine whether 3α-diol is intravenously self-administered. Second, there are concentration-dependent effects of androgens on behaviors, in addition to self-administration. T and other AAS, as well as alcohol consumption that increases T levels, are known to have aggression-enhancing effects in hamsters during adulthood and adolescence (Delville et al., 1996; Ferris et al., 1998; Grimes and Melloni, 2005; Harrison et al., 2000; Melloni et al., 1997; Melloni and Ferris, 1996). We have previously demonstrated that 3α-diol administration (1 mg to adult mice) can increase aggression in the resident intruder paradigm (Frye et al., 2002). Here 3α-diol availability had anti-aggression effects. As such, the oral availability of a single concentration of 3α-diol may well have limited effects that may have been observed otherwise with a broader range of dosages and/or availabilities. Third, androgens can produce dependence and/or tolerance. Effects of T and/or other AAS were attenuated in rats repeatedly administered them unless the dosages were increased (Bonson et al., 1994; DiMeo and Wood, 2004; Kochakian, 1950; Peters and Wood, 2005). Up to 18% of AAS users report tolerance (Brower et al., 1991). Withdrawal symptoms are also reported in rats given daily injections of T for 10, but not 3, weeks. For 2 weeks after T cessation, rats had tremors, ataxic effects and ptosis (Foltin, 1992). Fourth, the mesolimbic dopamine system also may be a common substrate for androgens and drugs of abuse. Testosterone appears to act through the mesolimbic dopamine system, which is involved in drugs of abuse. For example, T administration to the nucleus accumbens can produce CPP (Alexander et al., 1994; Frye et al., 2001, 2002; Packard et al., 1997, 1998; Rosellini et al., 2001) and these effects are blocked by administration of dopamine receptor antagonists or lesions to the nucleus accumbens with 6OHDA (Frye and Rhodes, 2006; Packard et al., 1998).

In addition to commonalities, there also seem to be differences in androgens' effects compared to that of other drugs of abuse. Rewarding effects of androgens may be less comparable to that of cocaine or heroin, but more analogous to that of mild reinforcers, such as benzodiazepines. Notably, 3α-diol in the concentrations produced here does not bind with high affinity for intracellular androgen receptors (Cunningham et al., 1979; Saartok et al., 1984; Verhoeven et al., 1975). Indeed, T self-administration did not increase androgen receptor immunoreactivity (DiMeo and Wood, 2006, in press). 3α-diol can bind to ERβ (Pak et al., 2005) and 3α-diol's anti-anxiety effects can be attenuated by blocking ERβ (Edinger and Frye, in press). To investigate further whether actions at ERβ are required for 3α-diol's rewarding effects, we are examining the ability of 3α-diol to have anti-anxiety effects and induce CPP in ERβ knockout versus wildtype mice. Another possible mechanism that may underlie the effects observed herein for 3α-diol are its agonist-like actions at GBRs (Frye et al., 1996a; Gee, 1988). Given that androgens and/or AAS differ in their abilities to alter GBR functioning and reinforcing effects, whether these differences are due to actions at GBRs is of interest. We are presently investigating whether formation of 3α-diol and/or actions of androgens at GBRs are required for its effects in the self-administration paradigm.

In summary, the present results demonstrate that 3α-diol is readily orally self-administered in male hamsters. Self-administration of 3α-diol increase locomotion and analgesia and decreases aggression. Furthermore, 3α-diol administration increases levels of 3α-diol in plasma and brain regions important for these behaviors. Thus, 3α-diol can have reinforcing, analgesic and anti-aggressive effects.

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References

Alexander G, Packard M, Hines M. Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. Behav Neurosci 1994;108:424–8.

Altschule MD, Tilletson KJ. The use of testosterone in the treatment of depression. N Engl J Med 1948;239:1036–8.

Arnedo MT, Salvador A, Martinez-Sanchez S, Gonzalez-Bono E. Rewarding properties of testosterone in intact male mice: a pilot study. Pharmacol Biochem Behav 2000;65:327–32.

Arvary D, Pope HG. Anabolic–androgenic steroids as a gateway to opioid dependence. N Engl J Med 2000;342:1532.

Ballard CL, Wood RI. Intracerebroventricular self-administration of commonly abused anabolic–androgenic steroids in male hamsters (*Mesocricetus auratus*): nandrolone, drostanolone, oxymetholone, and stanozolol. Behav Neurosci 2005;119:752–8.

Bahrke M, Yesalis C, Wright J. Psychological and behavioral effects of endogenous testosterone levels and anabolic–androgenic steroids among males—a review. Sports Med 1990;10:303–37.

Bahrke M, Yesalis C, Wright J. Psychological and behavioral effects of endogenous testosterone and anabolic–androgenic steroids—an update. Sports Med 1996a;22(6):367–90.

Bahrke M, Yesalis C, Brower. Anabolic–androgenic steroid abuse and performance-enhancing drugs among adolescents. Child Adolesc Psychiatr Clin N Am 1996b;1:821–38.

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;19 (1):39–51.

Bonson KR, Garrick NA, Murphy DL. Evidence for a withdrawal syndrome following chronic administration of an anabolic steroid to rats. Soc Neurosci Abstr 1994;20:1527.

Brower KJ, Blow FC, Young JP, Hill EM. Symptoms and correlates of anabolic–androgenic steroid dependence. Br J Addict 1991;86:759–68.

Buckley WE, Yesalis CE, Friedl KE, Anderson WA, Streit AL, Wright JE. Estimated prevalence of anabolic steroid use among male high school seniors. JAMA 1988;260:3441–5.

Caggiula AR. Analysis of copulation-reward properties of posterior hypothalamic stimulation in male rats. J Comp Physiol Psychol 1970;70:399–412.

Caldarone B, Stock H, Abrahamsen G, Boechler M, Svare B, Rosellini R. Nonassociative processes and place preferences conditioned by testosterone. The Psychol Rec 1996;46:373–90.

Campbell HJ. The effect of steroid hormones on self-stimulation, central and peripheral. Steridologia 1970;1:8–24.

Carroll ME, Lac ST. Acquisition of i.v. amphetamine and cocaine self-administration in rats as a function of dose. Psychopharmacology 1997;129(3):206–14.

Cunningham GR, Tindall DJ, Means AR. Differences in steroid specificity for rat androgen binding protein and the cytoplasmic receptor. Steroids 1979;33:261–76.

DeBeun R, Jansen E, Slangen JL, Van de Poll NE. Testosterone as appetitive and discriminative stimulus in rats: sex- and dose-dependent effects. Physiol Behav 1992;52:629–34.

Delville Y, Mansour KM, Ferris CF. Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. Physiol Behav 1996;60:25–9.

DiMeo AN, Wood RI. Circulating androgens enhance sensitivity to testosterone self-administration in male hamsters. Pharmacol Biochem Behav 2004;79:383–9.

DiMeo AN, Wood RI. ICV testosterone induces fos in male Syrian hamster brain. Psychoneuroendocrinology 2006;31:237–49.

DiMeo AN, Wood RI. Self-administration of estrogen and dihydrotestosterone in male hamsters. Horm Behav 2006;49:519–26.

Edinger KL, Frye CA. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5α-reduced metabolites in the hippocampus. Behav Neurosci 2004;118:1352–64.

Edinger KL, Frye CA. Testosterone's anti-anxiety and analgesic effects may be due in part to actions of its 5α-reduced metabolites in the hippocampus. Psychoneuroendocrinology 2005;30:418–30.

Edinger, KL, Frye, CA. Androgens' effects to enhance learning may be mediated through actions at estrogen receptor β; in press.

Edinger KL, Lee B, Frye CA. Mnemonic effects of testosterone and its 5α-reduced metabolites in the conditioned fear and inhibitory avoidance tasks. Pharmacol Biochem Behav 2004;78:559–68.

Faigenbaum AD, Zaichkowsky LD, Gardner DE, Micheli LJ. Anabolic steroid use by male and female middle school students. Pediatrics 1998;101:398–407.

Ferris CF, Shtiegman K, King JA. Voluntary ethanol consumption in male adolescent hamsters increases testosterone and aggression. Physiol Behav 1998;63:739–44.

Frye CA, Bayon LE. Mating stimuli influence endogenous variations in the neurosteroids 3α,5α-THP and 3α-diol. Neuroendocrinology 1999;11:839–47.

Frye CA, Edinger KL. Testosterone's metabolism in the hippocampus may mediate its anti-anxiety effects in male rats. Pharmacol Biochem Behav 2004;78:473–81.

Frye, CA, Rhodes, ME. Actions in the nucleus accumbens underlie some of androgens' rewarding effects Pharmacol Biochem Behav (submitted for publication).

Frye CA, Seliga AM. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. Cogn Affect Behav Neurosci 2001;1:371–81.

Frye CA, Duncan JE, Basham M, Erskine MS. Behavioral effects of 3α-androstanediol: II. Hypothalamic and preoptic area actions via a GABAergic mechanism. Behav Brain Res 1996a;79:119–30.

Frye CA, Van Keuren KR, Rao PN, Erskine MS. Analgesic effects of the neurosteroid 3α-androstanediol. Brain Res 1996b;709:1–9.

Frye CA, Park D, Tanaka M, Rosellini R, Svare B. The testosterone metabolite and neurosteroid 3α-androstanediol may mediate the effects of testosterone on conditioned place preference. Psychoneuroendocrinology 2001;26 (7):731–50.

Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3α,5α-THP. Pharmacol Biochem Behav 2000;67:587–96.

Frye CA, Rhodes ME, Walf A, Harney J. Testosterone enhances aggression of wild-type mice but not those deficient in type I 5alpha-reductase. Brain Res 2002;948:165–70.

Frye CA, Edinger KL, Seliga AM, Wawrzycki JM. 5α-reduced androgens may have actions in the hippocampus to enhance cognitive performance of male rats. Psychoneuroendocrinology 2004a;29:1019–27.

Frye CA, Walf AA, Rhodes ME, Harney JP. Progesterone enhances motor, anxiolytic, analgesic, and antidepressive behavior of wild-type mice, but not those deficient in type 1 5α-reductase. Brain Res 2004b;1004:116–24.

Foltin RW. The importance of drug self-administration studies in the analysis of abuse liability: an analysis of caffeine, nicotine, anabolic steroids, and designer drugs. Am J Addict 1992;1:139–49.

Gee KW. Steroid modulation of the GABA/benzodiazepine receptor-linked chloride ionophore. Mol Neurobiol 1988;2:291–317.

Grimes JM, Melloni Jr RH. Serotonin-1B receptor activity and expression modulate the aggression-stimulating effects of adolescent anabolic steroid exposure in hamsters. Behav Neurosci 2005;119:1184–94.

Harrison RJ, Connor DF, Nowak C, Nash K, Melloni Jr RH. Chronic anabolic–androgenic steroid treatment during adolescence increases anterior hypothalamic vasopressin and aggression in intact hamsters. Psychoneuroendocrinology 2000;25:317–38.

Haupt HA, Rovere GD. Anabolic steroids: a review of the literature. Am J Sports Med 1984;12:469–84.

Johnson LR, Wood RI. Oral testosterone self-administration in male hamsters. Neuroendocrinology 2001;73:285–92.

Kann L, Warren C, Harris W, Collins JL, Williams BI, Ross JG, et al. Youth risk behavior surveillance: US. J School Health 1996;10:365–77.

Katz DL, Pope Jr HG. Anabolic–androgenic steroid-induced mental status changes. NIDA Res Monogr 1990;102:215–23.

King BE, Packard MG, Alexander GM. Affective properties of intra-medial preoptic area injections of testosterone in male rats. Neurosci Lett 1999;269:149–52.

Kochakian CD. Comparison of protein anabolic property of various androgens in the castrated rat. Am J Physiol 1950;160:53–61.

Koob GF, Caine B, Markou A, Pulvirenti L, Weiss F. Role for the mesocortical dopamine system in the motivating effects of cocaine. NIDA Res Monogr 1994;145:1–18.

Kornetsky C, Esposito RU. Reward and detection thresholds for brain stimulation: dissociative effects of cocaine. Brain Res 1981;209:496–500.

Melloni Jr RH, Ferris CF. Adolescent anabolic steroid use and aggressive behavior in golden hamsters. Ann N Y Acad Sci 1996;794:372–5.

Miller KE, Hoffman JH, Barnes GM, Sabo D, Melnick MJ, Farrell MP. Adolescent anabolic steroid use, gender, physical activity, and other problem behaviors. Subs. Use Misuse 2005;40:1637–57.

Olds J. Effects of hunger and male sex hormone of self-stimulation of the brain. J Comp Phys Psychol 1958;51:320–4.

Packard M, Cornell A, Alexander G. Rewarding affective properties of intra-nucleus accumbens injections of testosterone. Behav Neurosci 1997;111:219–24.

Packard M, Schroeder J, Alexander G. Expression of testosterone conditioned place preference is blocked by peripheral or intra-accumbens injection of α-flupenthixol. Horm Behav 1998;34:39–47.

Pak TR, Chung WC, Lund TD, Hinds LR, Clay CM, Handa RJ. The androgen metabolite, 5alpha-androstane-3beta, 17beta-diol, is a potent modulator of estrogen receptor-beta1-mediated gene transcription in neuronal cells. Endocrinology 2005;146(1):147–55.

Peters KD, Wood RI. Androgen dependence in hamsters: overdose, tolerance, and potential opioidergic mechanisms. Neuroscience 2005;130:971–81.

Pope Jr HG, Cohane GH, Kanayama G, Siegel AJ, Hudson JI. Testosterone gel supplementation for men with refractory depression: a randomized, placebo-controlled trial. Am J Psychiatry 2003;160:105–11.

Rao PN, Khan AH, Moore Jr PH. Synthesis of new steroid haptens for radioimmunoassay: Part III. 15β-Carboxyetylmercaptosteroid-bovine serum albumin conjugates. Specific antisera for radioimmunoassay of 5α-dihydrotestosterone, 5α-androstane-3β,17β-diol and 5α-androstane-3α,17β-diol. Steroids 1977;29:171–84.

Rosellini RA, Svare BB, Rhodes ME, Frye CA. The testosterone metabolite and neurosteroid 3α-androstanediol may mediate the effects of testosterone on conditioned place preference. Brain Res Rev 2001;37:162–71.

Saartok T, Dahlberg E, Gustafsson JÅ. Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. Endocrinology 1984;114:2100–6.

Sahakian BJ, Robbins TW, Morgan MJ, Iversen SD. The effects of psychomotor stimulants on stereotypy and locomotor activity in socially-deprived and control rats. Brain Res 1975;84(2):195–205.

Schroeder JP, Packard MG. Role of dopamine receptor subtypes in the acquisition of a testosterone conditioned place preference in rats. Neurosci Lett 2000;282:17–20.

Stewart J, de Wit H, Eikelboom R. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 1984;91(2):251–368.

Taylor WN. Synthetic anabolic–androgenic steroids: a plea for controlled substance status. The Physician and Sports Med 1987;15:140–50.

Todtenkopf MS, Carlezon Jr WA. Contribution of drug doses and conditioning periods to psychomotor stimulant sensitization. Psychopharmacology (Berl) 2006;185(4):451–8.

Triemstra JL, Wood RI. Testosterone self-administration in female hamsters. Behav Brain Res 2004;154:221–9.

Ufer M, Dadmarz M, Vogel WH. Voluntary consumption of amphetamine, cocaine, ethanol and morphine by rats as influenced by a preceding period of forced drug intake and clozapine. Pharmacology 1999;58(6):285–91.

Verhoeven G, Heyns W, De Moor P. Ammonium sulfate precipitation as a tool for the study of androgen receptor proteins in rat prostate and mouse kidney. Steroids 1975;26:149–67.

Wood RI. Oral testosterone self-administration in male hamsters: dose–response, voluntary exercise, and individual differences. Horm Behav 2002;41:247–58.

Wood RI. Reinforcing aspects of androgens. Physiol Behav 2004;83:279–89.

Wood RI, Johnson LR, Chu L, Schad C, Self DW. Testosterone reinforcement: intravenous and intracerebroventricular self-administration in male rats and hamsters. Psychopharmacology 2004;171:298–305.

Yesalis C, Cowart VS. The steroid game. Champaign. Human Kinetics 1998.

Yesalis CE, Vicary JR, Buckley WE, Streit AL, Katz DL, Wright JE. Indications of psychological dependence among anabolic–androgenic steroid abusers. NIDA Res Monogr 1990;102:196–214.