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Mnemonic effects of testosterone and its 5α-reduced metabolites in the conditioned fear and inhibitory avoidance tasks

Kassandra L. Edinger[a,b], Bomi Lee[a,b], Cheryl A. Frye[a,b,c,*]

[a]Department of Psychology, The University at Albany-SUNY, Social Science 220, 1400 Washington Avenue, Albany, NY 12222, USA
[b]Department of Biological Sciences, The University at Albany-SUNY, 1400 Washington Avenue, Albany, NY 12222, USA
[c]The Center for Neuroscience Research, The University at Albany-SUNY, 1400 Washington Avenue, Albany, NY 12222, USA

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Abstract

Experiments were conducted to examine whether performance in hippocampally mediated learning tasks is influenced by testosterone (T) and/or its 5α-reduced metabolites, dihydrotestosterone (DHT) and 3α-androstanediol (3α-diol). Performance in the conditioned fear and inhibitory avoidance tasks were examined in intact and gonadectomized (GDX), androgen-replaced rats. In Experiment 1, the behavior of intact and GDX rats in the conditioned fear paradigm were compared. GDX rats spent more time freezing, an index of increased learning, in the context, hippocampally-mediated task, but not in the cued, amygdala-mediated task. In Experiment 2, GDX rats were administered T, DHT, 3α-diol, estrogen (E_2), or vehicle 1 mg/kg sc after training in the conditioned fear paradigm. T-, 3α-diol-, or E_2-, compared with vehicle-administered rats, spent significantly more time freezing in the contextual, but not the cued, condition. In Experiment 3, intact compared with GDX rats had significantly longer crossover latencies, indicating better performance, in the inhibitory avoidance task. In Experiment 4, T, DHT, 3α-diol, or vehicle 1 mg/kg sc was administered to GDX rats immediately following training in the inhibitory avoidance task. Rats administered T, DHT, or 3α-diol had significantly longer crossover latencies compared with vehicle controls. In Experiment 5, androgen levels in the hippocampus were elevated 1 h following administration, when androgen exposure is essential for consolidation. These data indicate that androgens effects to enhance learning may be mediated in part by actions of 5α-reduced metabolites in the hippocampus.

Keywords: Androgens; Nongenomic; GABA; Androgen receptors; Learning

1. Introduction

Androgens may have both organizational and activational effects on learning. There are modest sex differences in cognitive performance of people and animals. Men typically perform better than women do in measures of spatial cognition (Aster et al., 1998; Gallagher et al., 2000; Linn and Petersen, 1985; McGee, 1979). Male, compared with female, rodents show better spatial performance in the radial arm and Morris Water Mazes, both 1 week and 21 days following birth (Roof, 1993). In people, testosterone (T) levels are positively correlated with greater spatial and mathematical abilities (Gouchie and Kimura, 1991; Yaffe et al., 2002). Similarly, adult male deer mice that have increased androgen levels due to the breeding season show better performance in the Morris Water Maze than do nonbreeding male or female controls (Galea et al., 1996). These data suggest that androgens may have organizational and activational effects to mediate cognitive performance in people and animals.

Androgens may influence the cognitive performance of aging men. As men become older, androgen levels decrease, which may be associated with the decline in cognitive function that can occur with aging (Juul and Skakkebaek, 2002; Lund et al., 1999). Longitudinal studies of aging men have found age-dependent decreases in free-T levels. Cognitive tests administered over this period indicate that there may be a T-dependent decline in cognitive performance. T administration to aging men enhances spatial performance (Janowsky et al., 1994). Men with andropause, or androgen decline in the aging male (ADAM), also experience deficits

* Corresponding author. Department of Psychology, The University at Albany-SUNY, Social Science 220, 1400 Washington Avenue, Albany, NY 12222, USA. Tel.: +1-518-442-4836; fax: +1-518-442-4867.
E-mail address: cafrye@cnsunix.albany.edu (C.A. Frye).

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in cognitive performance (Lund et al., 1999; Sternbach, 1998; Tan, 2001), which can be ameliorated by T-replacement therapy (Juul and Skakkebaek, 2002; Lund et al., 1999). Alzheimer's disease (AD), which is associated with decreased cognitive function, may also be mediated by androgens. T replacement to men with mild cognitive impairment (MCI), a precursor to AD, mildly improves cognitive function and may delay the onset of AD (Tan et al., 2003). Furthermore, hypogonadal men have low physiological T levels and poorer cognitive performance than do eugonadal men: T replacement can reinstate androgen levels and spatial performance of hypogonadal men (Alexander et al., 2001; Howell and Shalet, 2001). These studies suggest that androgens may influence hippocampally-mediated cognitive performance of men.

Findings from animal models also illustrate androgens' cognitive-enhancing effects. Androgens have a dose-dependent effect to improve performance in the Morris Water Maze (Isgor and Sengelaub, 2003). Castration, or gonadectomy (GDX), of male rats decreases performance in the inhibitory avoidance (Frye and Seliga, 2001), object recognition (Ceccarelli et al., 2001), or Y-Maze tasks (Kritzer et al., 2001) compared with intact controls, and systemic T replacement can reinstate performance. These data suggest that cognitive deficits produced by GDX are due to androgen deficiency.

Metabolites of T may mediate some of its effects on learning and memory. T is metabolized by the 5α-reductase enzyme to dihydrotestosterone (DHT), a nonaromatizable androgen. T or DHT replacement to GDX rats enhances cognitive performance (Frye and Seliga, 2001; Frye et al., in press). However, DHT can be further metabolized by 3α-hydroxysteroid dehydrogenase (3α-HSD) to 5α-androstane, 17β-diol (3α-diol), which can also influence cognitive performance. For example, 3α-androstanediol (3α-diol) is more effective than is T or DHT administration to male rats to improve conditioned place preference (Frye et al., 2002; Rosellini et al., 2001). As well, 3α-diol administration improves performance in spatial tasks (Frye and Lacey, 2001) and limiting the formation of 3α-diol with a 3α-HSD inhibitor reduces performance in the inhibitory avoidance task of intact or GDX, DHT-replaced rats to that of GDX controls (Frye et al., in press).

It is also possible that androgen effects to enhance cognitive performance involve T's aromatization to estrogen (E_2; Ivanova and Beyer, 2000; Jacobs et al., 1999). E_2 can enhance the cognitive performance of ovariectomized rats in the inhibitory avoidance task or of males in the water maze (Frye and Rhodes, 2002; Packard et al., 1996; Rhodes and Frye, 2004a). Although the administration of the nonaromatizable androgen, DHT, to young male rats enhances inhibitory avoidance, it does not seem to improve performance in the radial water maze of 22-month-old male rats (Bimonte-Nelson et al., 2003; Frye et al., in press). These data indicate that T's metabolites may be important in mediating cognition.

Androgens may have actions in the hippocampus to improve cognitive performance. Performance in the inhibitory avoidance, water maze, and Y-maze tasks are dependent upon the integrity of the hippocampus (Bannerman et al., 2002; Martinez et al., 2002) and are influenced by androgen milieu (Frye and Seliga, 2001; Isgor and Sengelaub, 2003; Kritzer et al., 2001). The hippocampus is also a target of androgens. T administration to GDX rats increases hippocampal neuronal excitability (Smith et al., 2002). T administration to male or female rats increases dendritic spine density in the CA3 and CA1 regions of the dorsal hippocampus and improves spatial navigation in the water maze (Isgor and Sengelaub, 2003; Leranth et al., 2003). T and DHT are readily metabolized in the hippocampus to 3α-diol by the 5α-reductase and 3α-HSD enzymes localized there (Jacobs et al., 1999; Li et al., 1997; MacLusky et al., 1994; Pelletier et al., 1994). Interestingly, 3α-diol is more effective than its prohormones, T and DHT, to prevent adrenalectomy-induced apoptosis in the dentate and associated deficits in passive avoidance behavior (Frye and McCormick, 2000a,b). These data provide evidence that androgens effects to enhance learning may be occurring through metabolism to 3α-diol in the hippocampus.

To investigate the capacity of T and its 5α-reduced metabolites to mediate learning in hippocampally-mediated tasks, the following experiments were conducted. First, we hypothesized that if androgens' actions in the hippocampus mediate cognitive performance, then, intact rats would show better performance in the contextual conditioned fear and the inhibitory avoidance tasks. Second, we hypothesized that if T's 5α-reduced metabolites are important for androgens' effects to enhance learning, then, posttraining administration of T, DHT, or 3α-diol will similarly enhance learning in the conditioned fear and inhibitory avoidance tasks. Third, we hypothesized that if T's actions are independent of E_2, then, the administration of E_2 would not enhance performance, and T would not significantly increase plasma and hippocampal E_2 levels.

2. Methods

These methods were preapproved by the Institutional Animal Care and Use Committee at the University at Albany-SUNY.

2.1. Animals and housing

Male Long Evans rats ($N = 198$), approximately 55 days of age, were obtained from the colony at the University at Albany (original stock from Taconic Farms, Germantown, NY). Rats were group housed in polycarbonate cages (45 × 24 × 21 cm), in a temperature- (21 ± 1 °C) and humidity- (45–55%) controlled room that was maintained on a 12:12 reversed light cycle (lights off at 8:00 a.m.). Rats had

unrestricted access to Purina Rat Chow and tap water in their home cages.

2.2. Surgery

Rats were anesthetized with Rompun (12 mg/kg) and Ketaset (80 mg/kg) and were randomly assigned to sham surgery (intact) or GDX, 4 to 6 weeks prior to behavioral testing. We have previously demonstrated that within 4–6 weeks after GDX, endogenous androgen levels of adult male rats reach nadir (Frye et al., in press).

2.3. Hormone replacement

Rats in Experiment 2 ($n = 16$/group) received 1 mg/kg dosages of E_2 (17β-estradiol), T, DHT, 3α-diol, or vehicle suspended in oil immediately following training in the conditioned fear task. Rats in Experiment 4 ($n = 15$/group) received 1 mg/kg dosages of T, DHT, or 3α-diol immediately following training in the inhibitory avoidance task. We have previously found that these regimen produce circulating and central estrogen and/or androgen levels in a high, but physiological, range (Edinger and Frye, 2004; Frye, 2000; Rosellini et al., 2001). To further address concentrations produced by these regimen, plasma and hippocampal androgen levels were assessed in Experiment 5.

2.4. Behavioral testing

2.4.1. Conditioned fear

Rats were tested in the conditioned fear paradigm, according to previously published methods (Kim et al., 1993; Kjelstrup et al., 2002). On training day, the rats were placed in the apparatus for a habituation period of 4 min. Following habituation, a tone was sounded, followed by the administration of an electric shock (2-s duration, 0.5 mA). After 1 min, the tone and shock pairing was readministered until three training trials were received. Five days later, rats were tested in either the contextual or the cued condition. In the contextual condition, which is mediated by the hippocampus (Kim et al., 1993; Sanders et al., 2002), rats were placed in the original chamber without the tone and were habituated for 4 min. The freezing behavior, indicative of an association between the environment and the aversive stimuli, were observed for eight 1-min intervals. In the cued learning condition, which is mediated by the amygdala (Kim et al., 1993), a black insert was placed in the chamber, along with almond extract. After a 4-min habituation period, the tone was sounded, and freezing behavior was observed for eight 1-min trials following the tone.

2.4.2. Inhibitory avoidance

Rats were tested in the inhibitory avoidance task, which is used to assess spatial memory (Frye and McCormick, 2000b; Frye et al., in press). Rats were placed in a two-compartment (24 × 18 × 11 cm each) stainless steel box separated by a guillotine door. One chamber was white and brightly lit, while the other was painted black and covered

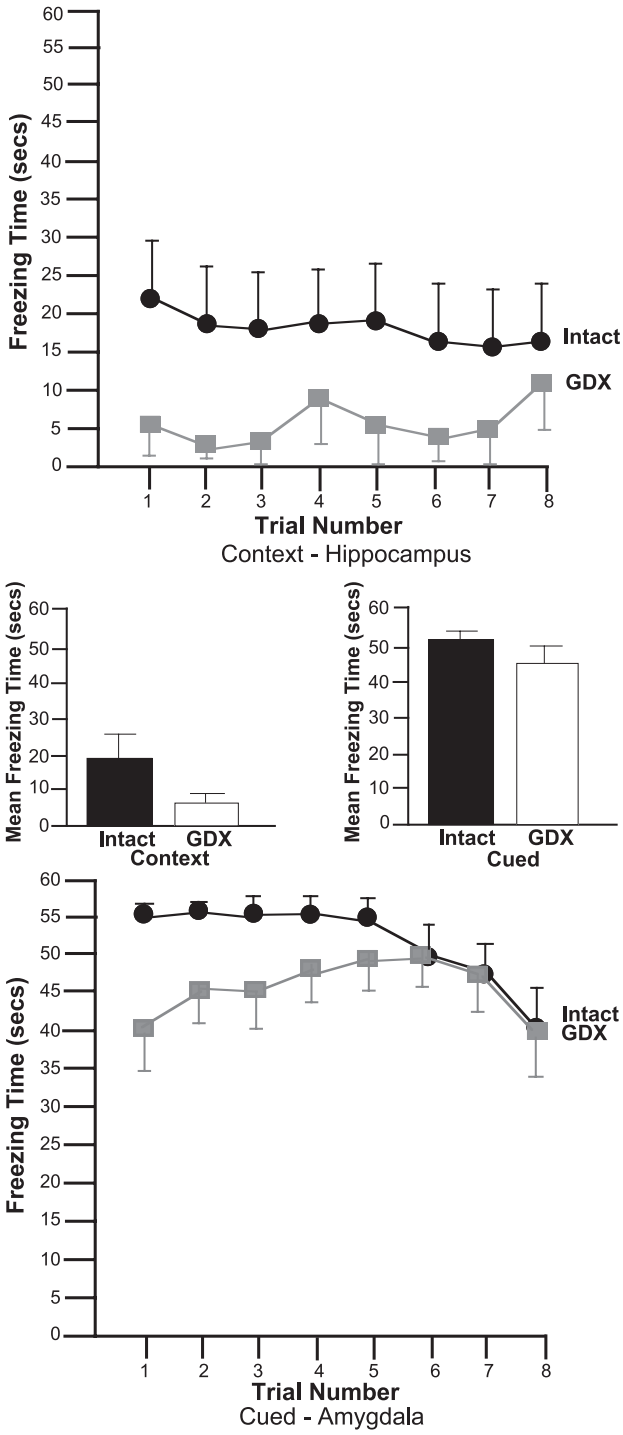


Fig. 1. Upper panel represents the mean duration of freezing (s ± S.E.M.) of intact (black circles) and GDX (gray squares) rats for each trial in the contextual portion of the conditioned fear task. Middle panels depicts the mean freezing time across all trials of intact (black) vs. GDX (white) rats in the contextual (left) and cued (right) tasks. Bottom panel represents the mean duration of freezing (s ± S.E.M.) of intact (black circles) and GDX (gray squares) rats for each trial in the cued trials of the conditioned fear task.

to block all light. On the first day, rats were placed in the white chamber and were allowed to explore for 2 min. Following the habituation of all animals, the first rat was again placed in the white chamber for 1 min. The door was lifted, and the crossover latency was recorded (maximum latency 20 min). The door was closed behind them, and a shock was administered (0.25 mA, 2-s duration). Twenty-four hours later, the rats were placed in the white chamber for 1 min. The door was lifted, and latency to move to the dark side was recorded, with a maximum latency of 5 min.

2.5. Tissue collection

A week following testing, a subset of the animals ($n = 7$/group) was readministered their assigned hormone condition, 1 mg/kg sc T, DHT, 3α-diol, E_2, or vehicle. Trunk blood and whole brains were collected after 1 h. Delay of exposure beyond the first hour after training obviates mnemonic effects (McGaugh, 1973, 1989; McGaugh and Petrinovich, 1966); thus, the hormone concentrations that were produced at this critical time for consolidation were of interest. Blood samples were centrifuged, and the hippocampus was dissected from whole brains on ice and all tissue samples were stored at −70 °C until radioimmunoassay.

2.6. Radioimmunoassay

2.6.1. Extraction

Androgens were extracted from plasma with diethyl ether and trace amounts of 3H ligand (purchased from New England Nuclear, Boston, MA). Brain tissue was homogenized with a glass/Teflon homogenizer in distilled water. Androgens were extracted from the homogenate with diethyl ether. Ether was evaporated, and the reconstituted extracts were separated using Celite column chromatography. Solvents of increasing polarity were used to elute the androgens: DHT (5% ethyl acetate/TMP), T and 3α-diol (15% ethyl acetate/TMP), E_2 (40% ethyl acetate/TMP). Fractions were dried in a Savant and then reconstituted in 0.1 M phosphate assay buffer (pH 7.4).

2.6.2. Assays

Plasma and hippocampal concentrations of T, DHT, 3α-diol, E_2, and corticosterone were measured according to previously published methods (Frye and Bayon, 1999; Frye et al., 1996a,c). The T antibody (T3-125; Endocrine Sciences, Calabasas Hills, CA) is moderately specific to T: There is modest cross reactivity with DHT and negligible binding to other androgens. The 1:20,000 dilution of this antibody binds between 60% and 65% of [^{3}H] T (NET-387: specific activity = 51.0 ci/mmol). The DHT antibody (DT3-351; Endocrine Sciences) is moderately specific to DHT: There is some cross reactivity with T and negligible binding to other androgens. The 1:10,000 dilution of this antibody binds between 60% and 65% of [^{3}H] DHT (NET-302: specific activity = 43.5 ci/mmol). 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 approximately 96% of [^{3}H] 3α-diol (NET-806: specific activity = 41.00 ci/mmol). The antibody for E_2 (Dr. Niswender, #244, Colorado State University, Fort Collins, CO) is highly specific to E_2 (Hotchkiss et al., 1971). The 1:30,000 dilution of this antibody binds approximately 90% of [^{3}H] E_2 (NET-317, 51.3 ci/mmol). The antibody for corticosterone (Endocrine Sciences: #B3-163) is highly specific, and in a 1:20,000 dilution, binds approximately 98% of [^{3}H] corticosterone (NET 182: specific activity = 48.2 ci/mmol ; New England Nuclear) with negligible cross reactivity for other steroids.

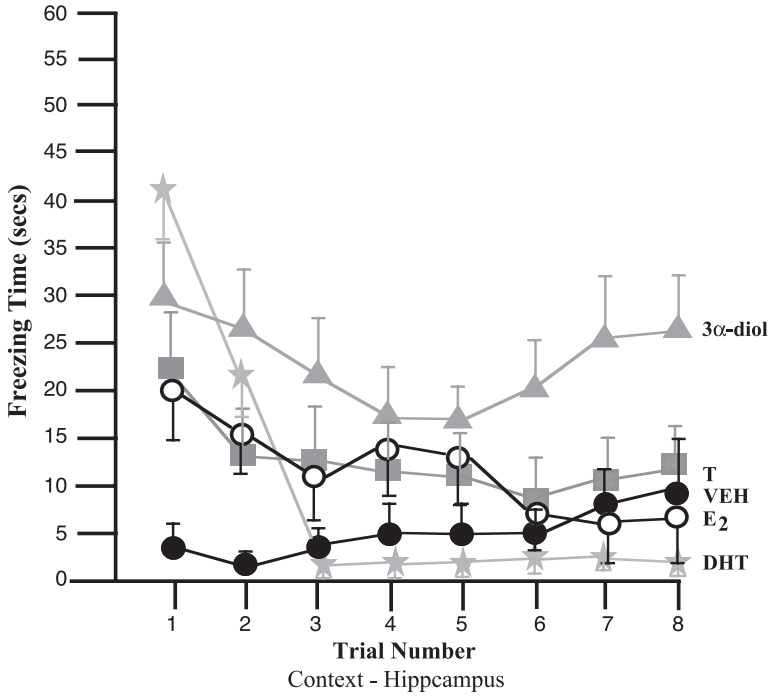
All standard curves are prepared in duplicate (range = 50–2000 pg). The standards are added to BSA assay buffer, followed by addition of the appropriate antibody and [^{3}H] steroid. T and DHT assays are incubated overnight at 4 °C; the 3α-diol assay is incubated overnight at room temperature. The E_2 and corticosterone assays were incubated at room temperature for 50 min.

Separation of bound and free was accomplished by the rapid addition of dextran-coated charcoal. Following incubation with charcoal, samples were centrifuged at 1200 × g. The supernatant was pipetted into a glass scintillation vial with scintillation cocktail. Sample tube concentrations were calculated using the logit-log method of Rodbard and Hutt (1974), interpolation of the standards, and correction for recovery. The intra- and interassay coefficients of variance were the following—T: 5% and 5%; DHT: 2% and 10%; 3α-diol: 9% and 10%, for E_2: 8% and 10%; and corticosterone: 2% and 5%.

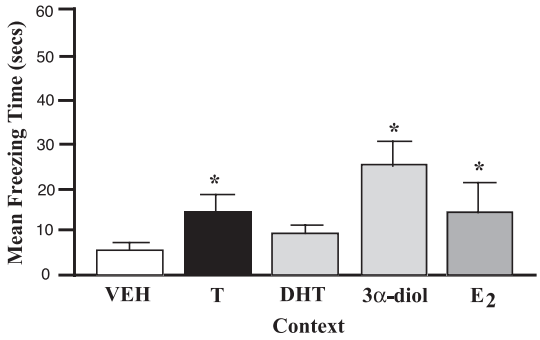
2.7. Statistical analyses

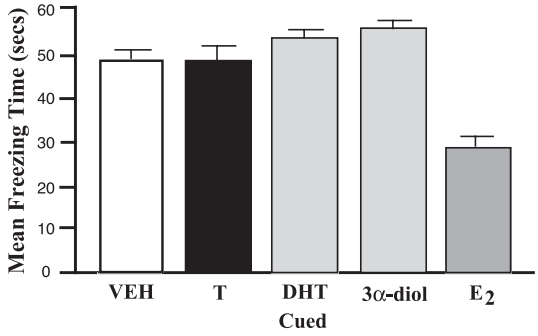
Analyses of variances (ANOVAs) were used to examine the differences between the groups in the behavioral measures recorded. The α level for statistical significance was $P < .05$. For Experiments 1 and 2, three-way ANOVAs, with the between-variable of androgen condition, one within-

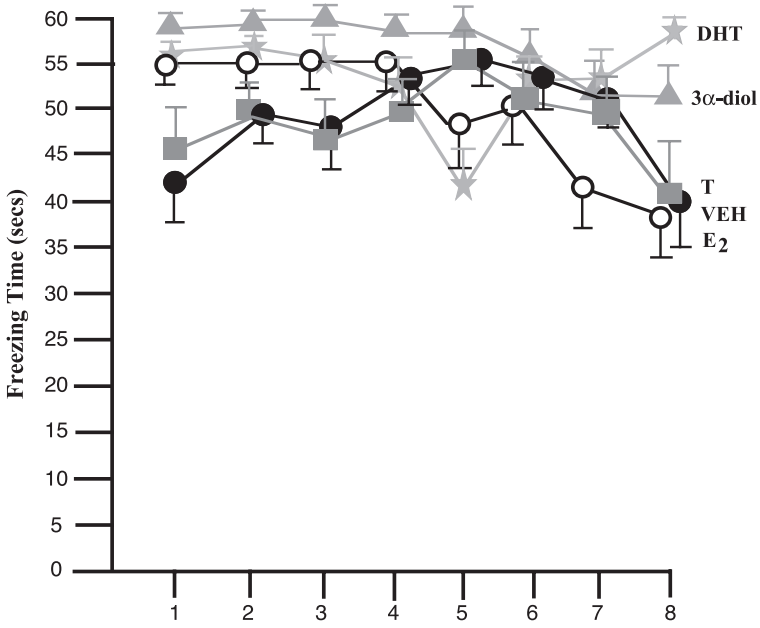
Fig. 2. Upper panel shows, for each trial, the mean duration of freezing behavior (s ± S.E.M.) of GDX rats administered posttraining 1 mg/kg sc T (gray square), DHT (gray star), 3α-diol (gray triangle), E_2 (white circle), or vehicle (black circle) in the contextual component of the conditioned fear paradigm. Middle panels depict the mean freezing time across all trials of GDX rats that received posttraining T (black bars), DHT (vertical stripes), 3α-diol (horizontal stripes), E_2 (gray bars), or vehicle (white) in the contextual (left) and cued (right) portions of the conditioned fear task. Bottom panel shows, for each trial, the mean duration of freezing behavior (s ± S.E.M.) of GDX rats administered posttraining 1 mg/kg sc T (gray square), DHT (gray star), 3α-diol (gray triangle), E_2 (white circle), or vehicle (black circle) in the cued component of the conditioned fear paradigm. * Significant difference ($P < .05$).



Context - Hippcampus







Cued - Amygdala

variable of trial and another of testing condition (context or cued), were utilized to examine effects on freezing. One-way ANOVAs were also used to compare mean freezing time across groups, irrespective of trials, in the contextual and cued environments. For Experiments 3 and 4, one-way ANOVAs compared crossover latencies between groups. NB: Training day latencies were not different cross groups. For Experiments 5, one-way ANOVAs examined the effects of androgen administration on plasma and hippocampal androgen levels. Where appropriate, Fisher's *post hoc* tests were used to determine differences between groups in all experiments.

3. Results

3.1. Experiment 1

Three-way ANOVAs revealed that the effect of androgens approached significance [$F(1,182)=3.519$, $P=.07$], and there was a significant main effect of test condition [$F(1,182)=123.596$, $P=.0001$], but no main effect of trials. There was also an interaction between androgen condition and trials [$F(7,182)=2.161$, $P=.04$; see Fig. 1, top and bottom panels].

One-way ANOVAs of the mean time spent freezing suggested apparent effects of androgen condition in the contextual, but not the cued, condition. Intact, compared with GDX, rats spent more time freezing in the contextual condition; however, this result was not statistically significant [$F(1,27)=3.337$, $P=.08$; see Fig. 1]. However, there were no significant differences in the freezing durations of intact and GDX rats in the cued condition (see Fig. 1, middle panels, left and right, respectively).

3.2. Experiment 2

Three-way ANOVAs revealed significant main effects of androgen [$F(4,525)=3.671$, $P=.008$], test [$F(1,525)=668.004$, $P=.0001$], and trial conditions [$F(7,525)=7.157$, $P=.001$]. There was also an interaction between androgens, test, and trials [$F(28,525)=2.262$, $P=.0003$; see Fig. 2, top and bottom panels].

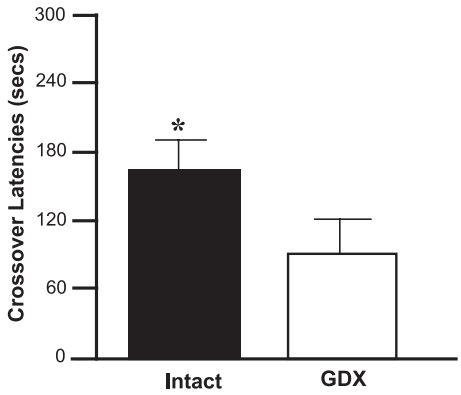


Fig. 3. Mean crossover latencies (s ± S.E.M.) of intact (black bar) and GDX (white bar) rats in the inhibitory avoidance task. * Significant difference ($P<.05$).

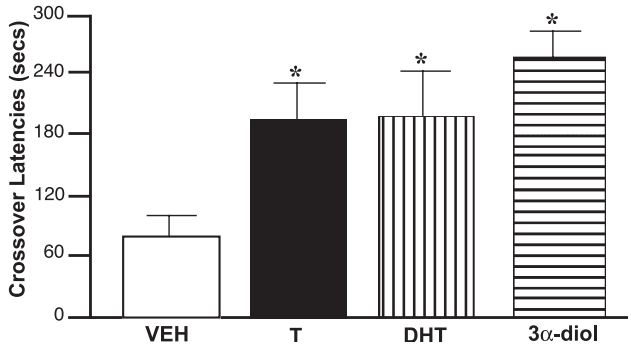


Fig. 4. Mean crossover latencies (± S.E.M.) of GDX rats administered posttraining 1 mg/kg sc T (black bar), DHT (vertical striped bar), 3α-diol (horizontal striped bar), or vehicle (white bar). * Significant difference ($P<.05$).

One-way ANOVAs of the mean time spent freezing indicated effects of androgen condition in the contextual [$F(4,75)=4.120$, $P=.004$], but not the cued, conditions. As Fig. 2 (middle panel) illustrates, 3α-diol-administered rats spent significantly more time freezing compared with all other groups in the context condition. T- and E_2-administered rats spent significantly longer freezing in the context condition than did the DHT-administered and control rats. There were no differences in mean freezing times among groups in the cued condition.

3.3. Experiment 3

Intact, compared with GDX, rats had significantly longer crossover latencies in the inhibitory avoidance task [$F(1,28)=7.649$, $P=.009$; see Fig. 3].

3.4. Experiment 4

The administration of T, DHT, or 3α-diol significantly increased crossover latencies in the inhibitory avoidance

Table 1
Mean plasma levels (± S.E.M.) of intact or GDX rats administered sc 1 mg/kg T, DHT, 3α-diol, or E_2

Plasma concentrations				
Hormone condition	T (ng/ml)	DHT (ng/ml)	3α-diol (ng/ml)	E_2 (pg/ml)
Intact	12.0 ± 4.3 *	2.6 ± 1.2 *	2.4 ± 0.5 *	8.0 ± 2.2 *
GDX	3.9 ± 1.6	0.3 ± 0.2	0.0 ± 0.0	1.9 ± 2.2
T	11.8 ± 2.0 *	3.6 ± 1.7 *	3.4 ± 0.5 *	6.2 ± 1.4 *
DHT	5.1 ± 1.4	3.6 ± 1.7 *	2.9 ± 0.6 *	0.4 ± 0.4
3α-diol	2.6 ± 0.6	0.2 ± 0.1	3.0 ± 0.6 *	0.4 ± 0.4
E_2	3.8 ± 1.2	0.5 ± 0.3	0.2 ± 0.2	23.8 ± 14.1 *

* Denotes significant difference from GDX control rats ($P<.05$).

Table 2
Mean hippocampal levels (± S.E.M.) of intact or GDX rats administered vehicle or 1 mg/kg sc T, DHT, 3α-diol, or E_2

Hippocampal concentrations				
Hormone condition	T (ng/mg)	DHT (ng/mg)	3α-diol (ng/mg)	E_2 (pg/mg)
Intact	2.2 ± 0.6 *	1.3 ± 0.3 *	2.5 ± 0.7 *	0.7 ± 0.4
GDX	0.1 ± 0.1	0.2 ± .09	0.0 ± 0.0	0.5 ± 0.5
T	2.0 ± 0.4 *	1.8 ± 0.7 *	1.9 ± 0.3 *	1.6 ± 0.8 *
DHT	0.09 ± 0.08	1.4 ± 0.1 *	0.7 ± 0.3	0.0 ± 0.0
3α-diol	0.2 ± 0.2	0.4 ± 0.1	3.0 ± 0.6 *	0.0 ± 0.0
E_2	0.2 ± 0.6	0.8 ± 0.8	0.07 ± 0.6	2.0 ± 0.5 *

* Denotes significantly greater than GDX control group ($P < .05$).

task compared with vehicle to GDX rats [$F(3,56) = 10.890$, $P = .0001$; see Fig. 4].

3.5. Experiment 5 (see Tables 1 and 2)

Intact and T-administered rats had significantly increased plasma [$F(3,56) = 3.686$, $P = .008$] and hippocampal [$F(3,56) = 9.923$, $P = .0001$] T compared with all other groups (Tables 1 and 2). Intact and T- or DHT-administered rats had nonsignificant increases in plasma [$F(3,56) = 2.193$, $P = .07$] and significantly greater hippocampal [$F(3,56) = 5.169$, $P = .001$] DHT levels. All intact and androgen-administered rats had significantly increased plasma 3α-diol [$F(3,56) = 11.096$, $P = .0001$] levels, and intact and T- or 3α-diol-administered rats had significantly increased hippocampal 3α-diol [$F(3,56) = 10.3878$, $P = .0001$] compared with all other groups. Only E_2 administration increased plasma E_2 concentrations [$F(3,56) = 2.319$, $P = .06$]; however, both T and E_2 administration significantly increased hippocampal E_2 levels [$F(3, 5) = 3.214$, $P = .01$]. There were no differences in plasma (0.30 ± 0.20 μg/dl) or hippocampal (0.02 ± 0.001 μg/dl) corticosterone levels across groups.

4. Discussion

The results of these experiments are, in part, consistent with our hypotheses. First, we hypothesized that if androgens are important for hippocampally-mediated learning, then, performance of intact rats would be better than that of castrated male rats. Indeed, intact rats spent more time freezing in the contextual conditioned fear task than did GDX rats. Although this effect only approached statistical significance ($P = .07$), it is notable that GDX rats were virtually unresponsive to the context portion of the task, indicating a lack of learning. In the inhibitory avoidance task, intact rats had significantly longer crossover latencies than did GDX rats. The increased duration of freezing in the conditioned fear task, and the longer crossover latencies in the inhibitory avoidance task of intact compared with GDX rats, suggests that androgens may enhance the recollection of aversive stimuli in these paradigms.

Second, we hypothesized that if 5α-reduced metabolites underlie androgen-enhanced learning, then, the administration of T, DHT, or 3α-diol to rats would enhance learning in hippocampally mediated tasks. The administration of T and 3α-diol, compared with vehicle, significantly increased the amount of time spent freezing in the hippocampally mediated contextual portion of the conditioned fear task. T, DHT, and 3α-diol significantly increased crossover latencies in the inhibitory avoidance task compared with GDX controls. These data suggest that 5α-reduced androgens may have mnemonic effects in these tasks.

Finally, we hypothesized that if aromatization to E_2 is not required for androgens' mnemonic effects, then, the administration of nonaromatizable androgens, DHT and 3α-diol, would enhance learning without increasing plasma or hippocampal E_2 levels. DHT and 3α-diol significantly enhanced learning in the inhibitory avoidance task, and 3α-diol had mnemonic effects in the conditioned fear task compared with all androgen groups. Furthermore, while T and 3α-diol administration increased hippocampal 3α-diol, only the subcutaneous administration of E_2 or T increased hippocampal E_2 levels. While E_2 enhanced freezing in the contextual fear task, both T and E_2 hormone regimens produced an increase in hippocampal E_2 levels. Thus, both T- and E_2-treated groups were not included in the inhibitory avoidance task. Posttraining administration of 3α-diol increased the duration of freezing in the conditioned fear task and crossover latencies in inhibitory avoidance more than any other androgen did, without increasing E_2 levels. These data suggest that 3α-diol may have actions in the hippocampus, independent of E_2, to enhance learning and retention; however, this does not preclude independent effects of E_2, which require further investigation.

The present findings confirm previous research indicating androgens may mediate learning and memory in rodents. First, the present study, as in previous inhibitory avoidance (Frye and Seliga, 2001; Frye et al., in press), object recognition (Ceccarelli et al., 2001), and Y-Maze (Kritzer et al., 2001) tasks, demonstrates that GDX can produce deficits in cognitive performance. As in previous studies (Frye and Seliga, 2001; Frye et al., in press; Kritzer et al., 2001), our findings indicate that GDX-induced deficits can be ameliorated through T-replacement. These data suggest that androgens may mediate cognitive behavior in male rodents. Second, our study indicates that DHT replacement to male rodents can restore GDX-induced deficits in cognitive performance. These findings are consistent with previous inhibitory avoidance studies (Frye et al., in press), which indicate that DHT replacement is equally effective as T is at enhancing cognitive performance. Finally, our studies suggest that 3α-diol administration to GDX rats is effective at enhancing cognitive performance, which is similar with results from previous findings indicating 3α-diol administration is effective at enhancing learning in the conditioned place preference and inhibitory avoidance tasks (Frye and McCormick,

2000b; Rosellini et al., 2001). 3α-Diol administration to GDX and adrenalectomized (ADX) male (Frye and McCormick, 2000b) and female (Frye and McCormick, 2000a) rats also prevents cell death in the dentate gyrus. Together, these findings suggest that T's effects to enhance cognitive performance may be due, in part, to the actions of its 5α-reduced metabolites.

Androgens' effects to enhance cognitive performance may be influenced by other androgen-mediated processes. Both the conditioned fear and inhibitory avoidance tasks utilize aversive stimuli as measures of learning. Previous studies have found that androgens enhance analgesia and affect (Bitran et al., 1993; Frye and Edinger, 2004; Frye and Lacey, 2001; Frye and Seliga, 2001; Frye et al., 1996c). In Experiments 1 and 3, there were differences between intact and GDX rats in performance and, presumably, androgen levels before and after training and at the time of testing. The behavior of intact and GDX rats was significantly different in the inhibitory avoidance task but only approached significance in the conditioned fear paradigm. These differences between tasks may reflect task demand. Performance in the inhibitory avoidance task may be easier and subject to greater influence by androgens, whereas the higher levels of shock and stimulation in the conditioned fear paradigm may more readily result in stress-induced central production of androgens, which may have influenced behavioral outcomes. Notably, in Experiments 2 and 4, androgens were administered posttraining, which would obviate androgen-mediated differences in the perception of the training stimulus. While previous studies have found that 5α-reduced metabolites may have actions to enhance cognitive performance (Frye et al., in press; Frye and McCormick, 2000b; Rosellini et al., 2001), better effects of posttraining androgens in the conditioned fear and inhibitory avoidance tasks suggest that 5α-reduced metabolites may not only have effects to enhance performance in cognitive tasks, but may also enhance the consolidation of memory.

Just as there were differences between tasks in the performance of intact and GDX rats, DHT administration produced variable effects within and between tasks. Overall, DHT was ineffective at enhancing performance in the conditioned fear task; however, there is a pronounced effect of DHT to increase freezing in the first two trials. In the inhibitory avoidance task, DHT had enhancing effects on performance that were similar with other androgens. It is possible that androgens produced similar effects in the inhibitory avoidance tasks because they were present 24 h after administration, when performance was being measured. Because conditioned fear testing takes place 5 and 6 days after androgen administration, it is unlikely that exogenous androgens are still present at the time of testing. It is also possible that the variable performance exhibited by DHT-administered rats is a result of withdrawal or the differential production of central androgens. Androgen withdrawal is associated with depressive-like symptoms, changes, perceptions, and attitudes (Hays et al., 1990). 5α-Reduced androgens are produced readily in the brain in response to stress (Erskine and Kornberg, 1992). DHT is very labile and rapidly converts to 3α-diol, which may account for some of the differences between task and trials produced by DHT. Notably, androgens enhanced conditioned fear, improved inhibitory avoidance, and produced androgen levels within the physiological range; whereas, comparisons of intact and GDX rats revealed more modest behavioral effects, despite differences in androgen levels. It is possible that the administration of specific androgens produce more salient behavioral effects because of their direct administration.

While T and 3α-diol administration had no effect on the amygdala-mediated aspects of the conditioned fear task, it did influence performance in the hippocampal aspects of the task. Furthermore, the administration of all androgen groups significantly increased learning behavior in inhibitory avoidance, which is a hippocampally-mediated task. The hippocampus is an important region for learning and memory (Bannerman et al., 2002) and may serve as a target for androgens actions (Collinson et al., 2002; Kerr et al., 1995; Sar et al., 1990; Smith et al., 2002). Previous findings indicate that limiting the production of 3α-diol through the administration of intrahippocampal indomethacin significantly decreases 3α-diol levels in the hippocampus and performance in the inhibitory avoidance task (Frye et al., in press). While our findings indicate that androgens can enhance cognitive performance in these tasks through actions in the hippocampus, this does not preclude androgens having mnemonic effects in other brain regions. For example, 3α-diol to the nucleus accumbens is more effective than T or DHT at improving place conditioning of male rats (Frye et al., 2002; Rosellini et al., 2001). It will be important to examine androgens' mnemonic effects in other tasks and via other brain regions.

Although T, DHT, and 3α-diol have actions at different substrates in the brain, all androgen administration enhanced inhibitory avoidance performance similarly, which suggests that they have a similar mechanism to effect behavior. While T and DHT bind with high affinity to androgen receptors (ARs; Roselli et al., 1987), 3α-diol typically does not. If 3α-diol is required for androgens' cognitive enhancing effects, then, actions at $GABA_A$ receptors, which 3α-diol has a high affinity for (GBRs; Frye et al., 1996b), may be important in mediating androgens' mnemonic effects. 3α-Diol may also be acting at other sites, including NMDA receptors or via signal transduction pathways (Rhodes and Frye, 2004b). Blocking metabolism to 3α-diol in intact and DHT-replaced rats produces deficits in cognitive performance (Frye et al., in press). Together, these findings suggest that androgens' effects on learning may be mediated by 3α-diol. It is also possible that androgens' effects to enhance cognitive performance are controlled by its effects on neuroplasticity. Androgen administration enhances neurogenesis in the hippocampus (Leranth et al.,

2003). While the present findings indicate that 5α-reduced androgens can have effects in the hippocampus to mediate learning and memory, further research is necessary to characterize androgens' effects and the mechanism of action to enhance cognitive performance. This may be particularly relevant for aging, which is associated with declining androgen levels and cognitive performance.

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