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Formation of Testosterone's 5alpha-Reduced, Versus Aromatized, Products Can Have Beneficial Cognitive and Anti-Anxiety Effects Without Negative Effects on Prostate or Sexual Behavior of Male Rats

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Formation of testosterone's 5α-reduced, versus aromatized, products can have beneficial cognitive and anti-anxiety effects without negative effects on prostate or sexual behavior of male rats.

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Abstract

Testosterone (T) can alter sexual, social, anxiety-like, and/or cognitive behavior of male rodents and exert trophic effects on the prostate. However, whether these effects are due to actions of T, or its 5α-reduced and/or aromatized metabolites, is of interest. We tested the hypothesis that T's effects to enhance prostate proliferation, sexual, social, cognitive and/or anti-anxiety-like behavior require formation of 5α-reduced and/or aromatized metabolites. Gonadectomized (GDX) or gonadally-intact rats were administered T-containing, or empty, silastic capsules in conjunction with a 5α-reductase inhibitor (finasteride; Experiment 1) or an aromatase inhibitor (formestane; Experiment 2). The performance of rats in sexual, cognitive (object recognition, object placement, water maze), anxiety-like (open field, elevated plus maze, light–dark transition, mirror maze, social interaction) tasks were examined. Prostate mass and concentrations of T and its metabolites were assessed. Rats that were GDX, compared to intact rats, had lower androgen levels, smaller prostates, longer latencies to initiate sexual contacts, had poorer cognitive performance in the object placement and water maze tasks, and demonstrated more anxiety-like behavior in the light/dark transition task and the mirror maze. Finasteride produced effects similar to GDX to decrease prostate weight and inhibit sexual behavior and spatial cognition, but not affective behaviors. Formestane did not alter prostate mass or sexual behavior, but did enhance cognitive performance in the object recognition task and tended to increase central entries in the open field, an indication of anti-anxiety behavior. Thus, shunting T's metabolism from aromatization to favor 5α-reduction had beneficial cognitive and anti-anxiety effects without negative effects on prostate or sexual behavior.

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Introduction

Aging men can experience decline in gonadal, sexual, cognitive, and affective function. For men, androgen levels begin to rise after puberty and remain high until midlife (Hiort, 2002), when a decade-by-decade decline in endogenous androgen levels occurs (Morley et al,. 1997). On average, this decline, involves a 0.4% reduction in total testosterone and a 1.2% reduction in biologically-free testosterone per year, causing mean total plasma testosterone levels decrease by about 35% between 25 and 75 years of age (Schatzl et al., 2003; Vermeulen, 2000). Some behavioral sequelae associated with aging include poorer performance in spatial tasks, greater anxiety and depression, and decreased sexual motivation (Davidson Kwan and Greenleaf, 1982; Basaria & Dobs, 2001; 2002; Morley et al., 2001; Janowsky Oviatt and Orwoll, 1994; Seidman, 2002). In addition to androgen-sensitive changes in behavior, there are physical changes, such as increased risk for benign prostate hyperplasia (BPH) and prostate cancer (Untergasser, Rumpold, Hermann, Dirnhofer, Jilg and Berger, 1999). These physical changes worsen with aging, as frequency of moderate and worse urinary symptoms related to BPH rise from 13% in the fifth to 28% in the eighth decade of life (Kaplan, 2005). Affective, cognitive, and physical decline associated with aging is of particular importance given that demographics suggest that older age groups are increasing as a percentage of the population (Nieschlag et al., 2006). Many men seek testosterone (T) replacement therapy (TRT) to combat these effects (Marks & Kaplan 2009; Kaufman & Seftel, 2004). TRTs have successfully been used to improve spatial cognition, libido and depression (Tenover, 1998; Janowsky, Oviatt, and Orwoll, 1994; Pope, Cohane, Kanayama, Siegel, and Hudson, 2003). However, TRTs are also associated with negative consequences

including increased risk for prostate cancer (Raynaud, 2006). Greater understanding is needed of the relative trophic effects of androgens on peripheral and central tissues.

In rodent models, decline in endogenous T can produce negative effects on sexual, cognitive, and anxiety-like, behavior. Older male rodents demonstrate similar behavioral decline to that of aging men: aged rodents display decreased sexual motivation and cognitive performance in spatial tasks (Chambers, Thornton, Roselli, 1991; Spruijt Meyerson, and Hodlund, 1989; Barnes, 1988). Extirpation of the testes, a primary source of androgens, can reduce plasma levels of androgens (Krey & McGinnis, 1990). Separate studies have shown that gonadectomy (GDX) of rodents produces behavioral effects similar to those seen with aging, including decreased sexual proceptivity, cognition, increased anxiety and depressive behavior (Adler, Vescovo, Robinson, and Kritzer, 1999; Hull & Dominguez, 2007; Aubele, Kaufman, Montalmant and Kritzer, 2008; Bernardi Genedani, Tagliavini, and Bertolini, 1989). Akin to hormone replacement therapy, administering T to rats reinstates sexual, cognitive, and affective, performance commensurate to that of gonadally-intact rats (Delhez, Hansenne, and Legros, 2003; Kritzer, Brewer, Montalman, Davenport, and Robinson, 2007), but coincident effects of prostate proliferation are of interest. Thus, utilizing a GDX model is useful for determining the effects of decreasing androgen levels with aging.

Some of T's psychological and physiological effects may be mediated, in part, through actions of its 5α-reduced and/or aromatized metabolites. Testosterone is metabolized by 5α-reductase enzymes to form 5α-dihydrotestosterone (DHT; Handa, Pak, Kudwa, Lund, and Hinds, 2008). In addition, T is metabolized by aromatase to form estradiol (E_2: Alejandre-Gomez, Garcia-Segura, Gonzalez-Burgos., 2007; Ellem &

Risbridger, 2009; Lephart Lund and Horvath, 2001; Séralini & Moslemi, 2001). Interestingly, among rats, levels of T and its 5α-reduced metabolites decline with aging (Frye et al., 2010). However, with aging among men, E_2 levels remain unchanged or increase, resulting in a decrease in the ratio of T to E_2 (Ellem & Risbridger; Vermeulen, 2000). Thus, the ratio of T to its 5α-reduced and/or aromatized metabolites in relation to effects on prostate and behavior is of interest.

The testes are a significant source of androgens, but T, its 5α-reduced, and aromatized metabolites, are also produced in the brain. Trophic effects of T in the brain may be in part dependant, and independent of, gonadal condition. For example, GDX reduces the number of aromatase-positive neurons in the hypothalamus, but not limbic areas (Jakab, Horvath, Leranth, Harada, and Naftolin, 1993). Within limbic regions, such as the hippocampus, androgen-mediated spine synapse density can be in part independent of systemic androgenic potency (MacLusky, Hajszan, Prange-Kiel, and Leranth, 2006). Interestingly, androgen-induced remodeling of spine synapses in the hippocampus and prefrontal cortex occur independently, and dependently, respectively of actions at androgen receptors (ARs; Hajszan, Milner, and Leranth, 2007). Thus, androgens can exert diverse effects and mechanisms for its trophic actions in the brain.

We investigated the relationship between T and its metabolites on reproductive, cognitive, and affective behavior as well as their proliferative effects in the prostate in two experiments. Rats were gonadally-intact, or GDX and implanted with silastic capsules that were empty or contained T. In Experiment 1, rats also had silastic capsules containing a 5α-reductase inhibitor, finasteride or control implants. In Experiment 2, rats received silastic capsules containing an aromatase inhibitor, formestane, or control

implants. All rats were tested for reproductive, cognitive, and affective behavior, in a 7 week-long battery. At the end of the battery, prostate was collected and wet weight was obtained as an indication of mass. Plasma and brains were collected for measurement of peripheral and central androgen concentrations. We anticipated that GDX and finasteride would decrease, while T and formestane would increase, sexual motivation, performance in cognitive tasks, anti-anxiety-like behavior, and prostate weights in correlation with formation of T's metabolites.

Materials and Methods

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

Animals & Housing

Male Long-Evans rats (N=250), approximately 2 months old at the start of the experiment, were obtained from our in-house breeding colony (original stock from Charles River Laboratories, Raleigh, NC). Rats were group-housed (3-4 per cage) in polycarbonate cages (45 X 24 X 21 cm) in the Laboratory Animal Care Facility of The Life Sciences Research Building at The University at Albany-SUNY. Subjects were housed in a temperature- (21 ± 1 °C) and humidity- (50 ± 5%) controlled room that was maintained on a 12:12 h reversed light cycle (lights off at 0800 h). Throughout the investigation, rats had continuous access to Purina Rat Chow and tap water in their home-cages.

Surgery: Gonadectomy and Implantation

Rats were either GDX or sham-surgerized under xylazine (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and ketamine (60 mg/kg; Fort Dodge Animal Health, Fort

Dodge, IA) anesthesia, 26-30 days before the start of behavioral testing. At the time of GDX or sham surgery, rats were randomly assigned to receive a silastic implant (1.57 mm inner diameter, 3.18 mm outer diameter; 10mm/rat) that was empty (vehicle), contained crystalline T, and/or one silastic that contained finasteride (Experiment 1) or formestane (Experiment 2) as modified from previous methods (Frye, Edinger, Seliga, Wawrzycki, 2004). Following surgery, and prior to testing, all rats were monitored for neurological status by monitoring for loss of weight, righting response, flank stimulation response, and/or muscle tone (Marshall & Teitelbaum, 1974). One rat (GDX with finasteride) did not pass these assessments and was immediately euthanized.

Behavioral Testing

Rats were tested in two tasks per week with a minimum of 2 days between tests. Behavioral data were collected by one trained, blinded, observer (DJD), using a video-tracking system (ANY-Maze- Stoelting, Inc., Wood Dale, IL). Unless otherwise indicated, testing was conducted in an open field (76 x 57 x 35 cm), situated in a brightly-lit room.

Sexual Behavior: Rats were tested for sexual behavior in a chamber (37.5 x 75 x 30 cm), per previously reported methods (Edinger & Frye, 2007). Briefly, each rat was placed in the chamber with a sexually-receptive female (ovariectomized rat administered supraphysiological levels of E_2) for 10 mins. Latencies to, and frequencies of mounts, intromissions and ejaculations were recorded. No significant differences were observed in mounting or ejaculatory behavior. Latencies to initial intromission are indicative of sexual capacity.

Social Interaction: Testing was conducted per previously reported methods (Frye Petralia and Rhodes, 2000). Rats were placed in the chamber with a novel male conspecific. Amount of time in social interaction (sniffing, grooming, crawling over/under, following with contact, engaging in rough and tumble interaction) was recorded. Time spent interacting is an index of social/affective behavior.

Object Recognition: This procedure was implemented as per prior methods (Ennaceur & Delacour 1988; Frye & Lacey, 2001; Paris & Frye, 2008; Luine, Mohan, Tu, and Efange, 2002; Walf, Rhodes, and Frye, 2006).

Training: Two identical sphere-shaped objects were placed into adjacent corners of the open field chamber. Rats were placed in a corner opposite the objects. Rats were allowed to freely explore the chamber for 3 minutes, and time spent interacting with each object was recorded. The rats were then single housed.

Testing: Four hours later, rats were placed back into the same chamber for another 3 minutes with one of the objects having been replaced with a novel cone-shaped object. Amount of time spent interacting with each object was recorded. Greater percentage of time spent with the novel, compared to the familiar, object was used as an indication of enhanced working memory.

Object Placement: Methods were as previously described (Ennaceur & Delacour 1988; Frye, Duffy, and Walf, 2007; Frye & Lacey, 2001; Luine et al., 2003; Paris & Frye, 2008). Training was the same as for object recognition and testing involved the same objects with one moved to the corner opposite its starting position. The amount of time spent with each object was recorded. Greater percentage of time spent with the object in

the novel/displaced location, compared to the object in the familiar location, was used as an indication of enhanced spatial cognition.

Morris Water Maze: This was conducted as described previously (Vongher & Frye 1999; Frye, Edinger, Lephart, and Walf, 2010).

Habituation: On day one, rats were allowed to swim for 60 secs, in a large circular tank (175 cm. diameter, 71 cm deep), with water (24-26^{o}C), that had white, non-toxic, tempera paint added to make it opaque.

Training: On day two, a clear Plexiglas platform, with a top that measures 5.3cm x 5.3cm, was placed in one of the four quadrants 30 cm from the side of the pool. The water level was filled so that it was 2.5cm above the top of the hidden platform. Rats were given two minutes to find the platform during two trials. Each trial was initiated from a different quadrant. If the rat did not reach the platform within 120 secs, it was guided to it and remained on the platform for 45 secs.

Testing: On day three, rats were allotted 120 secs to find the platform during each of four trials. Each trial was initiated from a different quadrant. The latency to find the hidden platform and the distance traveled were recorded. Shorter latencies and distances are considered indicative of better spatial performance.

Open Field: This was conducted per previous methods (Frye et al., 2000; McCarthy, Felzenberg, Robbins, Pfaff, and Schwartz-Giblin, 1995). A grid of 48 squares (9.5 cm width each) was superimposed on the floor of the test chamber by the video-tracking system. Entries into outer squares (those adjacent to the outer wall; n=24), central squares (all other squares apart from the outer; n=24), are recorded. The number of central square entries is used as an index of anti-anxiety-like behavior.

Light-Dark Transition: This task was conducted per previously described methods (Walf & Frye, 2005). Rats were placed on the light side of a two-chambered compartment (30 X 40 X 40 cm), which has white walls and floor, and is illuminated from above by a 40-watt light. The opposing side of the chamber is black and has a lid. The number of entries between the light and dark side of the chamber was recorded for 5 mins. An entry into the light side of the chamber is considered an indication of anti-anxiety behavior.

Mirror Maze: As per previous methods (Frye et al., 2006), the testing chamber is 52 x 57 x 52 cm. It has within it, a mirrored-compartment (52 x 5 x 52 cm) and an alleyway without mirrors. Rats are placed in the alley section, and the number of entries to the mirrored chamber were recorded. More entries to the mirror chamber is an indication of anti-anxiety behavior.

Tissue Collection

Immediately following testing, subjects were terminated by rapid decapitation. Trunk blood was collected and centrifuged (10 mins at 3000 x *g*). Brain and prostates were extracted, weighed, and flash frozen on dry ice. Brain, prostates, and plasma were stored at -80°C. Before radioimmunoassay, hypothalamus, hippocampus, frontal cortex, and midbrain, were grossly dissected on ice as previously described (Frye, Paris, and Rhodes, 2007).

Radioimmunoassay

T, DHT, and E_2 concentrations were assessed by radioimmunoassay as previously described (Edinger Lee and Frye, 2004; Frye & Bayon, 1999; Frye, McCormick, Coopersmith, and Erskine, 1996). Briefly, the T antibody (T3-125; Endocrine Sciences, Calabasas Hills, CA) was diluted 1:20, 000 and binds between 60% and 65% of [^{3}H] T

(NET-387: specific activity = 51.0 ci/mmol). The DHT antibody (DT3-351; Endocrine Sciences) was diluted 1:10,000 and binds between 60% and 65% of [^{3}H] DHT (NET-302: specific activity = 43.5 ci/mmol). The E_2 antibody (Dr. Niswender, #244, Colorado State University, Fort Collins, CO) was diluted 1:30, 000 and binds approximately 90% of [^{3}H] E_2 (NET-317: specific activity = 51.3 ci/mmol). Standard curves for all steroids were run in duplicate and ranged from 50 - 2000 pg in concentration. Standards were added to BSA assay buffer, followed by addition of the appropriate antibody and [^{3}H] steroid. The T and DHT assays were incubated overnight at 4°C. The E_2 radioimmunoassay was incubated at room temperature for 50 min. Separation of bound and free steroid was accomplished by the rapid addition of dextran-coated charcoal. Samples were centrifuged at 3000 X *g* for 10 min, following incubation with charcoal. Supernatant was decanted into 5 ml scintillation cocktail. The intra- and interassay coefficients of variance were: T = 0.09 and 0.04, DHT = 0.08 and 0.09, E_2 = 0.09 and 0.09.

5α-reductase activity

As per previously methods (Kellogg & Frye 1999), the turnover ratios of T to DHT were calculated and used as an index of 5α-reductase activity.

Statistical Analyses

Three-way analyses of variance (ANOVAs) with between-subjects factors of, gonadal status (intact, GDX), T condition (T-containing or empty silastics), and enzyme inhibitor (vehicle, 5α-reductase inhibitor, finasteride or aromatase inhibitor, formestane) were utilized for each measure examined. Interactions were assessed via one-way follow-up ANOVAs with alpha level conditions corrected for multiple comparisons. Where appropriate, Fisher's protected least significant differences *post-hoc* tests were utilized to

determine group differences. Simple regressions were used to assess the amount of variance that could be explained for each behavior by 5α-reductase activity in the hippocampus. The alpha level for statistical significance was $p < 0.05$. Trends towards significance are noted when $p \leq 0.10$. A few samples (< 10), were lost during radioimmunoassay causing slight variation in degrees of freedom.

Results

Procedure

Gonadally-intact and gonadectomized rats with silastic implants that were empty or containined T (Edinger & Frye 2006) had effects of 5α-reductase and aromatase enzyme inhibitors examined in Experiments 1 and 2, respectively. Sexual, social, cognitive, and anxiety behaviors were examined. Following completion of the testing battery, prostate, plasma and brains were collected for endocrine analyses

Experiment 1: The importance of T's 5α-reduced metabolites was examined with a 5α-reductase inhibitor, finasteride (Steraloids, Newport, RI). Finasteride blocks both type I and type II isoforms of the 5α-reductase enzyme, such that it can decrease metabolism of T by ~75% (Finn et al., 2006; Rittmaster, 1994; McConnell & Stoner, 2001). There were eight experimental groups: intact rats implanted with finasteride (n=20), intact rats implanted with control silastics (n=20), intact rats implanted with T (n=15), intact rats implanted with T and finasteride (n=15), GDX rats implanted with T (n=16), GDX rats implanted with finasteride (n=15), GDX rats implanted with both T and finasteride (n=17), and GDX rats implanted with control/empty silastics (n=14).

Experiment 2: The importance of T's aromatized, metabolites was examined with an aromatase inhibitor, formestane (Sigma, St. Louis, MO). Formestane is a selective steroidal aromatase inhibitor, such that it can decrease E_2 levels in circulation and brain (Martínez-Mota et al., 2008; Lenning et al., 2001). For Experiment 2, there were eight treatment groups: intact rats implanted with formestane (n=16), intact rats implanted with control silastics (n=18), intact rats implanted with T and formestane (n=15), intact rats implanted with T (n=15), GDX rats implanted with T (n=15), GDX rats implanted with formestane (n=13), GDX rats implanted with both T and formestane (n=13), and GDX rats implanted with control silastics (n=13).

Experiment 1

GDX decreased levels of T and DHT, while increasing E_2 levels.

There were significant main effects of GDX to decrease T and DHT levels in plasma [T: $F(1,123) = 335.28$, $p < 0.05$; DHT: $F(1,123) = 77.67$, $p < 0.05$], hypothalamus [T: $F(1,124) = 13.36$, $p < 0.05$; DHT $F(1,123) = 7.54$, $p < 0.05$], hippocampus [T: $F(1,123) = 20.37$, $p < 0.05$; DHT $F(1,123) = 34.80$, $p < 0.05$], cortex [T: $F(1,122) = 18.19$, $p < 0.05$; DHT: $F(1,123) = 12.60$, $p < 0.05$], and midbrain [T: $F(1,122) = 33.61$, $p < 0.05$; DHT: $F(1,122) = 51.86$, $p < 0.05$]. GDX increased E_2 levels in plasma [$F(1,123) = 58.01$, $p < 0.05$], hypothalamus [$F(1,123) = 7.45$, $p < 0.05$], and midbrain [$F(1,121)=13.92$, $p < 0.05$]. See table I.

T administration increased levels of, T and DHT E_2

T implants increased T levels significantly in plasma [$F(1,123) = 14.15$, $p < 0.05$] and tended to increase T levels in the cortex [$F(1,122) = 2.88$, $p < 0.10$. T implants

significantly increased DHT levels in the midbrain [$F(1,122) = 5.270$, $p < 0.05$]. T implants decreased E_2 levels in plasma [$F(1,123) = 16.664$, $p < 0.05$] and increased levels of E_2 in the hippocampus [$F(1,122) = 5.338$, $p < 0.05$]. See table I.

5α-reductase turnover, as indicated by T to DHT ratios, are in table I (bottom).

GDX and/or Finasteride Decreased Prostate Weight, Sex, and Social Behavior

Prostate weight was significantly decreased by GDX [$F(2,124) = 36.97$, $p < 0.05$] and finasteride [$F(1,124) = 18.37$, $p < 0.05$] and increased by T [$F(1,124) = 11.71$, $p < 0.05$] (Figure 1; top panel).

Latencies to initial intromission were significantly increased by GDX [$F(1,124) = 77.07$, $p < 0.05$] and finasteride [$F(1,124) = 4.129$, $p < 0.05$] (Figure 1; middle).

The duration of time spent in social interaction with a conspecific was decreased by GDX [$F(1,124) = 77.07$, $p < 0.05$] (Figure 1; bottom).

T improved working memory, GDX or Finasteride Decreased Spatial Performance

In the object recognition task, rats with T-containing compared to empty silastic implants spent significantly more time with the novel object [$F(1,124) = 3.87$, $p = 0.05$], an indication of better working memory (Figure 2; top).

In the object placement task, GDX compared to intact rats spent significantly less time with the displaced object [$F(1,124) = 5.14$, $p < 0.05$], a measure of poorer spatial memory (Figure 2; middle).

In the Morris Water Maze, finasteride-administered rats tended to have longer latencies to find the hidden platform [$F(1,124) = 2.80$, $p < 0.10$], a measure of poorer spatial memory (Figure 2; bottom).

GDX Produced Anxiety-Like Behavior in the Light/Dark and Mirror Mazes

In the light/dark transition task, GDX rats made fewer entries into the light chamber [$F(1,124) = 17.85$, $p < 0.05$], an indication of anxiety-like behavior (Figure 3; middle).

In the mirror maze, GDX rats spent less time in the mirrored chamber [$F(1,124) = 23.223$, $p < 0.05$], indicative of anxiety-like behavior (Figure 3; bottom).

No differences were seen in the open field task (Figure 3; top).

5a-reduction in the Hippocampus Accounts for Variable Effects in Behavior

Simple regressions revealed the 26% of the variance could be explained for all behaviors examined in total by 5α-reductase activity in the hippocampus (table II, left).

Results Experiment 2

GDX decreased levels of T and DHT, while increasing E_2 levels.

GDX decreased T and DHT levels in plasma [T: $F(1,112) = 176.46$, $p < 0.05$; DHT [$F(1,112) = 9.619$, $p < 0.05$], hypothalamus [T: [$F(1,112) = 17.10$, $p < 0.05$]; DHT [$F(1,112) = 63.454$, $p < 0.05$], hippocampus [T: [$F(1,112) = 65.47$, $p < 0.05$]; DHT [$F(1,112) = 35.66$, $p < 0.05$], cortex [T: [$F(1,112) = 44.38$, $p < 0.05$]; DHT: [$F(1,112) = 57.70$, $p < 0.05$], and midbrain [T: [$F(1,112) = 3.95$, $p < 0.05$]; DHT: [$F(1,112) = 51.01$, $p < 0.05$]. GDX increased E_2 levels in the hippocampus [$F(1,112) = 11.25$, $p < 0.05$] and midbrain [$F(1,112) = 9.93$, $p < 0.05$] (table III).

T administration increased levels of T and decreased levels of DHT.

T implants increased T levels significantly in hippocampus [$F(1,112) = 4.97$, $p < 0.05$] and decreased DHT levels in plasma [$F(1,112) = 7.377$, $p < 0.05$] See table III.

Formestane tended to increase central levels of T.

Formestane tended to increase T levels in the hippocampus [$F(1,112)=3.50$], $p < 0.1$] (table III).

GDX Decreased Prostate Weight, Sex, and Social Behavior

Prostate weight was significantly decreased by GDX [$F(1,111) = 369.43$, $p < 0.05$] and increased by T administration [$F(1,111) = 11.593$, $p < 0.05$] (Figure 4; top).

Latencies to initial intromission were significantly increased by GDX [$F(1,112) = 11.916$, $p < 0.05$] (Figure 4; middle).

The duration of time spent in social interaction with a conspecific was decreased by GDX [$F(1,112) = 12.823$, $p < 0.05$] (Figure 4; bottom).

Formestane improved working memory and GDX Decreased Spatial Performance

In the object recognition task, formestane increased time spent with the novel object [$F(1,112) = 5.72$, $p < 0.05$], indicating better cognitive performance (Figure 5; top).

In the Morris Water Maze, GDX tended to increase latencies to the hidden platform [$F(1,112) = 3.14$, $p < 0.10$], suggesting poorer spatial memory (Figure 5; bottom).

No differences were seen in the object placement task (Figure 5; middle)

Formestane increased, and GDX decreased, anti-anxiety like behavior

In the open field task, formestane tended to increase the number of central entries [$F(1,112) = 3.09$, $p < 0.10$], indicating more anti-anxiety-like behavior (Figure 6, top).

In the light/dark transition task, GDX rats made fewer entries into the light chamber [$F(1,112) = 31.67$, $p < 0.05$], an indication of anxiety-like behavior (Figure 6; middle).

In the mirror maze, GDX rats spent less time in the mirrored chamber [$F(1,112) = 46.5$, $p < 0.05$], indicative of anxiety-like behavior (Figure 6; bottom).

5a-reduction in the Hippocampus Accounts for Variable Effects in Behavior

Simple regressions revealed the 36% of the variance could be explained by 5α-reductase activity in the hippocampus (table II, right).

Conclusions

These findings partly supported our hypothesis that T's 5α-reduction mediates some of its behavioral and peripheral effects. We found that reducing androgens via GDX decreased prostate weight, sexual interest in the standard mating paradigm, cognitive performance in the object placement task, and anti-anxiety-like behavior in social interaction, light-dark transition, and mirror maze tasks. Systemic administration of T significantly increased prostate weight, and enhanced cognition in the object recognition task. Blocking T's metabolism to its 5α-reduced metabolites via systemic finasteride administration significantly decreased prostate weight, and increased latencies to sexual behavior supporting a role for trophic effects of 5α-reduced androgens. In addition, there were tendencies for finasteride to reduce cognitive performance in the water maze task. Blocking T's metabolism to E_2, via systemic formestane administration, did not significantly alter prostate mass or sexual behavior, but it did improve cognition in the object recognition task, and tended to decrease anxiety-like

behavior in the open field task. As such, these data support a role for gonadal status in sexual, affective, and cognitive behavior, and suggest that centrally routing T's metabolism toward 5α-reduction improves behaviors.

Castration reduced prostate proliferation while producing negative effects on behavior. As hypothesized, GDX significantly decreased prostate weight, consistent with past studies (Brooks Primka, Berman, Krupa, Reynolds, and Rasmusson, 1991; Borst et al., 2007), and this was associated with lower levels of DHT and T in plasma. Notably E_2 levels remained the same or were increased with GDX. We observed a decrease in sexual motivation in the present study similar to that of prior investigations (McGinnis, Mirth, Zebrowski, and Dreifuss, 1989; Hull & Dominguez, 2007; Davidson 1966). One structure that may be particularly important in this effect is the midbrain which is thought to mediate sexual behavior of rodents (Murphy, Rizvi, Ennis, and Shipley, 1999; Brackett & Edwards, 1984). Herein, we find that reduced androgen formation in the midbrain correlated with decreased sexual motivation. Indeed, decreases in androgen formation with GDX were also observed in hippocampus and cortex, regions that are critical for cognitive and anxiety-like behavior. (Aubele et al., 2008; Sandstrom, Kim, and Wasserman, 2006; Kritzer, McLaughlin, Smirls, and Robinson, 2001). In the present study, GDX decreased cognitive performance, which has been associated with androgen milieu in cortex and hippocampus (Kaut & Bunsey, 2001; Brown, Wilson, and Riches, 1987). Notably, androgens in the cortex and hippocampus are implicated in anxiety-like behaviors (Bannerman, Deacon, Offen, Friswell, Grubb, and Rawlins, 2002; Frye & Edinger 2004). In the present investigation, GDX increased anxiety-like behavior in the light/dark maze, mirror maze, and social interaction tasks supporting past findings of

GDX to increase anxiety-like behavior in rodents (Edinger & Frye; 2004, Bitran, Kellogg, and Hilvers, 1993; Frye & Seliga, 2001, Fernández-Guasti & Martínez-Mota, 2003; Adler et al., 1999), and reductions in these behaviors correlated with decreases in androgens in hippocampus and cortex. Thus, GDX decreased androgen levels, prostate weight, sexual performance, anti-anxiety behavior, and spatial cognition.

Administration of T increased prostate proliferation while improving cognitive behavior. Others have demonstrated that prostate mass is increased with T administration (Borst et al., 2008). We found that prostate mass correlates with increased levels of DHT and T found in plasma, indicating an important role for ARs in these effects. Androgens are often utilized to treat sequelae of aging, including decreased libido. While past studies have found that systemic T administration can reinstate sexual behavior commensurate to levels akin to gonadally-intact rats (McGinnis et al., 1989), we did not observe this enhancement. This may be due to the fact that our hormone regimen aimed to produce low physiological levels of T and its 5α-reduced metabolites in brain (Edinger & Frye, 2004), whereas others that have demonstrated sexual enhancement via T replacement to GDX rats have utilized regimen that are approximately twice what was used in the present study (McGinnis et al.). As such, even low levels of androgen reinstatement that do not readily produce expected behavioral enhancement may be relevant for prolific effects on prostate. Notably, it is rare to observe sexual behavior among GDX rats that did not have sexual experience prior to GDX, as was the case in this investigation (Retana-Marquez & Velazquez-Moctezuma, 1997) which likely contributed to the observed lack of sex effects in our model. Contrary to our hypothesis, administration of T did not have any effects on anxiety-like behavior. This dosage of T, 10mm silastic

implant, has been shown to not alter behavior in the open field and social interaction tasks (Frye & Seliga, 2001), which may be due to this regimen only producing low physiological levels of T and its 5α-reduced metabolites in brain (Edinger & Frye, 2004). Thus, we found clear effects of T-replacement to increase prostate mass and improve cognitive performance.

Finasteride decreased prostate proliferation and decreased sexual behavior, whereas formestane enhanced cognition without altering prostate weight. Finasteride, decreased prostate weight, as it has been shown to do in past studies (Borst et al., 2008); yet, formestane administration did not have any effect on prostate. Of interest, finasteride administration decreased sexual motivation in the standard mating paradigm, supporting past findings wherein administration of T's 5α-reduced metabolites have been shown to increase mounting behavior (Butera & Czaja, 1989). Men have also shown slightly decreased sex drive with finasteride administration, which were reversed when treatment was ceased (Amory et al., 2008). Formestane administration did not produce any significant effects on sexual behavior; however, formestane significantly increased time spent with an object in a novel location in the object recognition task. These beneficial effects observed with formestane administration may be due to its tendency to increase T produced by the hippocampus. Finasteride's effects on cognition tended to support this hypothesis in that there was a trend for increased latencies to find the hidden platform in the water maze task indicating poorer cognitive performance. We have previously observed T's 5α-reduced metabolites to increase cognition (Frye, Koonce, Edinger, Osbourne, and Walf, 2008). These findings suggest that the presence and importance of

androgens in the prostate, the putative effects of DHT's actions at these targets, and the effectiveness of finasteride in reducing prostate proliferation.

It important to understand the extent to which T's 5α-reduced, versus aromatized metabolites, mediate trophic effects in the brain and periphery, as these androgens can have different targets. While both T and DHT bind with high affinity to intracellular ARs, DHT binds with a higher affinity than does T (Roselli, Horton, and Resko, 1987). Among GDX rats, administration of DHT produces similar effects to administration of T, for reinstatement of cognitive, affective, and depressive performance to levels akin to those observed among intact rats (Edinger and Frye, 2005; Frye et al., 2004; Edinger and Frye, 2004; Frye & Walf, 2009). However, DHT is highly active in prostate where it may cause harmful proliferation (Tindall & Rittmaster, 2008). As such, some of the prostatic trophic effects of T's metabolites may outweigh their beneficial actions for sexual behavior, affect, and cognition. Because of the importance of DHT in prostate proliferation, 5α-reductase inhibitors have been used as a treatment for BPH and prostate cancer (Marks et al., 1999, Fleshner, Trachtenberg, Walsh, and Crawford, 1995; Brufsky, 1997). Finasteride's inhibition of T's 5α-reduction, and formestane's inhibition of aromatase, may alter actions at intra-cellular receptors (ARs and ERs), as well as at membrane receptors, due altered levels of DHT and E_2. In addition, past findings have demonstrated the importance of actions at these receptors, and other targets, in the brain in the mediation of cognitive, anxiety-like, and sexual behavior (Naghdi, Nafisy, and Majlessi, 2001; Frye et al., 2008; Edinger & Frye, 2006; Weiser, Foradori, and Handa 2008; Phillips-Farfán, Lemus, and Fernandez-Guasti, 2007). Of interest in future

investigations is the relative roles of T and its metabolites at these receptor targets in the brain and peripheral, androgen-sensitive tissues, such as the prostate.

In conclusion, these results suggest that some of T's behavioral and trophic effects may be mediated largely through its metabolites, and that shifting T's metabolism towards 5α-reduction can improve behaviors. This is supported by correlations seen between varying levels of these metabolites in the brain and behavior, and correlations between the reduction in prostate weight and the levels of these metabolites in plasma. This also suggests finasteride to be useful for treating BPH, and prostate cancer, because it produced much more salient effects on the prostate than on behavior, and it did not alter hormone levels in any brain regions. Concerning formestane, these results suggest that reducing aromatization may be beneficial because it produces positive effects on behavior without negative effects on the periphery. Formestane improved cognition, and tended to decrease anxiety like behavior. While TRT also improves behaviors, it produces negative effects on the prostate, which we did not see with formestane administration, suggesting the utility of formestane co-administration with androgen therapies. Castration-resistant prostate cancer, a leading cause of death among men, underscores the importance of understanding androgen actions and receptor mechanisms, as such cancers typically progress despite low levels of T (Mostaghel, Montgomery and Nelson, 2009).

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Figure Legends- Experiment 1

Figure 1: Depicts the average prostate weight (±SEM) and represents the sociosexual data. This includes the latency (± SEM) to intromission for the sex testing and the mean interaction time (± SEM) for the Social Interaction task for gonadectomized (GDX) or intact male rats (n=14-20/group) implanted with vehicle, finasteride, and/or testosterone. * indicates main effect of gonadal condition, $p < 0.05$, ^ indicates main effect of T condition, $p < 0.05$, # indicates main effect of inhibitor condition, $p < 0.05$.

Figure 2: Represents the cognitive data. This includes the percentage of time (± SEM) for the Object Recognition and Object Placement tasks and the mean average (± SEM) latency to the hidden platform in the Morris Water Maze. * indicates main effect of gonadal condition, $p < 0.05$, ^ indicates main effect of T condition, $p < 0.05$, ## indicates a tendency for main effect of inhibitor condition, $p < 0.05$.

Figure 3 Represents the anxiety data. This includes the mean central entries (± SEM) for the Open Field task, the mean white chamber entries (± SEM) for the Light/Dark Transition task, and the mean time spent in the mirror chamber (± SEM) for the Mirror Maze task. * indicates main effect of gonadal condition, $p < 0.05$.

Table I: Concentrations of testosterone, dihydrotestosterone, and estradiol in midbrain, hypothalamus, cortex, hippocampus, interbrain, and plasma, of gonadectomized (GDX) or intact male rats (n=14-20/group) implanted with vehicle, finasteride, and/or testosterone (mean±SEM). * indicates main effect of gonadal condition, $p < 0.05$, ^ indicates main effect of T condition, $p < 0.05$, ^^ indicates tendency for a main effect of T condition, $p < 0.1$

Gonad	**GDX**				**Intact**				Gonadal Condition	T Condition
T	Vehicle		Testosterone		Vehicle		Testosterone			
Inhibitor	Veh	Fin	veh	Fin	veh	Fin	veh	Fin		
T Levels										
Plasma	28.128	28.447	32.377	33.609	43.865	40.717	41.13	43.525	*	
± Std error	0.56	0.255	0.51	0.676	1.263	0.884	0.772	1.272		
Hypothalamus	3.966	3.977	3.958	4.09	4.46	4.322	4.26	4.373	*	
± Std error	0.163	0.099	0.105	0.053	0.122	0.059	0.253	0.161		
Hippocampus	1.633	1.593	1.641	1.744	1.806	1.844	1.703	1.833	*	
± Std error	0.028	0.035	0.024	0.042	0.041	0.04	0.078	0.062		
Cortex	1.545	1.646	1.637	1.692	1.866	1.752	2.106	1.806	*	^^
± Std error	0.03	0.026	0.034	0.034	0.032	0.026	0.236	0.04		
Midbrain	1.914	1.957	2.051	2.079	2.326	2.228	2.347	2.275	*	
± Std error	0.048	0.044	0.05	0.091	0.063	0.034	0.119	0.091		
DHT Levels										
Plasma	150.68	133.937	308.286	301.875	849.76	710.651	450.821	547.135	*	
± Std error	9.63	15.756	47.769	32.898	119.591	59.446	44.865	58.194		
Hypothalamus	95.086	46.365	56.34	39.919	90.757	107.877	100.787	91.857	*	
± Std error	41.424	14.486	18.786	3.905	18.01	22.705	24.1	12.263		
Hippocampus	21.775	30.598	20.642	33.068	67.674	62.954	66.648	71.315	*	
± Std error	6.778	11.006	4.203	9.915	8.815	13.669	4.834	6.4		
Cortex	29.345	16.955	30.453	26.459	45.703	49.792	48.646	42.583	*	
± Std error	10.17	5.116	11.019	5.445	6.16	9.46	5.364	4.009		
Midbrain	14.565	14.427	20.243	40.14	63.25	51.039	66.464	65.021	*	^
± Std error	3.407	2.746	3.232	13.297	9.613	6.328	5.622	5.468		
E2 Levels										
Plasma	17.913	17.153	17.357	17.391	16.175	16.306	14.063	13.632	*	^

± Std error	0.33	0.369	0.317	0.287	0.671	0.269	0.578	0.464		
Hypothalamus	2.507	2.694	2.584	2.712	2.206	2.146	2.102	2.503	*	^
± Std error	0.128	0.189	0.145	0.111	0.128	0.102	0.162	0.431		
Hippocampus	1.025	1.034	1.058	1.124	0.941	0.888	0.989	1.302		
± Std error	0.05	0.04	0.047	0.049	0.043	0.04	0.083	0.223		
Cortex	1.001	1.012	1.026	1.118	0.888	0.826	1.075	0.972		
± Std error	0.059	0.06	0.054	0.053	0.046	0.034	0.227	0.192		
Midbrain	1.286	1.347	1.357	1.337	1.116	1.071	0.97	1.212	*	
± Std error	0.084	0.072	0.087	0.088	0.055	0.061	0.08	0.166		
5α-reductase activity										
Hypothalamus	19.909	11.874	14.645	9.774	19.745	24.247	22.341	20.477		
Hippocampus	13.018	18.281	12.547	22.523	37.546	32.414	39.855	38.696		
Cortex	18.359	10.104	17.923	15.851	24.227	28.349	23.364	23.466		
Midbrain	7.882	7.274	9.611	18.344	26.527	22.508	28.17	28.578		

Table II: Simple regressions for all behaviors examined in total by 5α-reductase activity in the hippocampus for GDX or intact male rats (n=14-20/group) implanted with vehicle, finasteride, and/or testosterone (left), and GDX or intact male rats (n=13-18/group) implanted with vehicle, formestane, and/or testosterone.

Finasteride-Task vs. hippocampal T 5α Reduction	**R Squared**	**Regression P-Value**		**Formestane-Task vs. hippocampal T 5α Reduction**	**R Squared**	**Regression P-Value**
Lat to Intro	0.075	0.0015		Lat to Intro	0.226	<0.0001
Central entries	0.006	0.3824		Central entries	0.003	0.5287
Interaction Time	0.014	0.1812		Interaction Time	0.094	0.0007
Mirror Entries	0.071	0.002		Mirror Entries	0.005	0.442
White Entries	0.029	0.053		White Entries	0.021	0.1116
Obj Rec % Novel	0.042	0.019		Obj Rec % Novel	0.009	0.311
Obj Place % Novel	0.025	0.0736		Obj Place % Novel	9.83E-05	0.9148
H20 Avg Lat	0.001	0.6782		H20 Avg Lat	0.001	0.7356
FST Struggling	0.022	0.0833		FST Struggling	0.003	0.5287

Figure 1

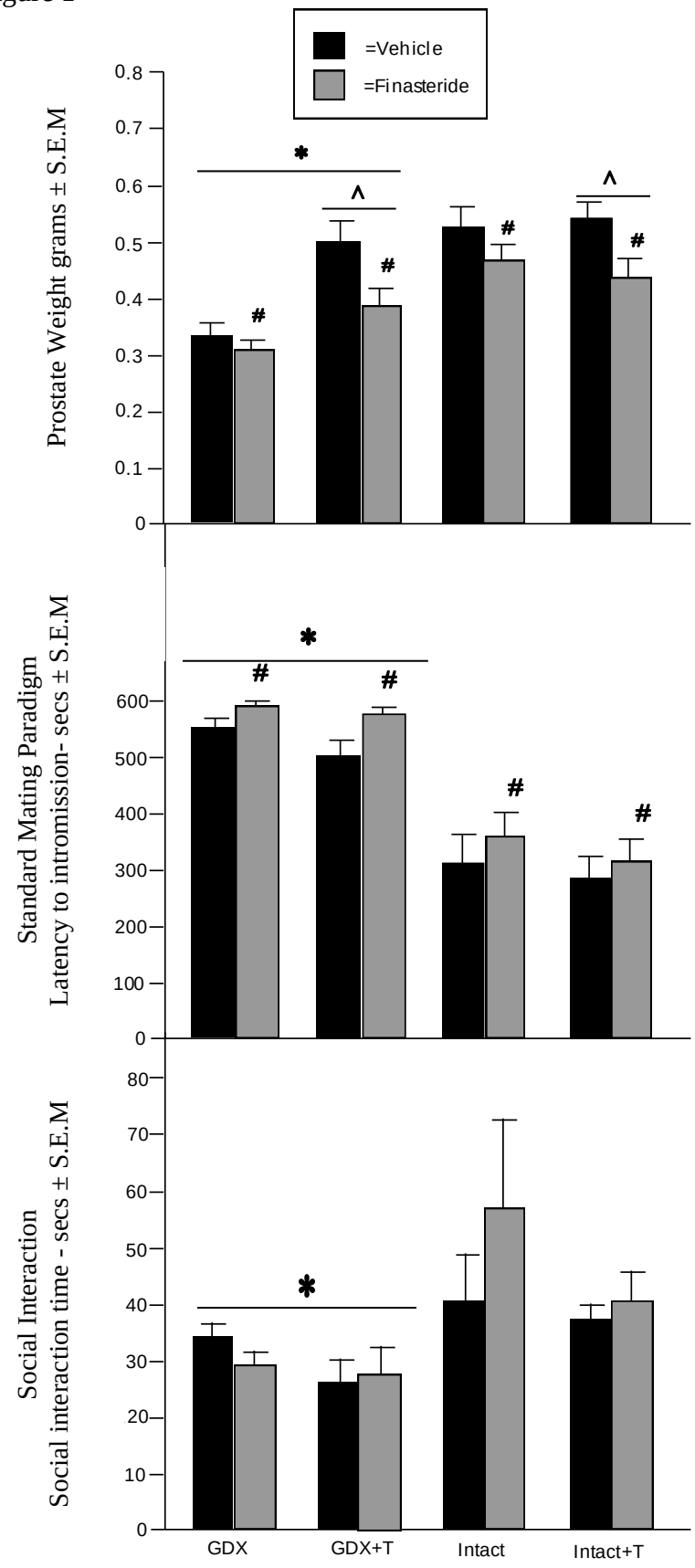

=Vehicle
=Finasteride
Prostate Weight grams ± S.E.M
Standard Mating Paradigm
Latency to intromission- secs ± S.E.M
Social Interaction
Social interaction time - secs ± S.E.M
GDX
GDX+T
Intact
Intact+T

Figure 2

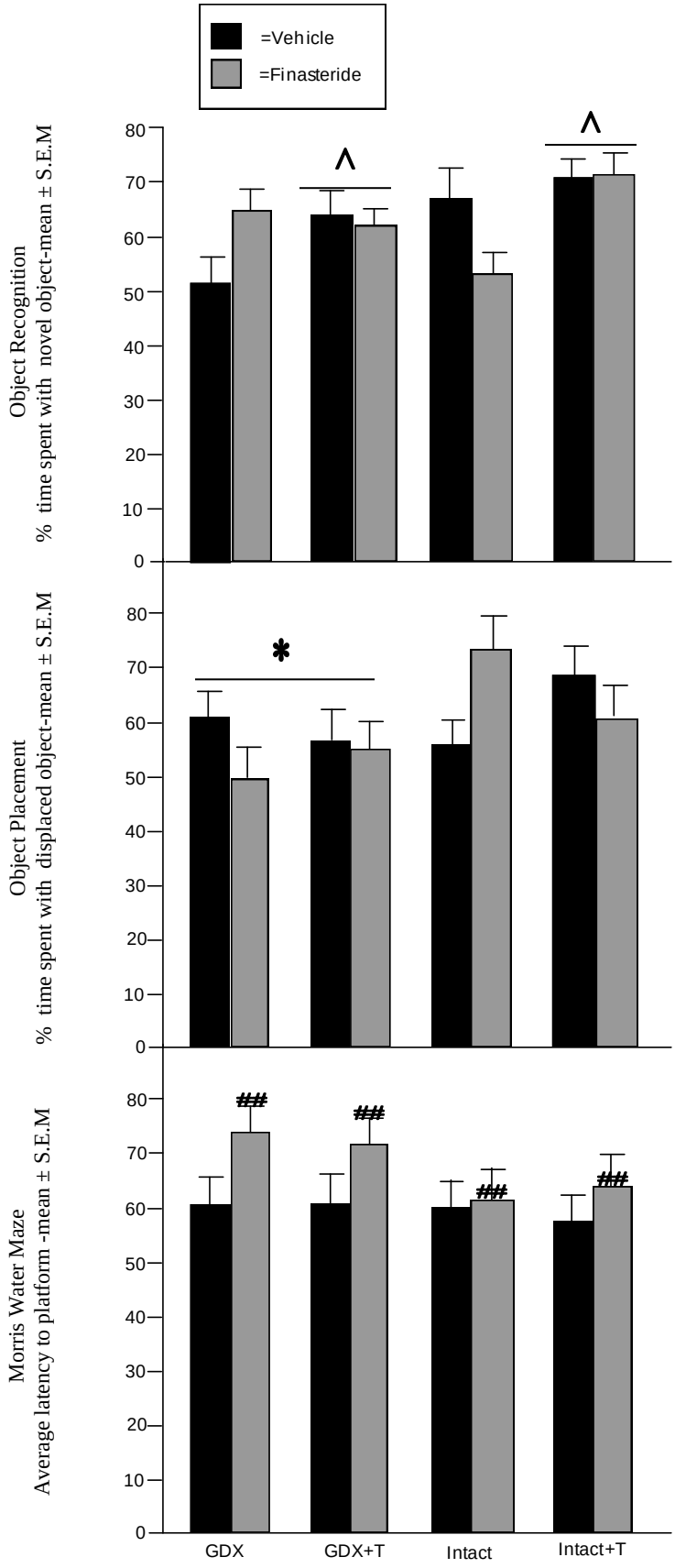

=Vehicle
=Finasteride
Object Recognition
% time spent with novel object-mean ± S.E.M
Object Placement
% time spent with displaced object-mean ± S.E.M
Morris Water Maze
Average latency to platform -mean ± S.E.M
^
*
##
GDX
GDX+T
Intact
Intact+T

Figure 3

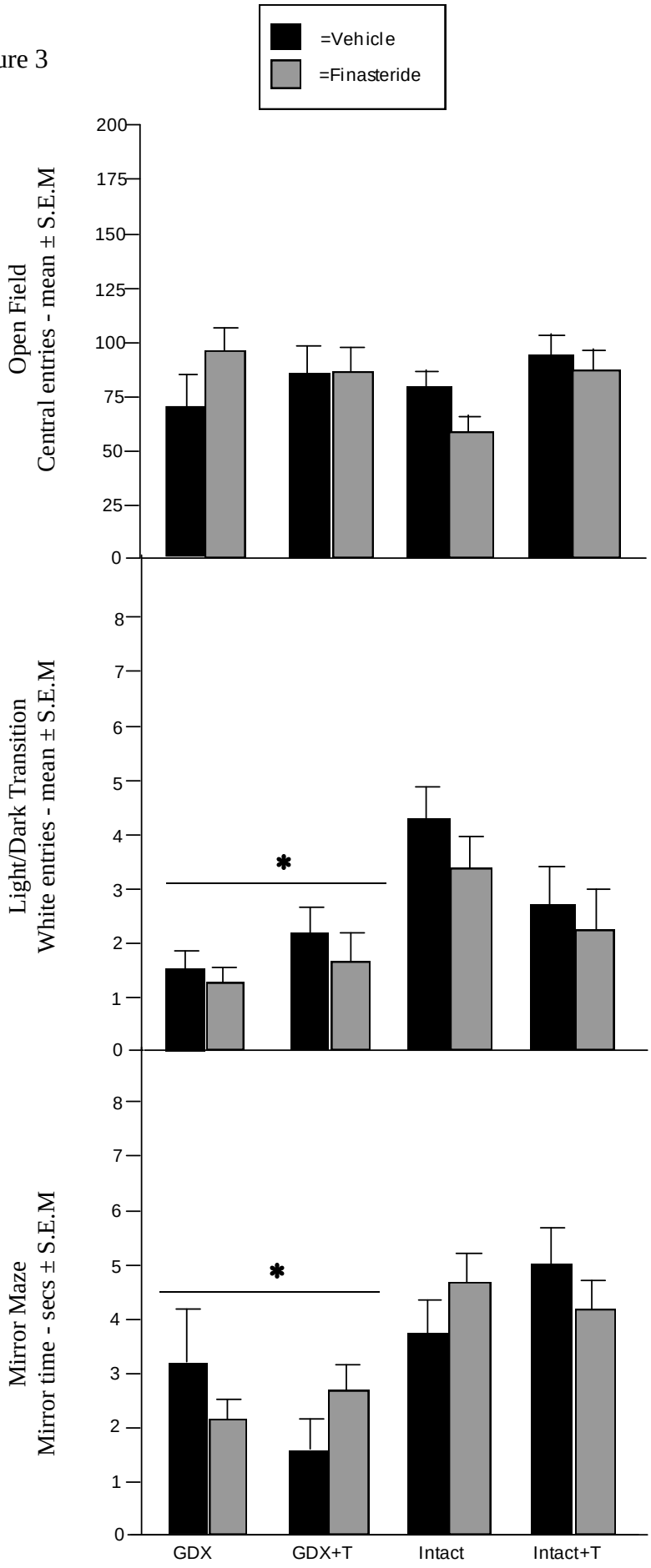

=Vehicle
=Finasteride
Open Field
Central entries - mean ± S.E.M
200
175
150
125
100
75
50
25
0
Light/Dark Transition
White entries - mean ± S.E.M
8
7
6
5
4
3
2
1
0
*
Mirror Maze
Mirror time - secs ± S.E.M
8
7
6
5
4
3
2
1
0
*
GDX
GDX+T
Intact
Intact+T

Figure Legends- Experiment 2

Figure 4: Depicts the average prostate weight (±SEM) and represents the sociosexual data. This includes the latency (± SEM) to intromission for the sex testing and the mean interaction time (± SEM) for the Social Interaction task for gonadectomized (GDX) or intact male rats (n=13-18/group) implanted with vehicle, formestane, and/or testosterone. * indicates main effect of gonadal condition, $p < 0.05$, ^ indicates main effect of T condition, $p < 0.05$.

Figure 5: Represents the cognitive data. This includes the percentage of time (± SEM) for the Object Recognition and Object Placement tasks and the mean average (± SEM) latency to the hidden platform in the Morris Water Maze. ** indicates a tendency for main effect of gonadal condition $p < 0.10$, # indicates a significant difference for the inhibitor condition $p < 0.05$.

Figure 6 Represents the anxiety data. This includes the mean central entries (± SEM) for the Open Field task, the mean white chamber entries (± SEM) for the Light/Dark Transition task, and the mean time spent in the mirror chamber (± SEM) for the Mirror Maze task. * indicates main effect of gonadal condition, $p < 0.05$ ## indicates a tendency for main effect of the inhibitor condition.

Table III: Concentrations of testosterone, dihydrotestosterone, and estradiol in midbrain, hypothalamus, cortex, hippocampus, interbrain, and plasma of gonadectomized (GDX) or intact male rats (n=13-18/group) implanted with vehicle, formestane, and/or testosterone (mean±SEM). * indicates main effect of gonadal condition, $p < 0.05$, ^ indicates main effect of T condition, $p < 0.05$, ^^ indicates tendency for a main effect of T condition, $p < 0.1$, and ## indicates tendency for a main effect of inhibitor condition, $p < 0.1$

Gonad	**GDX**				**Intact**				Gonadal Condition	T Condition	Inhibitor Condition
T	Vehicle		Testosterone		Vehicle		Testosterone				
Inhibitor	veh	Form	veh	Form	veh	Form	veh	Form			
T Levels											
Plasma	25.661	25.787	29.267	30.19	45.213	46.837	42.453	40.365	*		
± Std error	0.669	0.328	0.685	0.498	2.406	3.091	1.433	0.622			
Hypothalamus	3.134	3.396	3.375	3.641	3.849	3.965	4.419	3.798	*		
± Std error	0.286	0.133	0.152	0.15	0.175	0.102	0.332	0.266			
Hippocampus	0.01	0.011	0.011	0.012	0.014	0.013	0.014	0.014	*	^	##
± Std error	0.001	3.90E-04	4.79E-04	4.15E-04	3.67E-04	3.24E-04	0.001	3.77E-04			
Cortex	0.01	0.011	0.011	0.012	0.013	0.013	0.012	0.012	*		
± Std error	3.41E-04	4.11E-04	2.89E-04	2.12E-04	3.19E-04	2.68E-04	4.13E-04	4.47E-04			
Midbrain	1.803	1.798	1.75	1.86	1.957	2.089	1.867	1.777	*		
± Std error	0.069	0.084	0.08	0.037	0.061	0.063	0.073	0.161			
DHT Levels											
Plasma	2139.227	1503.359	1681.376	1670.884	2846.934	2724.256	2072.533	1773.293	*	^	
± Std error	275.988	391.94	269.121	385.3	194.511	147.792	242.12	172.43			
Hypothalamus	34.91	25.899	35.966	37.89	178.756	207.481	225.274	167.426	*		
± Std error	2.531	4.543	3.398	4.615	38.025	47.473	67.229	16.138			
Hippocampus	14.602	14.715	16.861	21.959	48.494	69.982	37.45	40.313	*		
± Std error	1.826	1.651	2.677	4.561	9.08	14.903	3.027	6.333			
Cortex	14.791	11.768	14.071	19.82	57.538	75.165	53.107	59.006	*		
± Std error	1.907	1.687	1.959	3.856	11.392	16.074	4.803	7.236			
Midbrain	14.638	14.841	18.237	19.146	59.497	93.98	58.189	53.064	*		
± Std error	1.706	1.948	2.741	2.286	11.901	20.799	3.545	5.393			
E2 Levels											
Plasma	18.187	18.407	17.892	18.284	18.433	17.976	20.52	20.321			

± Std error	0.282	0.376	0.387	0.33	0.55	0.283	1.256	1.559			
Hypothalamus	2.807	2.83	2.88	2.947	1.66	2.696	0.267	0.285			
± Std error	0.123	0.181	0.179	0.068	0.305	0.108	0.022	0.025		^^	
Hippocampus	1.185	1.195	1.156	1.068	0.932	1.081	0.749	0.929	*		
± Std error	0.054	0.071	0.063	0.042	0.084	0.026	0.045	0.22			
Cortex	1.102	1.159	1.095	1.081	1.047	1.073	0.992	1.018	*		
± Std error	0.048	0.059	0.038	0.033	0.054	0.029	0.123	0.11			
Midbrain	1.382	1.468	1.422	1.414	1.213	1.304	1.263	1.099	*		
± Std error	0.065	0.097	0.054	0.05	0.101	0.06	0.109	0.136			
5α-reductase activity											
Hypothalamus	10.564	7.762	10.802	10.559	45.703	50.942	45.684	43.873			
Hippocampus	1506.632	1332.824	1569.924	1882.994	3547.376	5109.681	2719.581	2795.789			
Cortex	1460.975	1114.081	1251.234	1707.829	4542.731	5432.153	4281.126	4746.839			
Midbrain	8.252	8.165	10.699	10.308	31.217	45.189	31.829	30.728			

Figure 4

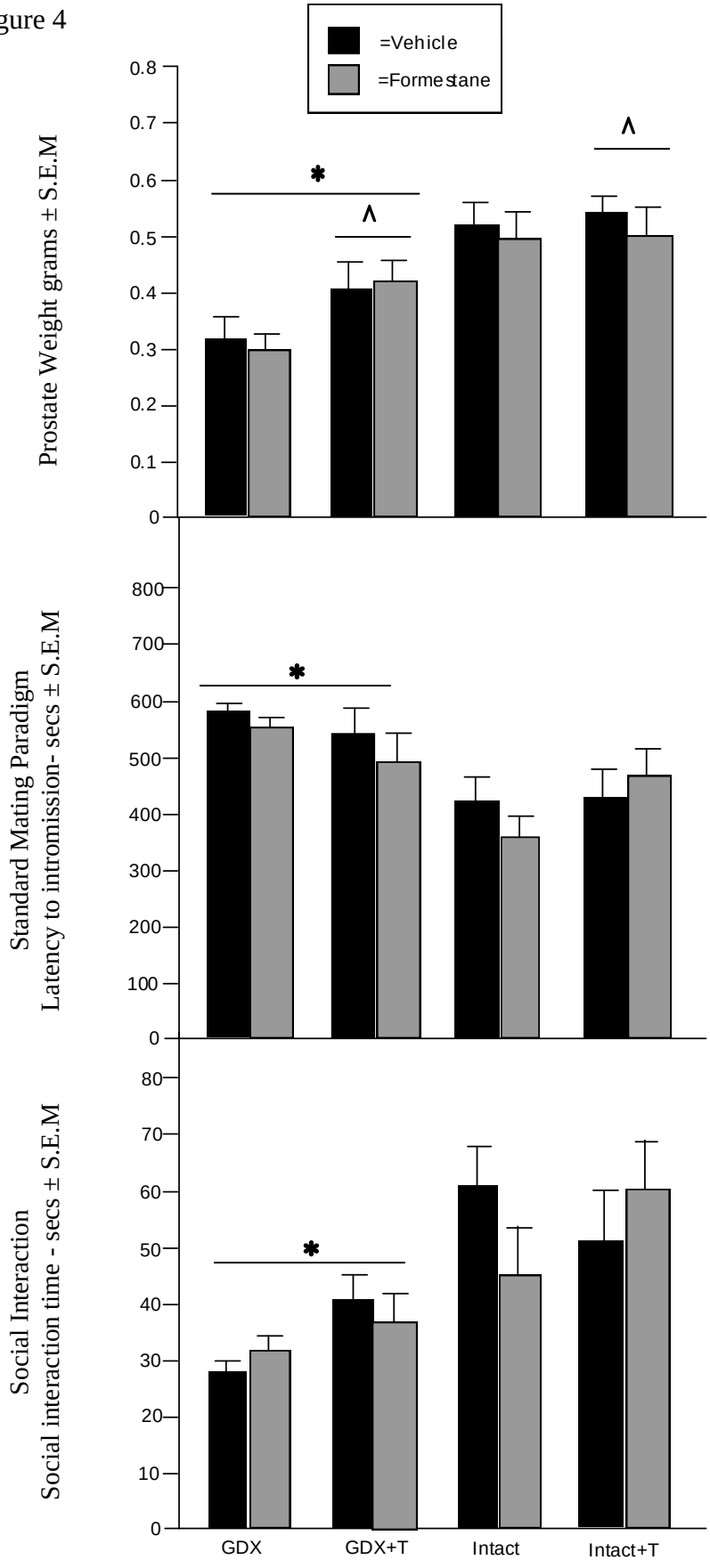

=Vehicle
=Formestane
Prostate Weight grams ± S.E.M
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
^
^
Standard Mating Paradigm
Latency to intromission- secs ± S.E.M
800
700
600
500
400
300
200
100
0
*
Social Interaction
Social interaction time - secs ± S.E.M
80
70
60
50
40
30
20
10
0
*
GDX
GDX+T
Intact
Intact+T

Figure 5

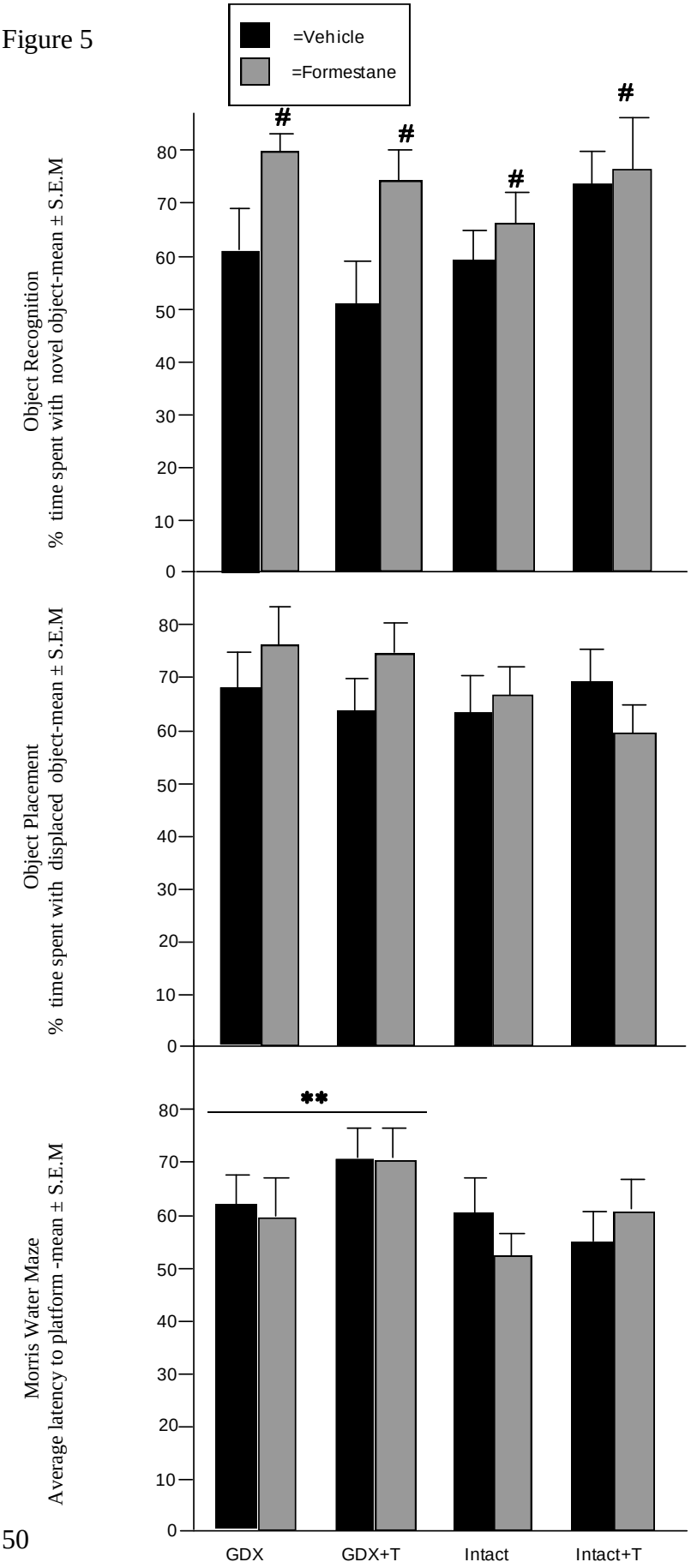
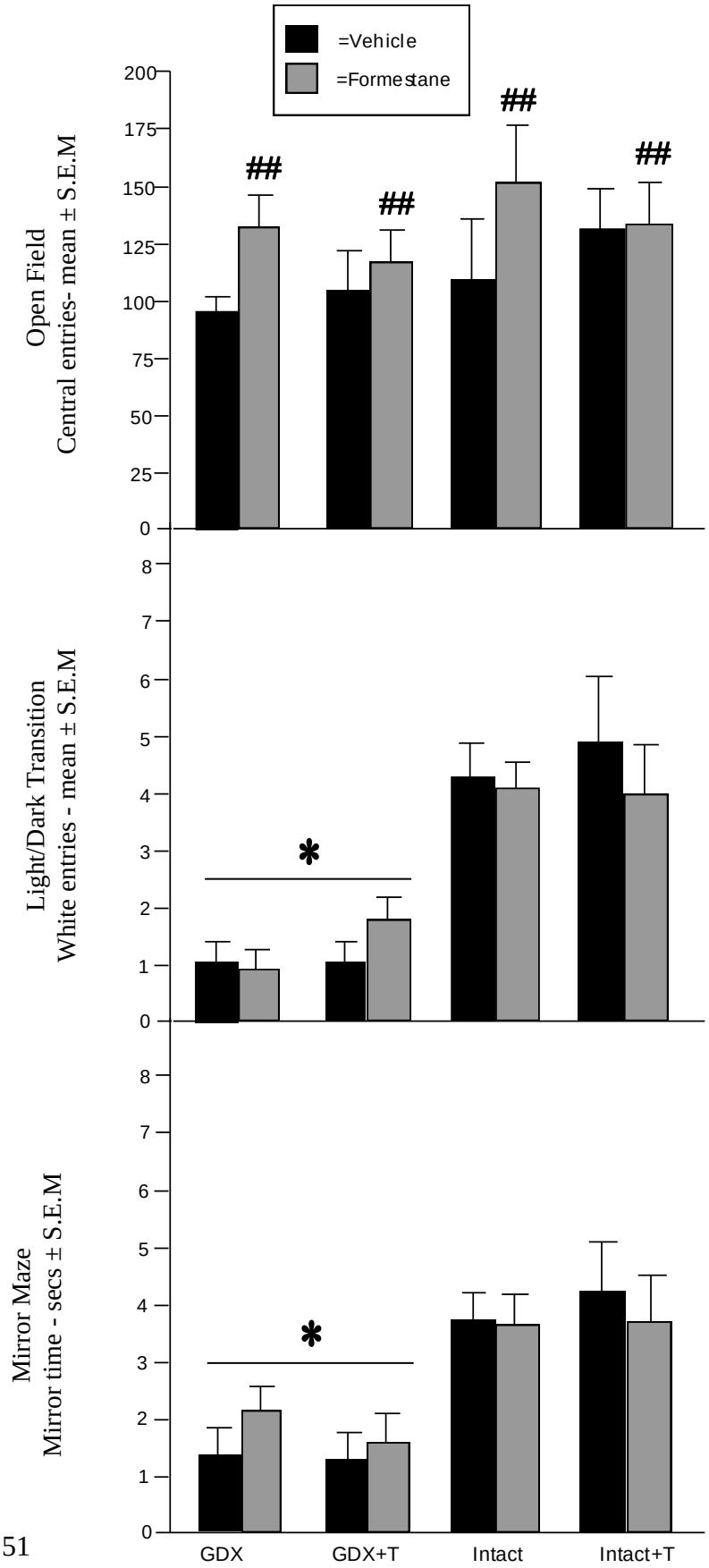

=Vehicle
=Formestane
Object Recognition
% time spent with novel object-mean ± S.E.M
Object Placement
% time spent with displaced object-mean ± S.E.M
Morris Water Maze
Average latency to platform -mean ± S.E.M
#
**
0
10
20
30
40
50
60
70
80
GDX
GDX+T
Intact
Intact+T

Figure 6


=Vehicle
=Formestane
Open Field
Central entries- mean ± S.E.M
Light/Dark Transition
White entries - mean ± S.E.M
Mirror Maze
Mirror time - secs ± S.E.M
##
*
GDX
GDX+T
Intact
Intact+T