The role of testicular hormones and luteinizing hormone in spatial memory in adult male rats

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Attempts to determine the influence of testicular hormones on learning and memory in males have yielded contradictory results. The present studies examined whether testicular hormones are important for maximal levels of spatial memory in young adult male rats. To minimize any effect of stress, we used the Object Location Task which is a spatial working memory task that does not involve food or water deprivation or aversive stimuli for motivation. In Experiment 1, sham gonadectomized male rats demonstrated robust spatial memory, but gonadectomized males showed diminished spatial memory. In Experiment 2, subcutaneous testosterone (T) capsules restored spatial memory performance in gonadectomized male rats, while rats with blank capsules demonstrated compromised spatial memory. In Experiment 3, gonadectomized male rats implanted with blank capsules again showed compromised spatial memory, while those with T, dihydrotestosterone (DHT), or estradiol (E) capsules demonstrated robust spatial memory, indicating that T’s effects may be mediated by its conversion to E or to DHT. Gonadectomized male rats injected with Antide, a gonadotropin-releasing hormone receptor antagonist which lowers luteinizing hormone levels, also demonstrated spatial memory, comparable to that shown by T-, E-, or DHT-treated males. These data indicate that testicular androgens are important for maximal levels of spatial working memory in male rats, that testosterone may be converted to E and/or DHT to exert its effects, and that some of the effects of these steroid hormones may occur via negative feedback effects on LH.

Introduction

For decades testicular hormones, primarily androgens, have been implicated in processes underlying learning and memory (Chambers, 1976; Janowsky, 2006; van Haaren et al., 1990). Several studies have identified androgen receptor mRNA and protein in various regions of the central nervous system in rodents and primates, including the hippocampus and cortex (Abdelgadir et al., 1999; Beyenburg et al., 2000; Choo et al., 1998; Kerr et al., 1995; Young and Chang, 1998), both of which subserve memory. Furthermore, androgens have been shown to influence spine formation in the hippocampus (Leranth et al., 2003), a process associated with memory formation (Leuner and Shors, 2004). Additionally, it has been noted that testicular hormones in males can affect performance on a plethora of learning and memory tasks including those that measure active and passive avoidance (Edinger and Frye, 2007b; Frye et al., 2004; van Haaren et al., 1990), taste aversion learning (Chambers, 1976; Chambers et al., 1981), lever-press behavior (van Haaren et al., 1990) and novel object recognition (Aubele et al., 2008; Ceccarelli et al., 2001). These molecular, cellular, and behavioral observations have built a foundation for the hypothesis that testicular androgens can influence learning and memory in males.

One of the most reliable memory differences noted between males and females is that males generally have an advantage over females in tests of spatial memory, both in rodents (Jonasson, 2005) and in humans (Linn and Petersen, 1985; Voyer et al., 1995). Attempts to address the role of testicular androgens in spatial memory have yielded contradictory results, however. For example, gonadectomy only affects performance in some spatial memory tasks. Gonadectomy of male rats has been shown to have only a minimal effect on the acquisition of reference memory in the Morris water maze task or 8 arm radial maze (Iggor and Sengelaub, 1998; Sandstrom et al., 2006; Spritzer et al., 2008), but significantly impairs spatial working memory (i.e. task-specific memory) in an operant T-maze, a delayed matching-to-place water maze task, and radial arm maze (Daniel et al., 2003; Kritzer et al., 2001; Kritzer et al., 2007; Sandstrom et al., 2006; Spritzer et al., 2008).

Administration of exogenous testosterone (T) to male rodents has produced no effect, a decrease, or an increase in spatial memory.
Studies that administered superphysiological levels of T to gonadally intact adult male rats found no effect or an impairment of performance in the Morris water maze (Clark et al., 1995; Goudsmit et al., 1990) and a radial arm maze task (Smith et al., 1996). When a high dose of T was injected into the hippocampus, it again impaired memory in the Morris water maze (Moradpour et al., 2006; Naghdii et al., 2001). Other studies have concluded that physiological levels of T improve spatial memory. Gonadectomized adult male rats were impaired in the Morris water maze and T-maze and had their performance rescued by supplemental, physiological levels of T (Kritzer et al., 2001; Sandstrom et al., 2006). Moreover, aged male rats treated with T performed better in the water radial arm maze compared to controls (Bimonte-Nelson et al., 2003).

In human males, there is a growing body of literature that supports the potential positive effect of physiological levels of testicular androgens on spatial memory. Although some studies have found no relationship between T and spatial ability or even a negative relationship (Goughie and Kimura, 1991), a number of studies have found that T levels are positively correlated with performance on a variety of spatial tasks including route learning (Cherrier et al., 2001; Choi and Silverman, 2002) and maze navigation (Driscol et al., 2005). Additionally, it is well established that androgen levels decrease with aging in men (Janowsky, 2006; Sherwin, 2003). T supplementation in elderly men improved spatial reasoning and memory (Cherrier et al., 2001; Janowsky et al., 1994). Moreover, men with Alzheimer’s disease have lower androgen levels than controls (Holland et al., 2011) and show improved spatial memory following T treatment (Cherrier et al., 2005b).

Although T is the primary androgen released from the testes, it can be converted to either estradiol (E) (by the enzyme aromatase) to act at estrogen receptors, or to the nonaromatizable androgen dihydrotestosterone (DHT) (by the enzyme 5α-reductase), which binds to androgen receptors. Aromatase, estrogen receptors, and 5α-reductase have been detected in the hippocampus and other brain regions (Hojo et al., 2004; Loy et al., 1988; MacLusky et al., 1994; Pelletier et al., 1994). Thus, testosterone may exert its effects on spatial memory by working as either an androgen or an estrogen.

Some studies support the hypothesis that T can enhance memory in males via its estrogenic metabolites. For example, intrahippocampally-injected E enhanced spatial memory in intact male rats in a water maze (Packard et al., 1996). Consistent with this, mice lacking the gene that codes for aromatase demonstrated impaired spatial reference memory in the Y-maze (Martin et al., 2003). Moreover, subcutaneous E capsules enhanced spatial working memory in a maze task in young adult and aged male rats (Lunie and Rodriguez, 1994). However, other studies have presented contrasting evidence. Administration of E to gonadectomized male rats had no effect on a delayed alternation task (Kritzer et al., 2007). E also impaired spatial memory performance in a water maze when it was injected into the hippocampus at high doses (Moradpour et al., 2006). Consistent with this, an aromatase inhibitor improved performance in the Morris water maze (Moradpour et al., 2006). Similarly, healthy, elderly men treated with either T alone or T with an aromatase inhibitor both demonstrated improved spatial memory compared to placebo-treated controls (Cherrier et al., 2005a).

There is evidence that DHT and possibly other reduced metabolites play a role in emotional memory (Edinger and Frye, 2007b; Frye et al., 2004), but it remains unclear whether or not they are important for spatial memory. In aged male rats, T improved working memory in a radial arm maze, and DHT-treated males generally scored between sham-treated and T-treated males (Bimonte-Nelson et al., 2003). Specifically, DHT-treated males showed a significant decrease in the number of working memory incorrect errors, similar to T-treated males, but DHT overall had only a small, usually nonsignificant, facilitatory effect on working memory in aged males.

Gonadal hormones can have indirect as well as direct actions on the brain. In males, gonadal hormones exert negative feedback effects keeping gonadotropin releasing hormone (GnRH) and Luteinizing Hormone (LH) levels low relative to those present in gonadectomized males. Recent work in female rats and mice has indicated that high levels of LH may have a detrimental effect on the hippocampus and spatial memory (Berry et al., 2008; Bryan et al., 2010; Casadesus et al., 2006; Casadesus et al., 2007; Ziegler and Thornton, 2010) but to our knowledge no research has yet been reported for male rodents. There are indications that high levels of LH may be harmful to the memory of human males as higher LH is correlated with poor memory recall in men (Hyde et al., 2010), and serum LH is significantly higher in individuals with Alzheimer’s disease compared toagematched controls (Bowen et al., 2000; Short et al., 2001).

Many factors may contribute to the conflicting effects of hormones that have been seen in previous studies. In addition to the type of hormone (e.g., T, DHT, or E) the level of hormone may be important; androgens may have differential effects depending on whether they are at physiological or superphysiological concentrations (Sandstrom et al., 2006). Moreover, the type of task and the type of spatial memory, be it spatial reference, or spatial working memory may be critical (Dohanich et al., 2009; Sandstrom et al., 2006). Most spatial memory tests require extensive training and rely upon food or water deprivation or an aversive stimulus (e.g. forced swimming in a pool of water) to motivate males to perform. It has been suggested that the behavioral effects of testicular hormones may differ whether the behavior is an aversively or positively motivated behavior (van Haaren et al., 1990) and there are known interactions between stress and gonadal hormones (Andreano and Cahill, 2005; Vial, 2002).

In the present studies we used the Object Location Memory Task (OLMT; Emaceur et al., 1997), which is unencumbered by potential stress effects from food or water deprivation or aversive stimuli, to examine the effects of physiological levels of testicular hormones and luteinizing hormone on spatial memory in adult male rats. First, to test the hypothesis that testicular secretions contribute to spatial memory, gonadectomized and sham-gonadectomized adult male rats were compared in the OLMT. Secondly, to test the hypothesis that the deficits observed in gonadectomized rats were due to the absence of T from the testes, gonadectomized rats were implanted subcutaneously with blank capsules or capsules containing T and tested for spatial memory. Finally, to examine whether T’s effect on spatial memory is mediated by its estrogenic or androgenic metabolites or by inhibition of high LH levels, gonadectomized males were tested for spatial memory after they were implanted with a capsule that contained either T, DHT, or E, or were injected with the LH-lowering compound Antide.

General methods

Animals

Adult male Sprague–Dawley rats, derived from the breeding of animals purchased from Hilltop Animal Laboratories, were weaned at four weeks of age and then housed in same-sex groups. After surgery, animals were housed in groups of 2–3 in 27.9 cm × 20 cm × 17.8 cm cages with ad libitum access to Purina Labdiet and water. All were kept on a 14 hour light: 10 hour dark cycle (7:00 pm lights off). The Oberlin College Institutional Animal Care and Use Committee approved all procedures.

Surgery and hormone implants

Rats were gonadectomized (GDX) or sham gonadectomized under anesthesia with 2–3% isoflurane, 1 l/min oxygen using aseptic technique. For gonadectomy a midline abdominal incision was made, testes were exposed, the vas deferens and accompanying blood vessels were ligated bilaterally and the testes were removed. The incised abdominal muscles were sutured together, and the skin incision was closed with surgical staples.
closed with wound clips. Sham gonadectomy was identical except the testes were exposed but not ligated or removed.

Males in Experiments 2 and 3 also received during GDX/sham surgery a hormone implant inserted subcutaneously through a small midline incision in the back near the neck. Implants contained either testosterone (T; Sigma), β-estradiol (E; Sigma), dihydrotestosterone (DHT; Sigma) or no hormone, and consisted of silastic tubing (1.57 mm i.d., 3.18 mm o.d.; Dow Corning) plugged at each end with 5 mm cut from a wooden applicator stick (Fisher Scientific). T, DHT, and blank capsules were 20 mm long and filled with 10 mm of hormone, and E capsules were 15 mm long with 5 mm of hormone. T, DHT, and E capsules of these sizes were found to produce circulating hormone concentrations at physiological concentrations (e.g. 6.05 ± 0.67 ng/ml: Sandstrom et al., 2006; 7.0 ± 0.2 ng/ml: Frye et al., 2004; and approximately 90 pg/ml: Luine and Rodriguez, 1994, respectively). The capsule ends were sealed with elastomer (Sylgard 184 silicone elastomer base and curing agent; Dow Corning) or rubber sealant (100% silicone rubber sealant; Dow Corning). The capsules were equilibrated by soaking in 0.9% saline solution for 24 h. Prior to implantation the capsules were rinsed with 70% ethanol.

Object location memory test

The test used was based on the one described by Ennaceur et al. (1997) and Berry et al. (2008). This test utilizes rats’ natural tendency to pay more attention to movements than to unmov ed objects. If a rat explores the moved object more than the unmoved object, it demonstrates good spatial memory. The test area was an open field: an 80 cm × 80 cm × 30 cm box with a grid of 10 cm × 10 cm squares. All testing was done during the rats’ dark phase and the testing room was dimly lit with red light. For visual cues, a white cross on a 70 cm × 54 cm black background was displayed on one wall.

Three-to-five days after surgery, rats underwent four days of habituation in the test area: on day 1 groups of 2–4 rats spent 20 min in the test box with wood shavings; on day 2 groups of 2–4 rats spent 20 min in the box without wood shavings; on day 3 rats explored the box with wood shavings individually for 5 min; on day 4 rats explored the box without wood shavings individually for 5 min, and testers noted the number of lines crossed on the floor grid as well as the amount of time the rats spent in the center of the arena, defined by the center 16 squares. There is an inverse relationship between activity and anxiety levels in rats, and differences in locomotor activity and time spent in the center of an open field have been used to determine differences in anxiety (Bronstein, 1972).

The OLM T involved two trials: an exposure trial and a test trial. In the exposure trial, two identical objects stood in two adjacent corners of the box, 20 cm from the nearest walls. A single rat was introduced into the test box, placed facing the wall farthest from the two objects, and was allowed to explore the test box with wood shavings for 5 min. Testers noted the amount of time the rat spent exploring each of the two objects. Exploration was recorded when the rat’s nose was more than 2 cm away from an object, as described by Ennaceur and Delacour (1988). Rats that failed to explore both objects during the exposure trial were excluded. After the exposure trial, the rat was returned to its home cage. Between trials the two objects were rinsed with 70% ethanol and the wood shavings were mixed to disrupt odor signals.

After a 30 min inter-trial interval one of the objects was moved to a new quadrant in one of two test configurations, counterbalanced for left and right bias (see Berry et al., 2008). The rat was returned to the test box for the three-minute test trial and the amount of time the rat spent exploring each of the two objects was recorded. Rats were tested 2 times with 4–7 days between tests, and the test results were averaged for each rat. Testers were blind to the treatment group scored.

Experiment 1

To determine if testicular hormones are important for spatial memory in male rats, adult males were either gonadectomized (n = 10) or sham gonadectomized (n = 10) and tested for spatial memory with the OLM T as described above.

Experiment 2

Because gonadectomy impaired spatial memory, and T is the primary testicular hormone, we hypothesized that T treatment would reverse the effect of gonadectomy and improve spatial memory. Rats were gonadectomized and implanted with either T capsules (n = 8) or blank capsules (n = 8) and were tested with the OLM T.

Experiment 3

T can be reduced to DHT or aromatized to E in the brain. To determine whether T, DHT, and/or E could reverse the effect of gonadectomy, males were gonadectomized and implanted with a T capsule (n = 12), DHT capsule (n = 12), E capsule (n = 11), or a blank capsule (n = 13). An additional group of gonadectomized males (n = 11) was injected with 1 ng/kg/ml of the GnRH receptor antagonist Antide (Bachem, Torrance CA) which was dissolved in sterile distilled water and injected sc 20–24 h prior to each test. This dose of Antide lowers serum LH levels within 6 h and maintains lowered LH for at least 96 h (Ziegler and Thornton, 2010). As a control for the Antide injection, approximately half of each steroid hormone treated group was injected with 1 ml/kg vehicle 20–24 h prior to each test. Because there were no differences between males injected with Antide vehicle or not injected, the data were combined. All subjects were tested with the OLM T.

Data analysis

For all experiments, the amount of time spent exploring the two objects in the exposure and test trials was analyzed with a mixed two-way analysis of variance (ANOVA) with a repeated measure and an independent factor: 2 repeated objects × number of independent treatment groups. A significant F value was followed by post hoc Bonferroni’s multiple comparison tests to compare exploration times for left versus right objects in the exploration trials. For locomotion data, either a two-tailed independent t-test (for two groups) or a one-way ANOVA (for more than two groups) was used to compare the number of lines crossed and amount of time spent in the center of the arena followed by Bonferroni’s post hoc tests if a significant effect was seen. Additionally, t-tests were used for planned comparison analysis when an a priori hypothesis was present. Because it was hypothesized that gonadally intact and hormone treated males would show better spatial memory than gonadectomized males, paired t-tests were used within a treatment group to compare the exploration times for the moved vs. unmoved objects. To compare across treatment groups in Experiment 3, difference scores were calculated as the difference between the amount of time spent exploring the moved and unmoved objects. Difference scores were then analyzed with one-way ANOVA and planned comparisons. Results are given as mean ± SEM and an alpha level of ≤ 0.05 was considered statistically significant.

Results

Experiment 1: effects of gonadectomy in males on object location memory

Gonadally intact males showed good spatial memory and gonadectomy significantly impaired object location memory in adult male rats. ANOVA indicated a significant effect of gonadectomy (F(1,18) =
9.86, p = 0.006), a non-significant effect of object (F(1,18) = 4.18, p = 0.056), and a significant interaction (F(1,18) = 5.88, p = 0.026). Gonadally intact males explored the moved object significantly more than the unmoved object during the test trial (moved = 14.2 ± 1.4 s, unmoved = 8.0 ± 1.7 s, p = 0.025), thus demonstrating good spatial memory. Gonadectomized (GDX) rats did not show good spatial memory since they did not explore the moved object more than the unmoved object (moved = 7.0 ± 0.9 s, unmoved = 7.6 ± 1.1 s, p = 0.742) (Fig. 1A).

Differences in spatial memory between gonadally intact and GDX males were not due to differences in activity or anxiety levels (Fig. 1B). GDX and gonadally intact males did not differ significantly in the number of lines crossed (GDX = 103 ± 16.0, intact = 88 ± 12.7, p = 0.487) or in the time spent in the center of the testing arena (GDX = 2.7 ± 0.7 s, intact = 2.6 ± 0.9 s, p = 0.933, data not shown) during activity/anxiety tests.

Gonadally intact and GDX rats also did not differ significantly in the amount of time they spent exploring the two objects during the exposure trials (GDX F(1,18) = 1.88, p = 0.187; interaction F(1,18) = 0.43, p = 0.520) however they did show a side preference (F(1,18) = 7.35, p = 0.014). Bonferroni post-hoc tests indicated that intact males did not demonstrate a significant preference toward either the right or the left object during the exposure trial (left = 19.4 ± 8.6 s, right = 17.0 ± 7.9 s, p = 0.273), though GDX rats spent more time exploring the left object (left = 15.5 ± 2.4 s, right = 11.6 ± 2.5 s, p = 0.006). Any location preferences were minimized in the test-trial by counterbalancing the side on which the moved object was placed.

**Experiment 2: effects of testosterone on object location memory**

T significantly enhanced object location memory in gonadectomized adult male rats. Using ANOVA, there was a non-significant effect of treatment (F(1,14) = 0.75, p = 0.401), a significant effect of object (F(1,14) = 8.44, p = 0.012) and a significant interaction (F(1,14) = 4.90, p = 0.044), indicating that spatial memory was not equivalent across groups. Rats implanted with T capsules explored the moved object significantly more than the unmoved object during the test trial (moved = 12.6 ± 3.2 s; unmoved = 4.8 ± 1.4 s; p = 0.023), thus demonstrating good spatial memory. Rats implanted with blank capsules did not show significant spatial memory in the test trial since they did not explore the moved object more than the unmoved object (moved = 7.1 ± 1.7 s; unmoved = 6.1 ± 1.3 s; p = 0.484) (Fig. 2A).

Differences in spatial memory were not due to differences in activity or anxiety levels or differential exposure to the objects. Rats with T capsules and rats with blank capsules did not differ significantly in time spent in the center of the testing arena (T = 4.4 ± 1.8 s; blank = 5.5 ± 0.9 s; p = 0.615, data not shown) or in the number of lines crossed on the arena’s floor grid (T = 144 ± 17.2 lines; blank = 134 ± 14.7 lines; p = 0.671) (Fig. 2B). ANOVA revealed that both groups explored the two objects similarly during the exposure trials (treatment F(1,14) = 2.19, p = 0.161; side F(1,14) = 2.96, p = 0.107; interaction F(1,14) = 0.35, p = 0.562) indicating that neither group demonstrated a strong preference toward either the right or the left object during the exposure trial (T: left = 13.6 ± 3.2 s, right = 16.0 ± 3.1 s, p = 0.147; blank: left = 9.3 ± 1.4 s, right = 10.5 ± 1.5, p = 0.449).

**Experiment 3: effects of T, DHT, E, and Antide on object location memory**

T, DHT, E and Antide significantly enhanced object location memory in gonadectomized adult male rats (Fig. 3A). Two-way ANOVA (5 groups×2 objects) indicated a significant effect of object (F(1,54) = 86.8, p < 0.0001) a nonsignificant treatment effect (F(4,54) = 0.95, p = 0.440), and a significant interaction (F(4,54) = 2.74, p = 0.038). Planned comparison analysis revealed that rats with T implants

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**Fig. 1. Gonadectomy impaired spatial memory in males. (A) In the test trial, gonadally intact rats spent more time exploring the moved object than the unmoved object, whereas gonadectomized (GDX) rats did not demonstrate a preference toward either object. *p = 0.025 compared with unmoved object in same treatment group. (B) Gonadectomy did not alter locomotor activity. The two groups did not exhibit a statistically significant difference in the number of lines crossed in an open field.**

**Fig. 2. Testosterone rescued spatial memory in gonadectomized males. (A) In the test trial, gonadectomized male rats implanted with T capsules demonstrated good spatial memory by spending more time exploring the moved object than the unmoved object, whereas rats implanted with blank capsules did not demonstrate a significant preference toward either object. *p = 0.023 compared with unmoved object in same treatment group. (B) T did not alter locomotor activity. The two groups did not exhibit a statistically significant difference in the number of lines crossed in an open field.**
again explored the moved object more than the unmoved object (moved = 21.6 ± 2.8 s, unmoved = 11.4 ± 1.3 s, p = 0.0006); and rats with blank capsules showed no significant memory (moved = 15.1 ± 1.5 s, unmoved = 12.0 ± 1.6 s, p = 0.125). Rats with DHT or E implants also explored the moved object significantly more than the unmoved object (DHT: moved = 17.9 ± 2.3 s, unmoved = 9.9 ± 1.0 s, p = 0.0046; E: moved = 21.8 ± 1.9 s, unmoved = 10.6 ± 1.4 s, p = 0.0004). Males administered Antide also showed strong spatial memory (moved = 20.5 ± 1.4 s, unmoved = 9.7 ± 1.2 s, p = 0.00001).

A comparison of difference scores across groups indicated that T, E and Antide all showed better spatial memory than control (blank) animals. A one-way ANOVA revealed a significant effect of treatment (F(4,54) = 2.74, p = 0.038). Planned comparison analysis indicated that T, E, and Antide groups were all significantly different from the blank group (p = 0.032, p = 0.016, p = 0.008, respectively) whereas the DHT group was not (p = 0.136). However the DHT group was also not significantly different from the T, E, or Antide groups (p = 0.302 to 0.491).

A one-way ANOVA suggested that there were differences in locomotion across the treatment groups (F(4,54) = 2.70, p = 0.040), however, although the Antide-treated group tended to be more active than other groups, posthoc Bonferroni pairwise comparisons were not statistically significant (Fig. 3B). Moreover, exploration during the exposure trial did not vary significantly across the treatment groups (Fig. 3C). Two-way ANOVA (5 groups x 2 objects) indicated a nonsignificant effect of treatment (F(4,54) = 2.48, p = 0.055), a nonsignificant side effect (F(1,54) = 0.26, p = 0.610), and a nonsignificant interaction (F(4,54) = 0.83, p = 0.509) indicating that the levels of exploration of the right and left objects were not significantly different within or across treatment groups (blank: left = 18.9 ± 3.0 s, right = 22.5 ± 3.3 s, p = 0.315; T: left = 28.0 ± 3.3 s, right = 28.8 ± 2.8 s, p = 0.700; DHT: left = 20.1 ± 2.8 s, right = 18.8 ± 2.5 s, p = 0.284; E: left = 25.0 ± 2.8 s, right = 26.3 ± 2.4 s, p = 0.585; Antide: left = 27.1 ± 2.0 s, right = 25.6 ± 2.0 s, p = 0.440).

Discussion

These studies have shown that gonadectomy of male rats impairs spatial memory in the object location memory task, and testosterone administration reverses this effect. Treatment with estradiol also improved the spatial memory of gonadectomized males, so it appears that T may exert its effects on spatial memory through its estrogenic metabolites, though this may not be T’s only means of influencing spatial memory, since treatment with dihydrotestosterone, an androgen that is not aromatized to estrogens, also improved performance. Luteinizing Hormone levels may also play a role as Antide, which lowers LH, enhanced spatial memory in gonadectomized male rats, as well.

These hormonal effects are unlikely to be due to effects on non-mnemonic variables such as activity or anxiety. In the present studies there were no reliable differences between gonadally intact and gonadectomized males administered T, E or DHT in the amount of locomotion or time spent in the center of an open field. This is consistent with other studies that revealed no difference in the amount of time on a rotord, or the amount of time spent ambulating, stationary or rearing, or in a reaction time test to assess motor ability whether males were gonadally intact, gonadectomized, or gonadectomized and given T or E (Aubele et al., 2008; Kritzer et al., 2001; Kritzer et al., 2007).

It is possible that Antide altered the animals’ activity levels, which could have affected their performance in the spatial memory tests. That is, the ANOVA on number of lines crossed in Experiment 3 detected a significant effect of treatment, and Antide-treated rats appeared to cross more lines in the open field than rats in other treatment groups. However, there were no statistically significant pairwise differences among the rats in any of the treatment groups. This apparent increase in activity in Antide-treated males could be due to random variability, since previous studies in our lab have not seen any effect of Antide treatment on rats’ activity levels even though a clear enhancement of spatial memory was observed (Ziegler and Thornton, 2010). Even if Antide were found to reliably increase locomotion this is unlikely to account for Antide’s effects on spatial memory. For example, when time spent exploring objects during the exposure trial was examined there were no statistically significant differences among groups and the values for E, T and Antide-treated males were all very similar. Moreover, Antide-treated rats’ exploration times in the test trial were comparable to those of T-, DHT-, and E-treated rats. Thus, the trend toward variation in activity levels is not likely to confound this study’s conclusions with regard to spatial memory performance.

Differences in amount of exposure to the objects are unlikely to account for the differences in spatial memory between treatment groups. There were no statistically significant differences between groups in the amount of time spent exploring the objects during the

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**Fig. 3. T, DHT, E, and Antide enhanced spatial memory in gonadectomized male rats.**

(A) In the test trial, gonadectomized rats treated with T, DHT, E or Antide spent significantly more time exploring the moved object than the unmoved object. Rats with blank capsules did not spend significantly different amounts of time exploring the two objects. *p < 0.01, **p < 0.001 compared with unmoved object in same treatment group. (B) T, DHT, E and Antide had no clear effect on locomotor activity (number of lines crossed in an open field). Although the overall ANOVA was significant, there were no significant pairwise comparisons (p > 0.05). (C) T, DHT, E and Antide did not affect exploration during exposure trials. There were no significant differences between groups in the amount of time spent exploring the left and right objects, and no group demonstrated a significant side preference.
Spatial memory tasks: superphysiological levels of hormones

Our data demonstrating that T can enhance spatial memory contrasts with studies that have given T in superphysiological amounts to males. Although one study reported better remembrance after a delay on a T-maze task (Gibbs, 2005), others have reported no discernible effects on spatial memory tasks (Clark et al., 1995), and numerous others have seen an impairment (Goudsmit et al., 1990).

For example, Moradpour et al. (2006) found that intrahippocampal injections of up to 80 μg of T enanthate (TE) into gonadectomized males impaired their performance in the Morris water maze. Naghdli et al. (2003) similarly observed a dose-dependent impairment when TE was administered into the amygdala in rats; high doses led to an increase in escape latency and travel distance when these rats were tested in the Morris water maze.

The amount of T may be critical in determining the nature of its effect for human males as well. A curvilinear relationship in the shape of an “inverted-U” between salivary T levels and performance on a spatial memory task has been found in men (Moffat and Hampson, 1996). In healthy older men, T injections resulting in physiological T levels enhanced spatial memory, whereas T injections resulting in supraphysiological T levels produced no significant enhancement (Cherrier et al., 2007). These combined observations may indicate a ceiling effect for androgens, such that physiological levels are required for optimal spatial memory, but supraphysiological levels do not improve spatial memory, and may instead be detrimental.

Spatial memory tasks: effects of physiological levels of testicular hormones on reference memory and working memory

Spatial memory has been categorized as spatial reference memory (i.e. memory across trials) and spatial working memory (i.e. task-specific memory). A number of studies have looked at the effects of physiological levels of testicular hormones on spatial reference memory. Studies that have examined the acquisition of reference memory (using the Morris water maze task or 8 arm radial maze) have generally seen at most a minimal effect of gonadectomy (e.g. Isgor and Sengelaub, 1998; Sandstrom et al., 2006; Spritzer et al., 2008), indicating that testicular hormones may not play a strong physiological role in reference memory.

On the other hand, physiological levels of testicular hormones do appear to have effects on spatial working memory tasks, which require animals to retain and manipulate spatial information. These spatial working memory tasks may be divided into ones that test the capacity or the retention of spatial working memory (Sandstrom et al., 2006). The OLMT has been considered to be a spatial working memory task that measures retention (Dohanich et al., 2009; Sandstrom et al., 2006). The present studies using the relatively non-stressful object location task support the results obtained from tasks such as water mazes and radial arm mazes that rely on aversive stimuli or food or water deprivation to increase motivation to complete the task, suggesting that any potential stress effects in those studies did not affect the responsiveness to gonadal hormones (Andreano and Cahill, 2009).

The present data are consistent with and extend the data that indicate that testicular hormones are important for working memory retention. Although Gibbs (2005), using a delayed matching-to-place (DMTP) task, did not see an effect of gonadectomy, Sandstrom et al. (2006) also used a DMTP water maze and found that gonadectomy resulted in declining retention scores as the retention interval increased (i.e. there was no effect of gonadectomy with a 10 min interval but there was with a 60 min interval). Similar to the present studies, they also found that T replacement to physiological levels in gonadectomized males reinstated retention scores to levels shown by gonadally intact males. Our results also support Luine and Rodriguez (1994) who found that gonadectomized males administered E showed better working memory retention than gonadectomized control males (gonadally intact males were not examined), in that study the E-treated males chose more correct arms in a maze trial that included a delay in the middle of the task. The current studies extend these data to indicate that DHT can also enhance spatial memory; gonadectomized DHT-treated males showed robust spatial memory whereas gonadectomized control males did not.

It is possible that DHT may be acting partly through estrogen-dependent mechanisms. T and small amounts of DHT may be converted to 5α-androstane 3α, 17β-diol (3α-diol; Jin and Penning, 2001) and to 5α-androstane 3β, 17β-diol (3β-diol; Torn et al., 2003). 3α-diol has weak activity and 3β-diol has strong activity at estrogen receptors (Wang et al., 2009) and it has been suggested that both may act at the estrogen receptor β to affect cognition (Edinger and Frye, 2007a). Both DHT and 3α-diol when administered to the hippocampus of GDX male rats improved inhibitory avoidance (Edinger and Frye, 2007a) and Osborne et al. (2009) demonstrated that either 3α-diol or 3β-diol administered to GDX adult male rats can enhance performance on a reference memory task using the Morris water maze. It is not known if these compounds play a role in the retention of spatial working memory.

The present studies may be relevant to the research on the role of testicular hormones and working memory capacity. Similarly to working memory retention, capacity is decreased by gonadectomy and restored with T, using either a maze alternation task (Kritzer et al., 2001; Kritzer et al., 2007), or radial arm maze (Daniel et al., 2003; Spritzer et al., 2008). Effects of reduced androgens were not explored, and gonadectomized male rats treated with E were not significantly different from gonadectomized control males (Kritzer et al., 2007). However the levels of E administered were in the low physiological range (25 pg/ml). Both the present studies and Luine and Rodriguez (1994) used implants that give levels in the high physiological range (approximately 90 pg/ml), so it is possible that slightly higher E could also affect working memory capacity.

Spatial memory tasks: effects of high levels of LH

Some of these gonadal hormone effects could occur via an interaction with LH. Following gonadectomy, LH levels in male rats increase within one day and reach maximal levels by about 4–7 days (Frager et al., 1981; Gay and Bogdanove, 1969; Gharib et al., 1986). T, DHT, and E can all feed back and inhibit LH levels in gonadectomized male rats (Frager et al., 1981; Gay and Bogdanove, 1969; Gharib et al., 1986). Consistent with the present studies in males, recent research in female rats and mice has shown that high levels of
LH (or its homologue human chorionic gonadotropin) can inhibit spatial memory, and lowering LH levels with Antide or leuprolide acetate can enhance spatial memory (Berry et al., 2008; Bryan et al., 2010; Casadesus et al., 2006; Casadesus et al., 2007; Ziegler and Thornton, 2010). Interestingly, T supplementation in older men decreased LH levels in addition to improving cognition (Janowsky et al., 1999) and higher LH is correlated with poor memory recall in men (Hyde et al., 2010).

Antide has been shown to induce a long-lasting decrease in LH in rats and non-human primates (Fallest et al., 1995; Weinbauer and Nieschlag, 1993). Although serum LH decrease was not confirmed in the Antide-treated rats in the present study, Antide is known to lower serum LH levels within 6–12 h in rats and non-human primates and maintain lowered LH for at least 96 h (Weinbauer and Nieschlag, 1993; Ziegler and Thornton, 2010).

Because Antide is a GnRH receptor antagonist the question arises whether any of the effects of Antide on spatial memory may be a function of Antide’s inhibition of the GnRH receptor rather than a reduction of LH. Consistent with the possibility that GnRH may have direct effects on hippocampal functioning, GnRH receptors have been identified in the hippocampus of mouse, rat, and sheep (Albertson et al., 2008; Badir and Pelletier, 1987), and Prange-Kiel et al. (2008) found that GnRH treatment increased spine density in hippocampal cultures, though this effect required E synthesis, as co-treatment with the aromatase inhibitor blocked the increase. Moreover, GnRH can alter the electrical and chemical properties of hippocampal neurons (see Skinner et al., 2009 for a review). However, the precise effects of GnRH on memory remain unclear. Allot et al. (1993) found that a GnRH analog improved working memory in aged female rats but this long-term GnRH treatment probably also decreased LH. Mora and Díaz-Velázquez (1985) found that GnRH had mixed effects on retention of active and passive avoidance responses, depending on the training conditions. Although further research is needed, our work in female rats indicates that GnRH does not display a consistent inverse relationship with spatial memory and hence is unlikely to be responsible for the changes seen. For example, gonadectomized female rats with suppressed GnRH showed either poor spatial memory (if GnRH was suppressed with an LH homologue and GnRH receptors were needed, our work in female rats indicates that GnRH does not display mixed effects on retention of active and passive avoidance responses, depending on the training conditions. Although further research is needed, our work in female rats indicates that GnRH does not display a consistent inverse relationship with spatial memory and hence is unlikely to be responsible for the changes seen. For example, gonadectomized female rats with suppressed GnRH showed either poor spatial memory if GnRH was suppressed with an LH homologue and GnRH receptors were needed, our work in female rats indicates that GnRH does not display mixed effects on retention of active and passive avoidance responses, depending on the training conditions.

Recently it has been suggested that rather than there being a simple negative relationship between LH and spatial memory (i.e. high levels of LH lead to low levels of spatial memory) that there might be an inverted U function (i.e. high levels of LH lead to low levels of spatial memory except when LH levels are very high after gonadectomy) (Acosta et al., 2009). Using middle-aged female rats it was suggested that when LH levels are at their highest after GDX they may no longer exert a suppressive effect on spatial memory, at least on reference memory. The present studies did not correlate individual LH levels with spatial memory in individual males so it remains to be determined if any of the variability in the retention of spatial working memory within a group of GDX males could be due to variations in their high levels of LH.

Conclusions

Gonadectomy impaired spatial memory in adult male rats. Treatment with T, DHT, E, or Antide (which lowers LH levels) rescued spatial memory in gonadectomized males, suggesting that T may exert its effects on memory via its androgenic or estrogenic metabolites, or by its inhibition of LH.

Considering the tremendous clinical implications of clarifying the role of testicular hormones in learning and memory in males and its connection to age-related cognitive decline and disease, further studies are strongly warranted, especially those targeting the mechanism and location of action of these hormones in the brain.

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References


