Castration Differentially Affects Spatial Working and Reference Memory in Male Rats

Mark D. Spritzer, Ph.D.,[1,2] Mandeep Gill, B.S.,[1] Alex Weinberg, B.S.,[1] and Liisa A.M. Galea, Ph.D.[1]

[1]Department of Psychology, The University of British Columbia, Vancouver, British Columbia, Canada.

[2]To whom correspondence should be addressed at Department of Biology, McCardell Bicentennial Hall, Middlebury College, Middlebury, Vermont 05753; e-mail: spritzer@psych.ubc.ca.

RUNNING HEAD: Effects of Castration on Spatial Memory in Male Rats

Correspondence and galley proofs:

Mark Spritzer, Ph.D.
Department of Biology
McCardell Bicentennial Hall
Middlebury College
Middlebury, VT 05753

ABSTRACT

A male advantage for spatial learning and memory tasks is well documented among humans and rodents. A possible physiological cause for this male advantage is activational effects of androgens among males. The spatial memory of 8 castrated and 8 sham-castrated adult male rats was compared using a working-reference memory version of the eight-arm radial arm maze followed by a reference memory version of the Morris water maze. After maze testing, blood was collected from each rat, and testosterone levels were determined using radioimmunoassay. In the radial arm maze, castrates committed significantly more working memory errors and significantly fewer reference memory errors than did shams. In the water maze, no statistically significant differences were found for acquisition or retention. There was a trend for shams with higher testosterone levels to have better retention in the water maze, but this seemed to be due to higher levels of perseverance rather than better reference memory. Castration may have affected performance in the radial arm maze but not the water maze because the radial arm maze was a more difficult task or because the water maze was aversively motivated while the radial arm maze was appetitively motivated. Our results indicate that androgens improve working memory and may impair reference memory, but the effects of androgens on reference memory seem to be task dependent.

KEY WORDS: spatial memory; androgens; radial arm maze; Morris water maze; rats.

INTRODUCTION

Spatial ability refers to the ability to encode, store, and retrieve knowledge about the position of environmental features relative to each other and the individual. A sex difference in spatial ability is one of the most well characterized human sex differences in cognition (Hampson, 2000; Linn & Petersen, 1985; Nyborg, 1983; Voyer, Voyer, & Bryden, 1995). Specifically, a male advantage has been well documented for a variety of spatial tasks, including mental rotation of objects (Collins & Kimura, 1997; Parsons et al., 2004), geographic knowledge (Dabbs, Chang, Strong, & Milun, 1998), route learning involving Euclidean strategies (Galea & Kimura, 1993; Holding & Holding, 1988; Postma, Jager, Kessels, Koppeschaar, & van Honk, 2004), and maze navigation (Astur, Ortiz, & Sutherland, 1998; Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005; Moffat, Hampson, & Hatzipantelis, 1998). It should be noted, however, that a female advantage has been documented for spatial tasks involving the memory of the relative position of objects within an array (Duff & Hampson, 2001; Silverman & Eals, 1992). A sex difference in spatial ability has also been documented in a variety of rodent species (Galea, Kavaliers, & Ossenkopp, 1996; Gaulin & FitzGerald, 1986; Gresack & Frick, 2003; Williams, Barnett, & Meck, 1990). In a recent meta-analysis, Jonasson (2005) concluded that male rats outperform females in water maze tasks (Effect size $d^* = 0.71$) as well as in radial-arm maze tasks (Effect size $d^* = 0.78$).

Studies with rodents and humans indicate that sex differences in spatial ability are partially due to the organizational effects of androgens during development (e.g., Hines et al. 2003; Isgor & Sengelaub 1998; Roof & Havens 1992). Growing evidence indicates that sex differences in spatial ability are also partially caused by the activational effects of androgens upon adult male cognition. Testosterone levels in men have been positively correlated with

mental rotation ability (Christiansen & Knussmann, 1987; Hooven, Chabris, Ellison, & Kosslyn, 2004; Silverman, Kastuk, Choi, & Phillips, 1999), route learning (Choi & Silverman, 2002), and maze navigation (Driscoll et al., 2005). In addition, older men given testosterone supplements showed improved spatial ability (Cherrier et al., 2001; Janowsky, Oviatt, & Orwoll, 1994). However, some studies using various spatial tasks have found no relationship between testosterone levels and spatial ability among men (McKeever & Deyo, 1990; McKeever, Rich, Deyo, & Conner, 1987), others have found a negative relationship (Gouchie & Kimura, 1991), and some have shown a negative parabolic relationship (Moffat & Hampson, 1996; Neave, Menaged, & Weightman, 1999). These contradictory results may be due, in part, to differences between studies in the range of subjects' testosterone levels or due to the use of different cognitive tests in which performance depends on different forms of memory.

Some studies have compared the performance of castrated and intact male rats in spatial working and reference memory tasks. Working memory refers to memories that are trial-unique and therefore require behavioral flexibility (Olton & Papas, 1979). Reference memory refers to long-term memories that are constant from trial to trial and require little or no behavioral flexibility (Olton & Papas, 1979). Daniel, Winsauer, and Moerschbaecher (2003) found that intact males committed fewer working memory errors than did castrated males in a working memory version of the eight-arm radial arm maze. Similarly, intact males committed fewer working memory errors than castrated males in a spatial T-maze (Kritzer, McLaughlin, Smirilis, & Robinson, 2001). In contrast, castrated and sham-castrated males showed no significant differences in retention or acquisition in the reference memory version of the Morris water maze (Sandstrom, Kim, & Wasserman, 2005).

Other studies have used exogenous manipulations of testosterone to examine the activational effects of testosterone on working and reference memory among male rats. Testosterone implants, resulting in high physiological levels of testosterone, led to impaired retention but had no effect on acquisition in the Morris water maze (Goudsmit, Van De Poll, & Swaab, 1990). In contrast, supra-physiological injections of testosterone (2.5 mg/rat) given to castrated males improved their performance during acquisition trials in the Morris water maze (Khalil, King, & Soliman, 2005). Bimonte-Nelson et al. (2003) tested intact males in a water radial arm maze and found that testosterone implants, resulting in low physiological levels of testosterone, led to improved working memory but had no effect on reference memory. Similarly, Kritzer et al. (2001) found that implants that restored castrates to physiological levels of testosterone led to improved working memory in a spatial T-maze. High doses of testosterone injected into the hippocampus impaired acquisition in the water maze (Naghdi, Nafisy, & Majlessi, 2001). In summary, testosterone supplementation seems to improve working memory, but the effects of testosterone on reference memory remain unclear and may depend on dose.

Past research indicates that testosterone can influence spatial memory among male rats. However, the results are somewhat contradictory and seem to depend upon the memory task employed as well as the dose of hormone manipulations. The primary goal of this study was to further clarify the effects of androgens upon spatial memory. This was done by comparing the performance of castrated and intact males on two spatial memory tasks. Rats were first tested with a working-reference memory version of the radial arm maze and then with a reference memory version of the Morris water maze. Circulating testosterone levels were determined for the intact males to examine correlations between testosterone levels and spatial memory.

METHOD

Participants

A total of 16 adult male Sprague Dawley rats (260-285 g; approximately 60 days old) were obtained from the University of British Columbia Animal Care Facility. Animals were housed individually in opaque polyurethane bins with aspen-chip bedding in a temperature controlled room (21 ± 1 ºC) with a 12:12 h light/dark cycle (lights on at 0700 h). Tap water and rodent chow (Lab Diet #5012; Jamieson) were provided *ad libitum* throughout the experiment.

Measures and Procedures

Surgery

Surgeries were conducted one week after rats arrived in the colony using aseptic procedures. Under halothane anesthesia (4% in oxygen during induction, 2% in oxygen during maintenance), eight males were bilaterally castrated and eight males received sham-castrations. For castrations, both testes were extracted through a small incision made at the posterior tip of the scrotum and were ligated with a silk suture. Sham operations involved incisions into the skin and muscle layers of the scrotum that were sutured without removing the testes. One week was allowed for recovery from surgery prior to any behavioral testing.

Radial Arm Maze

Previous studies have shown that male rats out-perform females in the radial arm maze (e.g., Roof 1993; Seymoure, Dou, & Juraska 1996). Testing was conducted on an eight-arm radial arm maze elevated 70 cm above the floor, with arms (56 cm × 10 cm) projecting at equal angles from a central platform (33 cm diameter). The maze was located in a dimly lit room with multiple visual cues (posters, a chair, and a small table). The maze, all cues, and the experimenter were in the same location for all testing, and testing was conducted at approximately the same time each day (1100-1400 h). At the start of each day, the maze was

rotated at random, while remaining in the same relative position in the room, to minimize intramaze cues. Fruit Whirls (Glenmont Distributors, Calgary, Alberta, Canada) were used as the food reward, and all rats received five whole Fruit Whirls in their home cages each day during the habituation and shaping phases of testing. Rats were maintained at 90% of their free-feeding body mass during all days of the radial arm maze procedure.

Our protocol consisted of 3 habituation days, 3 shaping days, and 30 training days. Four arms of the maze were assigned at random to be baited, and these arms remained consistent throughout the experiment for each rat. During the first day of habituation, rats were placed in the center of the maze and allowed to freely explore the maze for 5 min. During the second and third days of habituation, the time on the maze was extended to 10 min. During shaping, four pieces of Fruit Whirl were placed at equidistant intervals along the assigned arms, and the rats were allowed to freely explore the maze for 10 min. During training days, a single piece of Fruit Whirl was placed at the end of the assigned arms in a recessed cup, where it could not be seen by the rats until they reached the end of the maze arm. Training trials were ended when the rat either recovered all four Fruit Whirls or 10 min had elapsed. The task was not completed within the allotted 10 min during 7 trials involving 4 rats (2 castrates and 2 shams). For these cases, we assigned each rat the average number of errors made on the trial before and on the trial after the incomplete trial. An arm entry was scored if the rat's hind legs passed from the center of the maze into an arm. Rats could make at least two types of errors during training: Reference memory errors (RME) were defined as first entries into non-baited arms, and working memory errors (WME) were defined as repeat entries into baited or non-baited arms. Average time spent on the maze per arm choice was calculated as a measure of motivation and motor ability.

Morris Water Maze

Previous studies have shown that male rats out-perform females in the Morris water maze (e.g., Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996; Roof, 1993). Our water maze was a white circular pool (180 cm diameter, 60 cm height) that was filled to a depth of 22 cm with water (20 ± 2 °C) that was made opaque with nontoxic white paint. The pool was divided geographically into four quadrants with four equidistant release points designated N, W, S, E. The goal platform (10 cm diameter) was submerged 2 cm under the water so that it could not be used as an intra-maze cue. Water maze testing was conducted in a separate room from that used for radial arm maze testing. Various spatial cues (posters, camera, chalkboard, chairs, etc.) were kept constant throughout testing. The experimenter was out of view behind a barrier while trials were being run. All trials were video recorded using a camera mounted above the maze. Videos were analyzed using an HVS tracking system (HVS Image, Hampton, UK), which calculated path length to the platform, escape latency (time to reach the platform), swim speed, and time spent in each quadrant.

To allow rats to return to their free-feeding body mass, 60 days of *ad libitum* feeding elapsed between the final day of radial arm maze training and the first day of water maze testing. Three days prior to testing, black hair dye (Lady Clairol™, #124) was applied to the top of each rat's head so that their behavior could be scored by the tracking system. For all trials, rats were released near the edge of the pool facing the edge. Trials were conducted over eight consecutive days between 1200 h and 1400 h. A single free-swim trial was conducted the first day, which involved releasing rats into the pool for 90 s, without the platform present, to measure their baseline swimming speed. The next five days of testing involved blocks of four acquisition trials per day. The position of the platform was assigned at random to one of the four quadrants for each rat and the platform was placed consistently in the same quadrant for all trials involving a

particular rat. Rats were allowed to remain on the platform for 15 s after locating it. If a rat did not locate the platform within 90 s, it was guided to the platform and allowed to remain there for 15 s. During each day of acquisition, each rat was released from all four release points around the pool in a random order. Rats were gently towel dried and placed in a holding cage during 45 s inter-trial intervals. A single probe trial was conducted on the day following the final day of acquisition trials. For the probe trial, the platform was removed and the amount of time rats spent swimming in each quadrant was monitored for 90 s. On the day after the probe trials, four reversal-learning trials were conducted. For reversal learning, the platform was placed in the quadrant opposite where it had been during the acquisition trials, and rats were released from all four release points as for the acquisition trials.

Testosterone Assay

Testosterone levels for all males were assayed for two reasons: to verify that castration had eliminated most of the circulating testosterone, and to analyze the relationship between testosterone levels and performance on the spatial tasks among the sham-castrated males. After water maze testing was completed, all rats were euthanized in a CO_2 chamber. Just prior to death, blood samples (1-2 ml) were collected via cardiac puncture and samples were stored overnight at 4 °C. Samples were centrifuged at 10,000 rpm for 15 min, and serum was decanted and stored at -20 °C. Serum testosterone for each rat was assayed using the ImmuChem Coated Tube RIA Kit (MP Biomedicals Inc., Costa Mesa, CA) with [^{125}I]testosterone as the tracer. The detection range for the kit was 0.2-20.0 ng/ml. The testosterone antibody has some cross-reactivity with 5α-dihydrotestosterone (7.8%), 5α-androsterone-3β, 17β-diol (2.2%), and 11-oxytestosterone (2.0%), but has no cross-reactivity with progesterone, estrogens, or glucocorticoids (all < 0.01%). All samples were run in duplicate and the intra-assay coefficient of variation was 8.3%.

Statistical Analyses

The radial arm maze data were divided into six five-day blocks for analyses. Number of working memory errors, number of reference memory errors, and average time per arm choice were analyzed using repeated measures analysis of variance (ANOVA), with five-day blocks as the within-subjects factor and treatment group (sham or castrate) as the between-subjects factor. To further examine reference memory in the radial arm maze, the percentage of times that the first arm entry was correct during each five-day block was determined for each rat. This percentage was compared between treatment groups using repeated-measures ANOVA and was compared to chance levels (50%) using one-sample *t* tests. Repeated-measures ANOVA was also used to analyze the total number of working memory and reference memory errors for the entire 30 days. For this analysis, the within-subjects factor was error type (working or reference memory) and the between-subjects factor was treatment group. For the water maze, independent *t* tests were used to compare the performance of the two treatment groups during the free swim and reversal learning trials. Repeated-measures ANOVAs were used to analyze escape latencies and path lengths during the acquisition trials, with testing block as the within-subjects factor and treatment group as the between-subjects factor. One-sample *t* tests were used to compare the amount of time rats spent swimming in the target quadrant during the probe trials to the amount of time that would be expected if rats had swam at random (22.5 s). Linear regressions were used to determine the relationship between testosterone levels and performance of the sham-castrated males in both the radial arm maze and the water maze. Neuman-Keuls procedure was used for all post hoc comparisons, and effect size (η^2) is reported for some analyses. Statistica 6.1 (Statsoft, Inc., Tulsa, OK) was used for all analyses.

RESULTS

Radial Arm Maze

Castrates committed significantly more WME than did shams, $F(1, 13) = 6.14$, partial $\eta^2 = 0.32$, $p = .028$ (Fig. 1A). All males committed fewer errors on later five-day blocks, resulting in a significant main effect of block, $F(5, 65) = 9.08$, $p < .001$, and post hoc tests showed that males committed more WME during the first 5-day block than during any of the other blocks, all $p < .001$. There was no significant condition × block interaction for WME.

In contrast, Shams committed more RME on the radial arm maze than did castrates during some of the days of testing, but the main effect of treatment just failed to reach statistical significance, $F(1, 13) = 3.98$, $p = .067$ (Fig. 1B). There was no significant main effect of block on RME and no significant condition × block interaction. To further examine reference memory, the percentage of correct choices on the first arm entry was examined (Fig. 2). The main effects of block and treatment as well as the interaction effect were not significant for percentage of correct first choices. Furthermore, none of the 5-day blocks for castrates or shams were significantly different from chance levels (50% correct).

Insert Figure 1 and 2 about here

There was a significant main effect of block for average time per arm choice, $F(5,65) = 5.29$, $p < .001$, with rats spending significantly more time per arm choice during the first 5-day block than during any of the other blocks, all $p < .01$. There was no significant main effect of condition and no significant condition × block interaction for average time per arm choice. This indicates that castrates and shams did not differ in motor ability and/or motivational level during testing.

For the total number of errors committed over the 30 days of testing, there was a significant condition × error type interaction, $F(1, 13) = 11.42$, partial $\eta^2 = 0.47$, $p = .005$ (Fig.

3). Post hoc tests revealed that shams committed significantly more total RME than did castrates, $p = .015$, and that there was a trend for castrates to commit more total WME than did shams, $p = .072$. There was also a significant main effect of error type, with total number of RME significantly greater than the total number of WME, $F(1, 13) = 656.9$, $p < .005$, but no significant main effect of condition for total number of errors.

Insert Figure 3 about here

Morris Water Maze

Castrates and shams did not statistically differ in swim speed during the free-swim trials, which indicates that the treatment groups did not differ in their swimming abilities and/or motivation. Castrates and shams performed at similar levels during the acquisition trials. During the five blocks of acquisition trials, there was a significant main effect of block, $F(4, 56) = 17.56$, $p < .0001$ (Fig 4A). However, there was no significant difference between the castrates and sham groups for path length to reach the platform. There was also no significant block × condition interaction for path length. The escape latencies to reach the platform showed similar trends to those for path lengths (Fig. 4B). Escape latencies showed a significant main effect of block, $F(4, 56) = 13.00$, $p < .0001$, but no significant main effect of condition and no significant block × condition interaction.

Insert Figure 4 about here

During the probe trials, both shams and castrates preferentially swam in the quadrant where the platform had previously been ($M \pm SEM$, castrates = 40.78 s ± 3.05 s; shams = 35.65 s ± 3.21 s). Castrates spent significantly more time in the target quadrant than would be expected by a random swim path, $t(7) = 6.00$, $p < .001$, as did shams, $t(7) = 4.10$, $p = .005$. Castrates and

shams did not differ significantly in the amount of time spent in the target quadrant during the probe trials.

Castrates and shams did not differ in the mean path lengths to reach the platform during reversal-learning trials. Similarly, the groups did not differ in their mean escape latencies during reversal learning. However, castrates did spend significantly longer than shams swimming in the quadrant where the platform had been during acquisition trials, $t(14) = 3.06$, partial $\eta^2 = 0.40$, $p = .008$ (castrates, $M \pm SEM = 22.28$ s $\pm$ 2.19 s; shams, $M \pm SEM = 12.63$ s $\pm$ 2.27 s). This suggests that castrates had higher levels of perseverance for the old platform location than did shams.

Testosterone Levels

Radioimmunoassay revealed no detectable testosterone in the serum of the castrated males, indicating that they all had testosterone levels at least below the lower limit of the assay (0.2 ng/ml). Serum testosterone concentrations were 1.57 ng/ml $\pm$ 0.35 ng/ml for the sham group.

Testosterone concentration among shams was not predictive of performance in the radial arm maze. Specifically, testosterone concentration was not related to total reference memory errors or total working memory errors. Testosterone concentrations among the shams were also not predictive of average path length or escape latency during any of the blocks of acquisition trials in the water maze. Interestingly, there was a trend for shams with higher testosterone levels to spend more time swimming in the target quadrant during probe trials in the water maze, $r^2 = 0.48$, , $p = .058$ (Fig. 5). Shams with higher testosterone concentrations also displayed higher levels of perseverance during reversal learning in the water maze. Specifically, males with higher testosterone levels had longer path lengths, $r^2 = 0.63$, $p = .018$, had longer escape latencies, $r^2 =$

0.60, $F(1, 6) = 9.06$, $p = .024$, and spent significantly more time in the quadrant where the platform had previously been, $r^2 = 0.55$, $F(1, 6) = 7.22$, $p = .036$ (Fig. 6).

Insert Figures 5 and 6 about here

DISCUSSION

Castration resulted in changes in male performance in two maze tasks, suggesting that androgens have activational effects on spatial ability. Castrates committed more working memory errors in the radial arm maze, particularly during earlier blocks of training, than did sham-castrated male rats. Castrates also committed fewer total reference memory errors in the radial arm maze than did sham-castrated male rats. These results suggest that androgens may improve working memory while impairing reference memory. In contrast, castration had no effect on male performance during acquisition and probe trials in the Morris water maze, which suggests that androgens did not have a strong influence on the components of spatial ability tested in this particular task. However, shams with higher testosterone levels tended to have better retention, which suggests that testosterone may improve retention in the water maze. We did not find significant differences between castrates and shams for average time per arm choice in the radial arm maze or for average swim speed in the water maze, which indicates differences in performance were unlikely to be due to motor or motivational differences.

Past studies support our findings from the radial arm maze that castration impairs working memory. Similar to our study, Daniel et al. (2003) found that, although castrated male rats were capable of learning in a working memory version of the radial arm maze, they consistently performed more errors than did intact males. Kritzer et al. (2001) also found that castration resulted in impaired working memory, but testosterone implants reversed this deficit. Bimonte-Nelson et al. (2003) also found that testosterone implants resulted in reduced working

memory errors in a water escape radial arm maze. Our results also support human studies that have shown that testosterone improves spatial working memory in older men (Cherrier et al. 2001; Janowsky et al. 1994). Choi & Silverman (2002) observed a positive correlation between testosterone and route learning in humans. Surprisingly, we did not find a relationship between testosterone levels and total working memory errors among shams. This could be due to at least three reasons: 1) blood in the present study was collected 69 days after radial arm maze testing was completed and, therefore, may not have been indicative of testosterone levels during testing, 2) the sham group had relatively low levels of testosterone (0.6–3.4 ng/ml), which could have limited our ability to detect a relationship between working memory and testosterone level, and 3) our sample size for sham-castrated males was relatively low ($N = 8$). Indeed, mean values of testosterone were over 3 ng/ml in previous studies that found a positive relationship between testosterone and working memory (Bimonte-Nelson et al., 2003; Kritzer et al., 2001) compared to a mean of 1.57 ng/ml in the present study.

Compared to working memory, the effects of androgens upon reference memory remain less clear. Ours is the first study to examine the effects of androgens upon reference memory in males using an appetitively motivated spatial task. Castration seemed to improve reference memory in the radial arm maze, with castrates performing fewer total reference memory errors than did shams. An important caveat to this result was that both shams and castrates showed little change in the number of reference memory errors committed each day, and it was only the compiled errors for the entire 30 days that showed a significant interaction between error type and treatment group. Furthermore, both castrated and intact males were making reference memory errors at near chance levels for their first arm choice in the maze even during the last 5-day block of testing. In contrast, all rats clearly demonstrated learning in a reference memory

version of the Morris water maze. Hence, the radial arm maze may have been more difficult to learn than the water maze in terms of reference memory demands (Hodges 1996). Our finding that castration had no significant effect on acquisition or retention in the water maze was supported by some previous studies. A recent study found that castration had no effect on acquisition or retention in a reference memory version of the Morris water maze (Sandstrom et al. 2005). Similarly, Bimonte-Nelson et al. (2003) found that testosterone implants had no effect on male reference memory in a water-escape version of the radial arm maze. Goudsmit et al. (1990) also found that testosterone implants had no effect on acquisition in the Morris water maze, but, unlike other studies, they found that testosterone impaired retention. Jones and Watson (2005) found that androgen insensitive rats performed more poorly in the Morris water maze than did control males, but the effect was subtle with differences occurring on only one out of five days of testing. Hence, the effects of androgens on reference memory seem to be minor and may be task dependent, but further studies are needed to support this idea.

Although males with higher testosterone levels tended to have better retention in the water maze, they also had higher levels of perseverance during reversal-learning trials. This indicates that better retention by rats with higher testosterone levels may reflect perseverance rather than better reference memory per se. Specifically, during reversal learning trials, sham-castrated males with higher testosterone levels had longer path lengths, longer escape latencies, and more time spent in the quadrant where the platform had previously been. Similarly, a previous study showed that testosterone implants given to female rats resulted in increased levels of perseverance on a lever-pressing task (van Hest, van Haaren, & van de Poll, 1989). A positive relationship between testosterone levels and perseverance is contradicted, however, by our finding that castrates spent more time in the old target quadrant during reversal-learning than did

shams. One, highly speculative, explanation for this contradiction is that there is a curvilinear relationship between testosterone levels and perseverance, which results in high perseverance among males with both high and low testosterone levels. To clarify the relationship between testosterone and perseverance, future studies should use rats with a wider range of testosterone levels than those in our study and use tests specifically designed to examine behavioral flexibility (e.g., Stefani & Moghaddam 2005).

The fact that castration influenced male performance in the radial arm maze but not the Morris water maze highlights the importance of not drawing general conclusions based on a single spatial task (Hodges 1996). One explanation for the different results obtained using the two procedures is that the motivation in the Morris water maze is avoidance of an aversive stimulus (water), whereas the radial arm maze is an appetitively motivated task. Some past research indicates that the magnitude of the sex differences in learning and memory may vary depending on whether the task is negatively or positively motivated (van Haaren, van Hiest, & Heinsbroek, 1990). Another explanation for the different results obtained with the two tasks is that they measure different forms of spatial memory. Whereas the version of the radial are maze that we used distinguished between working memory and reference memory, the Morris water maze measured only reference memory. The effect of castration upon reference memory observed using the radial arm maze was subtle (i.e., only detected for total reference errors over 30 days) and may not have been detectable using only four days of acquisition in the water maze. It should also be mentioned that we did not counter-balance the procedures, and therefore testing rats in the radial arm maze may have in some way influenced their performance in the water maze. Sixty days elapsed between the two tasks, and this increased time since castration may have induced neuroendocrine changes (e.g., Gupta, Rager, Zarzycki, & Eichner 1975) that

influenced the performance of the castrated males in the water maze. Furthermore, one could argue that the increased age of the intact males could have resulted in both low testosterone levels and reduced performance in the water maze (Bimonte-Nelson et al., 2003; Goudsmit, Van De Poll, & Swaab, 1990), but this seems unlikely given that our subjects were approximately 6 months old at the time of water maze testing and males do not show a decrease in androgen levels until 20 months of age (Wang, Leung, & Sinha-Hikim 1993). Finally, as previously mentioned, the Morris water maze is an easier task to learn than the radial arm maze and this may partially explain the different findings with the two tasks. These ideas could be tested using different water maze tasks designed specifically to distinguish between reference and working memory (e.g., Baldi, Efoudebe, Lorenzini, & Bucherelli, 2005; Bimonte-Nelson et al. 2003).

Our finding that androgens improved working memory and seemed to impair reference memory in the radial arm maze may be due to varying effects of androgens upon different brain regions. Lesions to both the hippocampus and various cortical regions have been shown to impair performance in spatial tasks (de Bruin, Swinkels, & Brabander, 1997; Eichenbaum, Stewart, & Morris 1990; Olton & Papas, 1979; Pothuizen, Zhang, Jongen-Rêlo, Feldon, & Yee, 2004; Seamans, Floresco, & Phillips, 1995; Seamans & Phillips, 1994). The hippocampus and prefrontal cortex are two major areas that influence spatial working memory (Kesner, Hunt, Williams, & Long, 1996; Lee & Kesner 2003), which suggests that androgens could influence processing between or within these regions to improve working memory. In support of this idea, androgen receptors have been isolated from both the hippocampus and prefrontal cortex of male rats (Kerr, Allore, Beck, & Handa, 1995; Kritzer 2004). Within the hippocampus, testosterone influences neuronal activity (Harley, Malsbury, Squires, & Brown, 2000; Smith, Jones, & Wilson, 2002), increases spine density (Leranth, Petnehazy, & MacLusky, 2003), and may

increase adult neurogenesis (Spritzer & Galea 2005). In support of our finding that castration improved reference memory, high doses of testosterone injected into the hippocampus were shown to impair reference memory in the water maze (Naghdi et al., 2001; Naghdi, Majlessi, & Bozorgmerhr, 2005).

Although testosterone may influence spatial ability directly, it can also be aromatized to estradiol (Chung & Hu, 2002). Numerous studies conducted with humans and rodents have shown that estradiol can have activational effects on spatial ability among females (e.g., Hampson, 1990; Holmes, Wide, & Galea, 2002; Luine, Richards, Wu, & Beck, 1998; Postma, Winkel, Tuiten, & van Honk, 1999). Some evidence suggests that estradiol can also have activational effects on spatial memory among male rats. Two studies showed that estradiol implants improved working memory among castrated males (Gibbs, 2005; Luine & Rodriguez, 1994). This suggests that our finding that castration impaired working memory may be primarily due to a reduction in estradiol rather than a reduction in androgens. In addition, Gibbs (2005) found that increasing the delay between trials in a working memory version of the T-maze impaired testosterone-implanted males to a lesser degree than among estradiol-implanted males, suggesting that estradiol and testosterone have different effects on working memory. Some past studies suggest that estradiol improves reference memory among males (Packard, Kohlmaier, & Alexander, 1996; Martin, Jones, Simpson, & van den Buuse, 2003), which suggests that our finding that castrates had better reference memory than shams did not involve changes in estadiol levels.

The current study demonstrated that androgens can have activational effects upon spatial working and reference memory among males, and these effects may be part of the physiological cause of the male advantage in many spatial tasks that has been well documented among rodents

and humans. We found that castration impaired working memory in the radial arm maze, which suggests that higher androgen levels in males compared to females may underlie sex differences in spatial working memory. Our results for the effects of castration on reference memory are less clear, but suggest that androgens may impair spatial reference memory. Therefore, it seems unlikely that the male advantage for spatial reference memory is due to activational effects of androgens.

ACKNOWLEDGEMENTS

We thank Lucille Hoover and Stephanie Lieblich for technical assistance with behavioral procedures. We also thank Jaspreet Kambo for assistance with surgeries. This work was funded by CIHR and NSERC operating grants to LAMG and a CIHR training grant to MDS.

REFERENCES

Astur, R. S., Ortiz, M. L., & Sutherland, R. J. (1998). A characterization of performance by men and women in a virtual Morris water task: A large and reliable sex difference. *Behavioral Brain Research,* 93, 185-190.

Baldi, E., Efoudebe, M., Lorenzini, C.A., & Bucherelli, C. (2005). Spatial navigation in the Morris water maze: Working and long lasting reference memories. *Neuroscience Letters, 378,* 176-180.

Bimonte-Nelson, H. A., Singleton, R. S., Nelson, M. E., Eckman, C. B., Barber, J., Scott, T. Y., et al. (2003). Testosterone, but not nonarmoatizable dihydrotestosterone, improves working memory and alters nerve growth factor levels in aged male rats. *Experimental Neurology, 181,* 301-312.

Cherrier, M. M., Asthana S., Plymate, S., Baker, L., Matsumoto, A. M., Peskind, E.,et al. (2001). Testosterone supplementation improves spatial and verbal memory in healthy older men. *Neurology, 57,* 80-88.

Choi, J., & Silverman, I. (2002). The relationship between testosterone and route-learning strategies in humans. *Brain and Cognition, 50,* 116-120.

Christiansen, K., & Knussmann, R. (1987). Sex hormones and cognitive functioning in men. *Neuropsychobiology, 18,* 27-37.

Chung, B., & Hu, M-C. (2002). Androgen biosynthesis and degradation. In C. Chang, & G. Wipple (Eds.), *Androgens and andogen receptor: Mechanisms, functions, and clinical applications* (pp. 1-15). Boston, MA: Kluwer Academic Publishers.

Collins, D. W., & Kimura, D. (1997). A large sex difference on a two-dimensional mental rotation task. *Behavioral Neuroscience, 111,* 845-849.

Dabbs, J. M., Chang, E., & Strong, R. A. (1998). Spatial ability, navigation strategy, and geographic knowledge among men and women. *Evolution and Human Behavior, 19,* 89-98.

Daniel, J. M., Winsauer, P. J., & Moerschbaecher, J. M. (2003). Castration in rats impairs performance during acquisition of working memory task and exacerbates deficits in working memory produced by scopolamine and mecamylamine. *Psychoneuroendocrinology, 170,* 294-300.

de Bruin, J. P. C., Swinkels, W. A. M., & de Barbander, J. M. (1997). Response learning of rats in Morris water maze: Involvement of the medial prefrontal cortex. *Behavioral Brain Research, 85,* 47-55.

Driscoll, I., Hamilton, D. A., Yeo, R. A., Brooks, W. M., & Sutherland, R. J. (2005). Virtual navigation in humans: The impact of age, sex, and hormones on place learning. *Hormones and Behavior, 47,* 326-335.

Duff, S. D., & Hampson, E. (2001). A sex difference on a novel spatial working memory task in humans. *Brain and Cognition,* 47, 470-493.

Eichenbaum, H., Stewart, C. & Morris, R. G. M. (1990). Hippocampal representation in place learning. *Journal of Neuroscience, 10,* 3531-3542.

Galea, L. A. M., Kavaliers, M., & Ossenkopp, K.-P. (1996). Sexually dimorphic spatial learning in meadow voles *Microtus pennsylvanicus* and deer mice *Peromyscus maniculatus*. *Journal of Experimental Biology, 199,* 195-200.

Galea, L. A. M., & Kimura, D. (1993). Sex differences in route-learning. *Personality and Individual Differences, 14,* 53-65.

Gaulin, S. J. C., & FitzGerald, R. W. (1986). Sex differences in spatial ability: An evolutionary hypothesis and test. *The American Naturalist, 127,* 74-88.

Gibbs, R. B. (2005). Testosterone and estradiol produce different effects on cognitive performance in male rats. *Hormones and Behavior, 48,* 268-277.

Gouchie, C., & Kimura, D. (1991). The relationship between testosterone levels and cognitive ability patterns. *Psychoneuroendocrinoogy, 16,* 323-334.

Goudsmit, E., Van De Poll, N. E., & Swaab, D. F. (1990). Testosterone fails to reverse spatial memory decline in aged rats and impairs retention in young and middle-aged animals. *Behavioral and Neural Biology, 53,* 6-20.

Gresack, J. E., & Frick, K. M. (2003). Male mice exhibit better spatial working and reference memory than females in a water-escape radial arm maze task. *Brain Research, 982,* 98-107.

Gupta, D., Rager, K., Zarzycki, J., & Eichner, M. (1975). Levels of luteinizing hormones, follicle stimulating hormone, testosterone and dihydrotestosterone in the ciculation of sexually maturing intact male rats and after orchidectomy and experimental bilateral cryptoorchidism. *Journal of Endocrinology, 66,* 183-193.

Hampson, E. (1990). Estrogen-related variations in human spatial and articulatory-motor skills. *Psychoneuroendocrinology, 15,* 97-111.

Hampson, E. (2000). Sexual differentiation of spatial functions in humans. In A. Matsumoto (Ed.), *Sexual differentiation of the brain* (pp. 279-300). Boca Raton, FL: CRC Press.

Harley, C. W., Malsbury, C. W., Squires, A., & Brown, R. A. M. (2000). Testosterone decreases CA1 plasticity in vivo in gonadectomized male rats. *Hippocampus, 10,* 693-697.

Hines, M., Fane, B.A., Pasterski, V. L., Mathews, G.A., Conway, G. S., & Brook, C. (2003). Spatial abilities following prenatal androgen abnormality: targeting and mental rotations performance in indviduals with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 28,* 1010-1026.

Hodges, H. (1996). Maze procedures: the radial-arm and water maze compared. *Cognitive Brain Research, 3,* 167-181.

Holding, C. S., & Holding, D. H. (1988). Acquisition of route network knowledge by males and females. *The Journal of General Psychology, 116,* 29-41.

Holmes, M. M., Wide, J. K., & Galea, L. A. M. (2002). Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. *Behavioral Neuroscience, 116,* 928-934.

Hooven, C. K., Chabris, C. F., Ellison, P. T., & Kosslyn, S. M. (2004). The relationship of male testosterone to components of mental rotation. *Neuropsychologia, 43,* 782-790.

Isgor, C. & Sengelaub, D. R. (1998). Prenatal gonadal steroids affect adult spatial behavior, CA1 and CA3 pyramidal cell morphology in rats. *Hormones and Behavior, 34,* 183-198.

Janowky, J. S., Oviatt, S. K., & Orwoll, E. S. (1994). Testosterone influences spatial cognition in older men. *Behavioral Neuroscience, 108,* 325-332.

Jonasson, Z. (2005). Meta-analysis of sex differences in rodent models of learning and memory: A review of behavioral and biological data. *Neuroscience and Biobehavioral Reviews, 28,* 811-825.

Jones, B. A., & Watson, N. V. (2005). Spatial memory performance in androgen insensitive male rats. *Physiology and Behavior, 85,* 135-141.

Kerr J. E., Allore R. J., Beck S. G., & Handa, R. J. (1995). Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. *Endocrinology, 136,* 3213-3221.

Kesner, R. P., Hunt, M. E., Williams, J. M., & Long, J. M. (1996). Prefrontal cortex and working memory for spatial response, spatial location, and visual object information in the rat. *Cerebral Cortex, 6,* 311-318.

Khalil, R., King, M. A., & Soliman, M. R. I. (2005). Testosterone reverses ethanol-induced deficit in spatial reference memory in castrated rats. *Pharmacology, 75,* 87-92.

Kritzer, M. (2004). The distribution of immunoreactivity for intracellular androgen receptors in the cerebral cortex of hormonally intact adult male and female rats: Localization in pyramidal neurons making corticocotical connections. *Cerebral Cortex, 14,* 268-280.

Kritzer, M. F., McLaughlin, P. J., & Smirlis, T. (2001). Gonadectomy impairs T-maze acquisition in adult male rats. *Hormones and Behavior, 39,* 167-174.

Lee, I., & Kesner, R. P. (2003). Time-dependent relationship between dorsal hippocampus and the prefrontal cortex in spatial memory. *Journal of Neuroscience, 223,* 1517-1523.

Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *The Journal of Neuroscience, 23,* 1588-1592.

Linn, M. C., & Petersen, A. C. (1985). Emergence and characterization of sex differences in spatial ability: A meta-analysis. *Child Development, 56,* 1479-1498.

Luine, V. N., Richards, S. T., Wu, V. Y., & Beck, K. D. (1998). Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Hormones and Behavior, 34,* 149-162.

Luine, V., & Rodriguez, M. (1994). Effects of estradiol on radial arm maze performance of young and aged rats. *Behavioral and Neural Biology, 62,* 230-236.

Martin, S., Jones, M., Simpson, E., & van den Buuse, M. (2003). Impaired spatial reference memory in aromatse-deficient (ArKO) mice. *Neuroreport, 14,* 1979-1982.

McKeever, W. F., & Deyo, R. A. (1990). Testosterone, dihydrotestosterone, and spatial task performance of males. *Bulletin of the Psychonomic Society, 28,* 305-308.

McKeever, W. F., Rich, D. A., Deyo, R. A., & Conner, R. L. (1987). Androgens and spatial ability: Failure to find a relationship between testosterone and ability measures. *Bulletin of the Psychonomic Society,* 25, 438-440.

Moffat, S. D., & Hampson, E. (1996). A curvilinear relationship between testosterone and spatial cognition in humans: Possible influence of hand preference. *Psychoneuroendocrinology, 21*, 323-337.

Moffat, S. D., Hampson, E., & Hatzipantelis, M. (1998). Navigation in a "virtual" maze: sex differences and correlation with psychometric measures of spatial ability in humans. *Evolution and Human Behavior, 19,* 73-87.

Naghdi, N., Majlessi, N., Bozorgmehr, T. (2005). The effect of intrahippocampal injection of testosterone enanthate (an androgen receptor agonist) and anisomycin (protein synthesis inhibitor) on spatial learning and memory in adult male rats. *Behavioral Brain Research, 156,* 263-268.

Naghdi, N., Nafisy, N., & Majlessi, N. (2001). The effects of intrahippocampal testosterone and flutamide on spatial localization in the Morris water maze. *Brain Research, 897,* 44-51.

Neave, N., Menaged, M., & Weightman, D. R. (1999). Sex differences in cognition: the role of testosterone and sexual orientation. *Brain and Cognition, 41,* 245-262.

Nyborg, H. (1983). Spatial ability in men and women: Review and new theory. *Advances in Behavior Research and Theory, 5,* 89-140.

Olton, D. S., & Papas, B. C. (1979). Spatial memory and hippocampal function. *Neuropsychologia, 17,* 669-682.

Packard, M. G., Kohlmaier, J. R., & Alexander, G. M. (1996). Posttraining intrahippocampal estradiol injections enhance spatial memory in male rats: Interaction with cholinergic systems. *Behavioral Neuroscience, 110,* 626-632.

Parsons, T. D., Larson, P., Kratz, K., Thiebaux, M., Bluestein, B., Buckwalter, J. G., et al. (2004). Sex differences in mental rotation and spatial rotation in a virtual environment. *Neuropsychologia, 42,* 555-562.

Perrot-Sinal, T. S., Kostenuik, M. A., Ossenkopp, K.-P., & Kavaliers, M. (1996). Sex differences in performance in the Morris water maze and the effect of initial nonstationary hidden planform training. *Behavioral Neuroscience, 110,* 1309-1320.

Postma, A., Jager, G., Kessels, R. P. C., Koppeschaar, H. P. F., & van Honk, J. (2004). Sex differences for selective forms of spatial memory. *Brain and Cognition, 54,* 24-34.

Postma, A., Winkel, J., Tuiten, A., & van Honk, J. (1999). Sex differences and menstrual cycle effects in human spatial ability. *Psychoneuroendocrinology, 24,* 175-192.

Pothuizen, H. H., Zhang, W.-N., Jongen- Rêlo, A. L., Feldon, J., & Yee, B. K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working memory. *European Journal of Neuroscience, 19,* 705-712.

Roof, R.L. (1993). Neonatal exogenous testosterone modifies sex difference in radial arm and Morris water maze performance in prepubescent and adult rats. *Behavioural Brain Research, 53,* 1-10.

Roof, R. L., & Havens , M.D. (1992). Testosterone improves maze performance and induces development of a male hippocampus in females. *Brain Research, 572,* 310-313.

Sandstrom, N. J., Kim, J. H., & Wasserman, M. A. (2005). Testosterone modulates performance on spatial working memory task in male rats. *Hormones and Behavior, 50,* 18-26.

Seamans, J. K., Floresco, S. B., & Phillips, A. G. (1995). Functional differences between the prelimbic and anterior cingulated regions of the rat prefrontal cortex. *Behavioral Neuroscience, 109,* 1063-1073.

Seamans, J. K., & Phillips, A. G. (1994). Selective memory impairments produced by transient lidocaine-induced lesions of the nucleus acumens in rats. *Behavioral Neuroscience, 108,* 456-468.

Seymore, P., Dou, H., & Juraska, J. M. (1996). Sex differences in radial maze performance: influence of rearing environment and room cues. *Psychobiology, 24,* 33-37.

Silverman, I., Eals, M. (1992). Sex difference n spatial abilities: Evolutionary theory and data. In J. Barkow, L. Cosmides, & J. Tooby (Eds.), The adapted mind: Evolutionary psychology and the generation of culture (pp. 533-549). Oxford, UK: Oxford University Press.

Silverman, I., Kastuk, D., Choi, J., & Phillips, K. (1999). Testosterone levels and spatial ability in men. *Psychoneuroendocrinology, 24,* 813-822.

Smith, M. D., Jones, L. S., & Wilson, M. A. (2002). Sex differences in hippocampal slice excitability: Role of testosterone. *Neuroscience, 109,* 517-530.

Spritzer, M. D., & Galea, L. A. M. (2005). Androgens enhance cell survival but not cell proliferation in adult male rats. *Hormones and Behavior, 48,* 129.

Stefani, M. R., & Moghaddam, B. (2005). Systemic and prefrontal cortical NMDA receptor blockade differentially affect discrimination learning and set-shift ability in rats. *Behavioral Neuroscience, 119,* 420-428.

van Haaren, R., van Hiest, A., & Heinsbroek, R. P. W. (1990). Behavioral differences between male and female rats: Effects of gonadal hormones on learning and memory. *Neuroscience & Biobehavioral Reviews, 14,* 23-33.

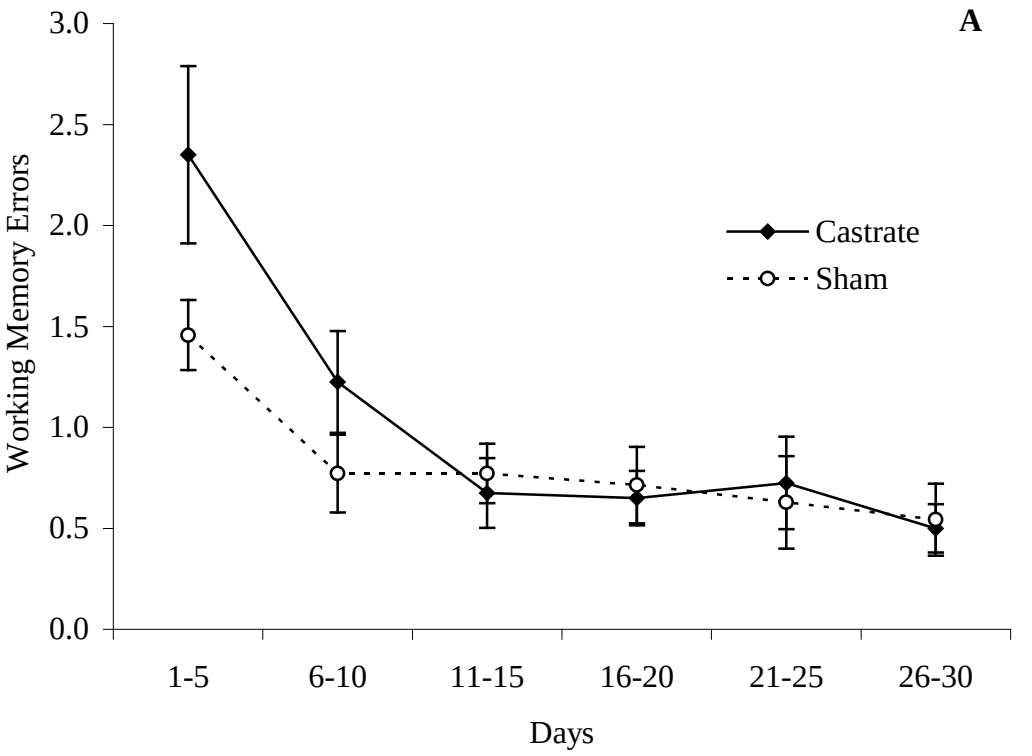
van Hest, A., van Haaren, F., van de Poll, N. E. (1989). Perseverative responding in male and female Wistar rats: Effects of gonadal hormones. *Hormones and Behavior, 23,* 57-67.

Voyer, D., Voyer, S., & Bryden, M. P. (1995). Magnitude of sex differences in spatial abilities: A meta-analysis and consideration of critical variables. *Psychological Bulletin, 117,* 250-270.

Wang, C., Leung, A., & Sinha-Hikim, A. P. (1993). Reproductive aging in the male brown-Norway rat: A model for the human. *Endocrinology, 133,* 2773-2781.

Williams, C. L., Barnett, A. M., & Meck, W. H. (1990). Organizational effects of early gonadal secretions on sexual differentiation in spatial memory. *Behavioral Neuroscience, 104,* 84-97.

Fig. 1. Mean number (± *SEM*) of RME (A) and WME (B) performed by castrated (*n* = 8) and sham-castrated (n = 7) male rats during 5-day blocks over 30 days of testing on the radial arm maze. There was a trend for shams to commit more RME than castrates, and castrates committed more WME than did shams.


A
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Working Memory Errors
Castrate
Sham
1-5
6-10
11-15
16-20
21-25
26-30
Days

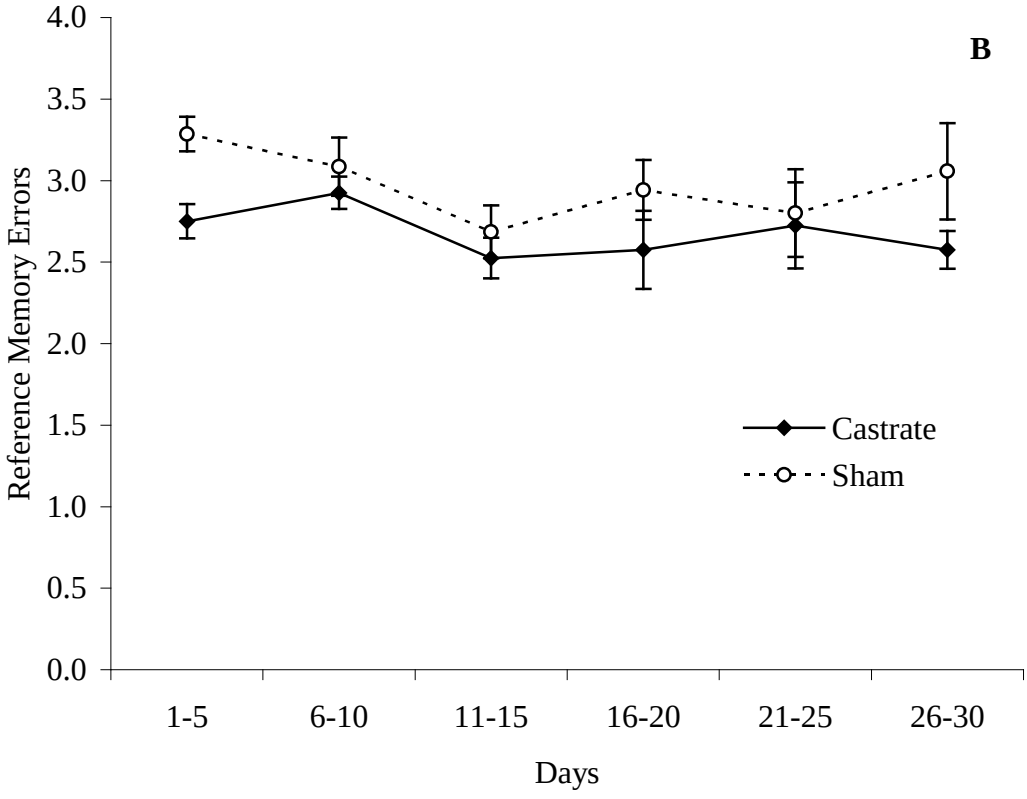

B
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Reference Memory Errors
Castrate
Sham
1-5
6-10
11-15
16-20
21-25
26-30
Days

Fig. 2. Mean percentage (± *SEM*) of first arm choices that were correct for male rats during 5-day blocks over 30 days of testing on the radial arm maze. An incorrect choice for the first arm entry was a RME. No differences were found between castrated and shams, and none of the 5-day blocks differed from chance level shown by dashed line (50% correct).

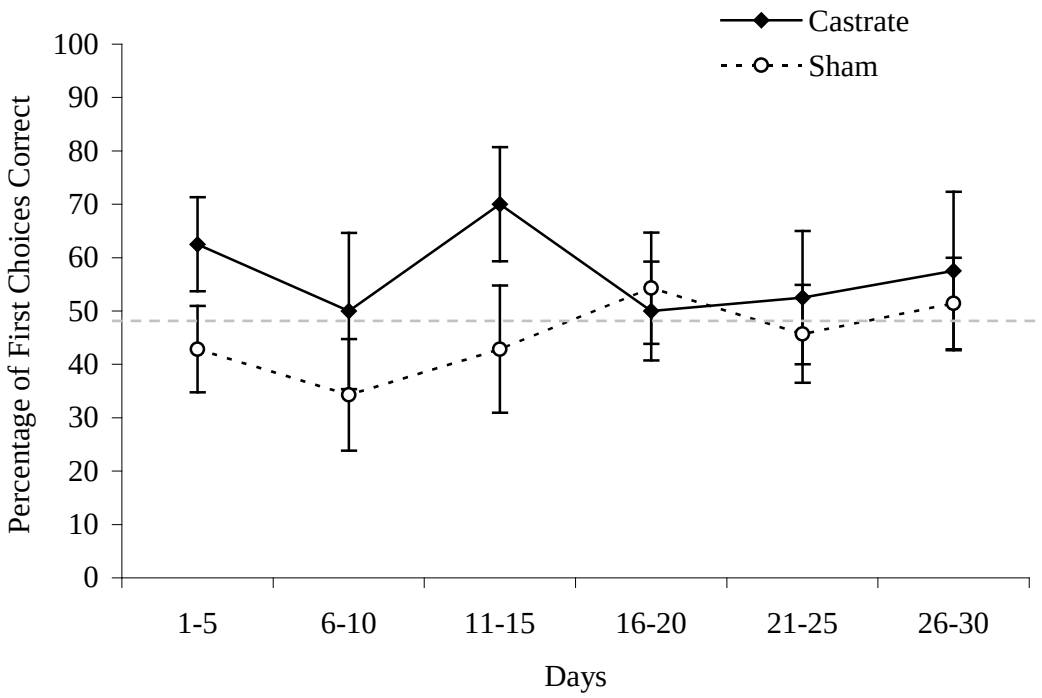

Castrate
Sham
Percentage of First Choices Correct
100
90
80
70
60
50
40
30
20
10
0
1-5
6-10
11-15
16-20
21-25
26-30
Days

Fig. 3. Total number (+ *SEM*) of WME and RME committed by castrated (*n* = 8) and sham-castrated (n = 7) male rats over 30 days of testing on the radial arm maze. Shams committed more total RME than did castrates, and there was a trend for castrates to commit more total WME than shams.

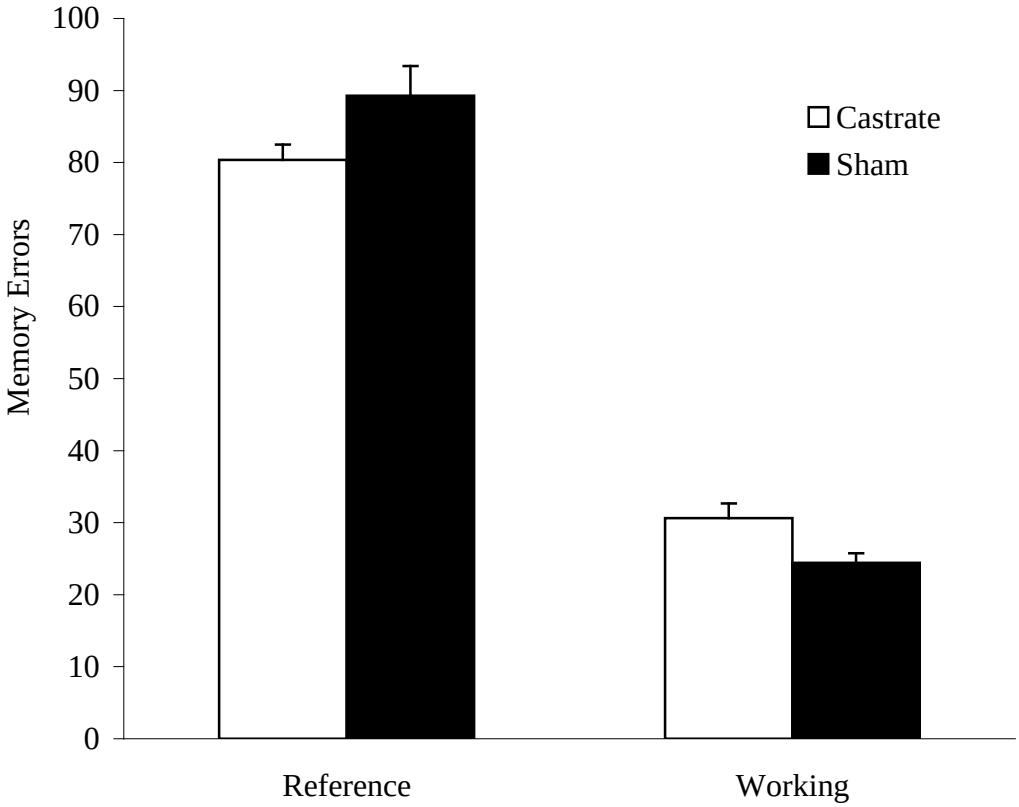
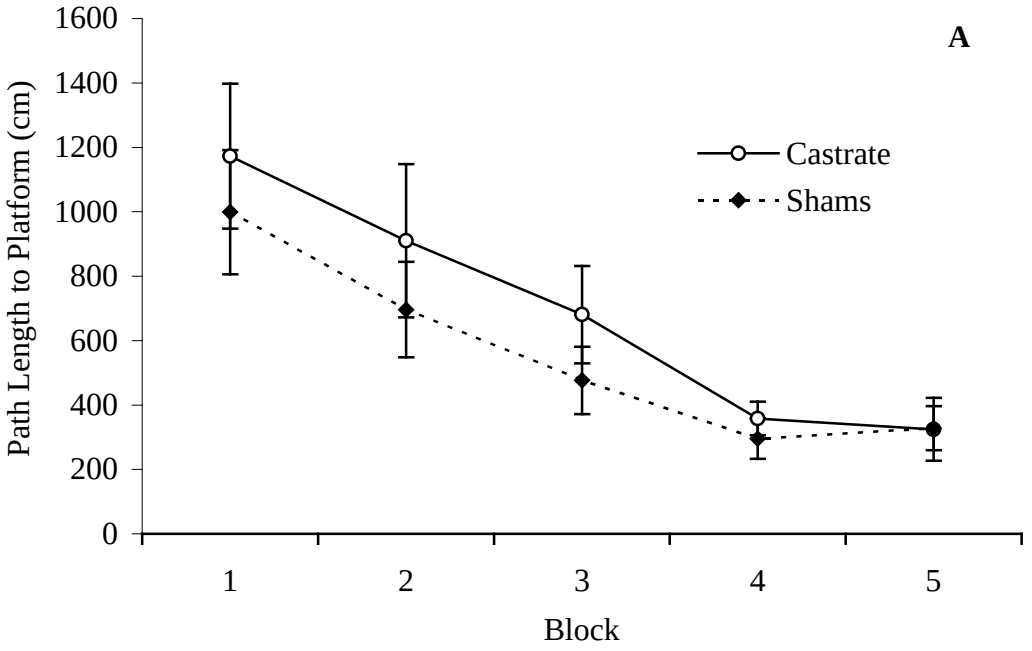

100
90
80
70
60
50
40
30
20
10
0
Memory Errors
Castrate
Sham
Reference
Working

Fig. 4. Mean (± *SEM*) escape latency (A) and path length (B) to reach platform for castrated (*n* = 8) and sham-castrated (*n* = 8) male rats during five blocks of four acquisition trials in the Morris water maze. No statistically significant differences were observed between castrates and shams.


A
Path Length to Platform (cm)
1600
1400
1200
1000
800
600
400
200
0
Castrate
Shams
1
2
3
4
5
Block

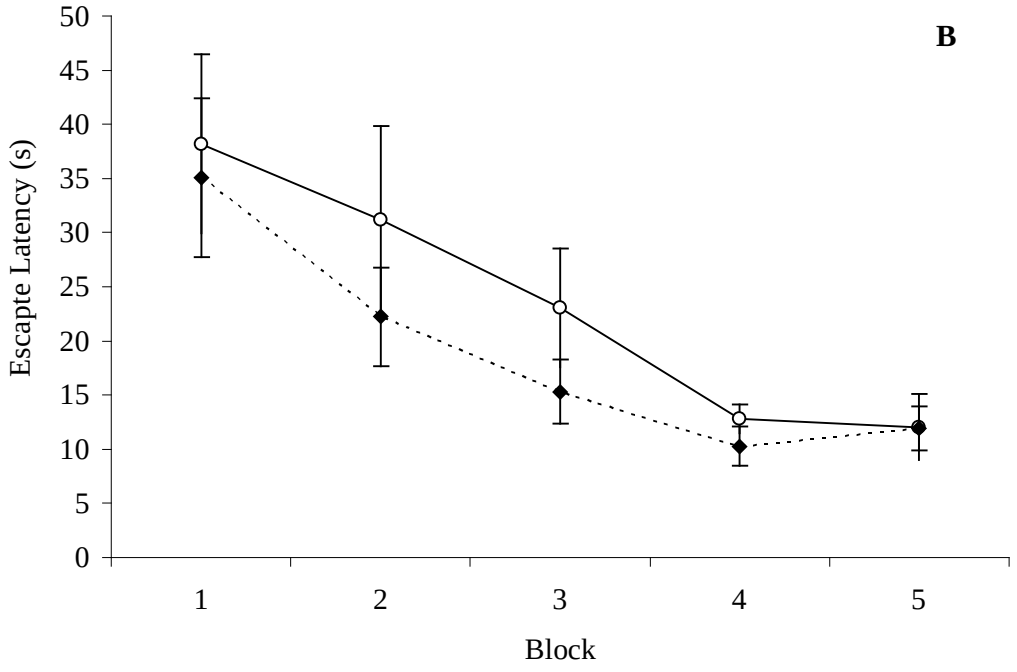

B
Escapte Latency (s)
50
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
Block

Fig. 5. Linear regression of time spent in the target quadrant during probe trials in the Morris water maze against serum testosterone concentrations at the time of perfusion among sham-castrated males (n = 8). There was a trend for males with higher testosterone levels to spend more time in the target quadrant.

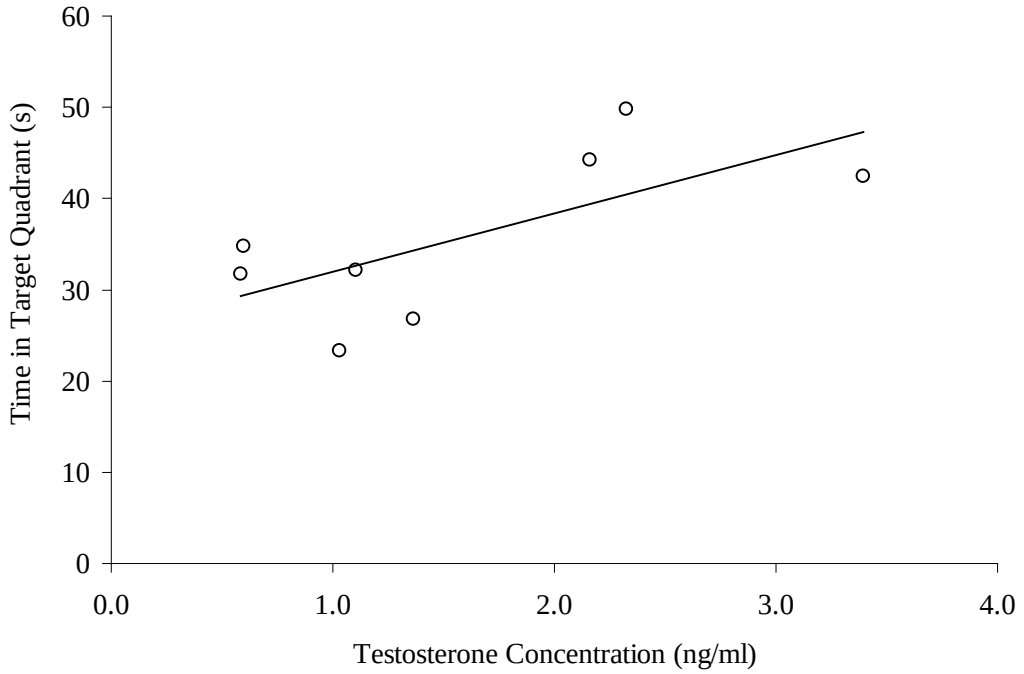
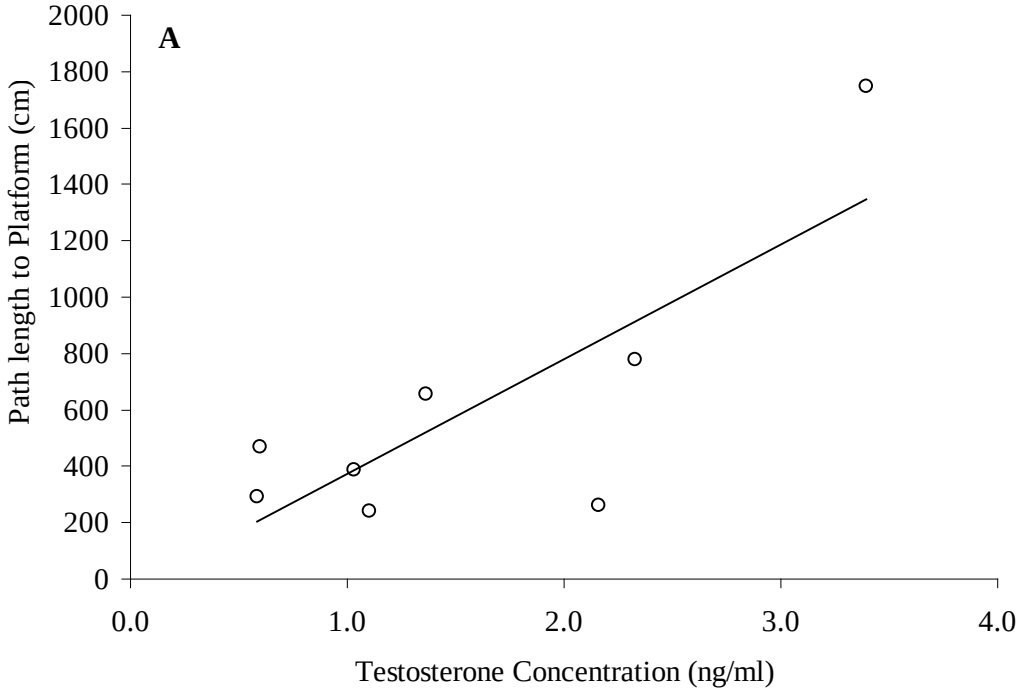
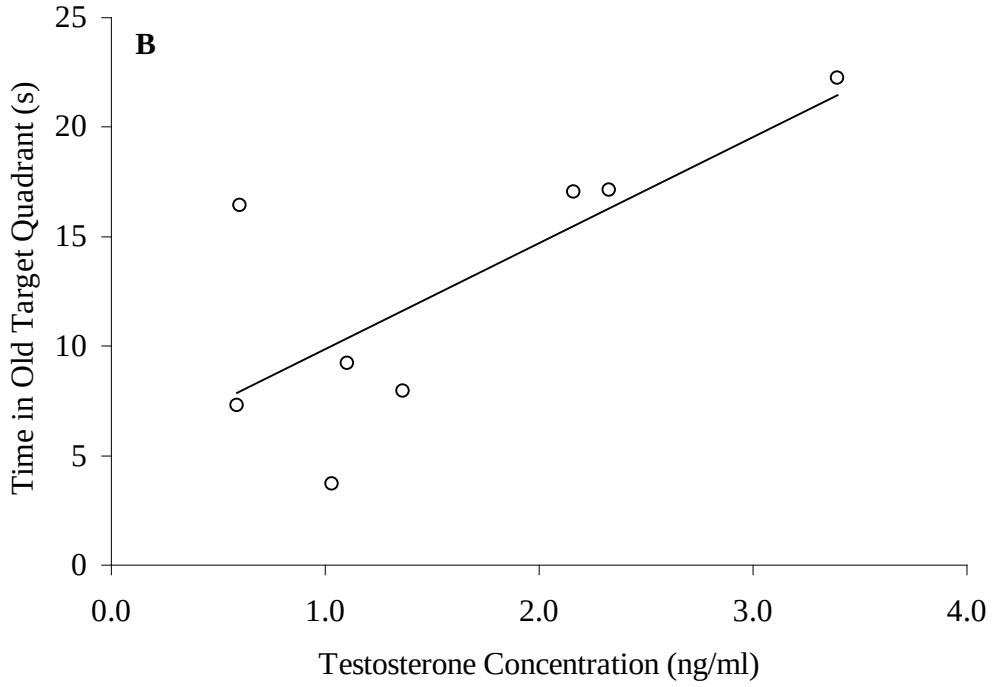

60
50
40
30
20
10
0
Time in Target Quadrant (s)
0.0
1.0
2.0
3.0
4.0
Testosterone Concentration (ng/ml)

Fig. 6. Performance of sham-castrated males (n = 8) during the reversal learning trials in the Morris water maze. Linear regressions are shown for path length to the platform (A) and time spent in the quadrant where the platform had been during acquisition trials (B) against testosterone concentrations at the time of perfusion. Males with higher testosterone levels had longer path lengths and spent more time in the quadrant where the platform had previously been.


A
Path length to Platform (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0.0
1.0
2.0
3.0
4.0
Testosterone Concentration (ng/ml)


B
Time in Old Target Quadrant (s)
25
20
15
10
5
0
0.0
1.0
2.0
3.0
4.0
Testosterone Concentration (ng/ml)