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Effects of testosterone on spatial learning and memory in adult male rats

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ABSTRACT

A male advantage over females for spatial tasks has been well documented in both humans and rodents, but it remains unclear how the activational effects of testosterone influence spatial ability in males. In a series of experiments, we tested how injections of testosterone influenced the spatial working and reference memory of castrated male rats. In the eight-arm radial maze, testosterone injections (0.500 mg/rat) reduced the number of working memory errors during the early blocks of testing but had no effect on the number of reference memory errors relative to the castrated control group. In a reference memory version of the Morris water maze, injections of a wide range of testosterone doses (0.0625–1.000 mg/rat) reduced path lengths to the hidden platform, indicative of improved spatial learning. This improved learning was independent of testosterone dose, with all treatment groups showing better performance than the castrated control males. Furthermore, this effect was only observed when rats were given testosterone injections starting 7 days prior to water maze testing and not when injections were given only on the testing days. We also observed that certain doses of testosterone (0.250 and 1.000 mg/rat) increased perseverative behavior in a reversal-learning task. Finally, testosterone did not have a clear effect on spatial working memory in the Morris water maze, although intermediate doses seemed to optimize performance. Overall, the results indicate that testosterone can have positive activational effects on spatial learning and memory, but the duration of testosterone replacement and the nature of the spatial task modify these effects.

Introduction

Spatial ability is broadly defined as the ability to perceive, encode, store, retrieve, transform and integrate spatial information drawn from two- or three-dimensional space (Ecuyer-Dab and Robert, 2004). Men outperform women on a variety of spatial tasks including mental rotation of objects (Kaufman, 2007; Parsons et al., 2004), route-learning (Holding and Holding, 1988; Postma et al., 2004), and maze navigation (Astur et al., 1998; Moffat et al., 1998). A male advantage for a variety of spatial learning and memory tasks has also been documented for meadow voles (Gaulin and FitzGerald, 1986), deer mice (Galea et al., 1994), and various strains of laboratory mice and rats (Jonasson, 2005), which suggests that this sex difference may be a common phenomenon among mammals possibly shaped at the ultimate level by various selective pressures (Ecuyer-Dab and Robert, 2004; Gaulin and FitzGerald, 1986). Among rats, specifically, males have been shown to have better working and reference memory based on performance in the Morris water maze (Harris et al., 2008; Markowska, 1999; Roof and Stein, 1999) and 17-arm radial maze (Gibbs and Johnson, 2008; Seymoure et al., 1996). Working memory is defined as a form of short-term memory that involves storage of information from a particular task only for as long as it is useful to complete the task, and reference memory is defined as the long-term storage of memories that are used from one task to the next (Olton and Papas, 1979).

At the proximate level, there is considerable evidence that sex steroids (estrogens and androgens) play at least some role in causing sex differences in spatial ability. Evidence from experiments with humans and rats indicates that testosterone has organizational effects upon the brain early in development that enhance spatial learning and memory (Hines et al., 2003; Isgor and Sengelaub, 1998; Mueller et al., 2008; Roof and Havens, 1992; Williams et al., 1990). Whether androgens have activational effects that enhance spatial ability in adults remains less clear. Recent reviews have concluded that there is no consistent evidence that elevated circulating testosterone levels improve spatial ability in men (Puts et al., 2010; Ulubaev et al., 2009). In spite of inconsistency, however, there is some evidence that testosterone can improve mental rotation ability (Christiansen and Knussmann, 1987; Hooven et al., 2004; Silverman et al., 1999), route-learning (Choi and Silverman, 2002), and performance of a block design task (Thilers et al., 2006) among younger men. A causal link

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between age-related declines in testosterone and memory has been demonstrated by some longitudinal and cross-sectional studies (Moffat et al., 2002), but other studies have found no relationship between testosterone and spatial ability among older men (Martin et al., 2007; Matousek and Sherwin, 2010). Clinical studies testing the effects of exogenous testosterone replacement therapy on the cognitive abilities of healthy older men have also produced mixed results, with some studies showing improved spatial ability in men receiving testosterone (Cherrier et al., 2001, 2005; Janowsky et al., 1994), while other studies have shown no effect of testosterone on cognition (Emmelot-Vonk et al., 2008; Vaughan et al., 2007; Wolf et al., 2000). Additionally, testosterone supplementation given to men with Alzheimer's disease has been shown to be effective in improving spatial memory (Cherrier et al., 2005; Tan and Pu, 2003). Thus, although there is considerable evidence that testosterone supplementation may restore cognitive function (specifically spatial ability) in men with age-related memory loss, contradictory results render the benefits of such intervention questionable.

The contradictory results obtained with human studies highlight the need for the use of rodent models to experimentally test the effect of testosterone upon spatial ability. Unfortunately, the results of rodent studies are also inconsistent, but some general trends have begun to emerge. In various versions of the radial arm maze and T-maze, castration has been shown to impair spatial working memory (Daniel et al., 2003; Gibbs and Johnson, 2008; Hasegawa and Mochizuki, 2009; Kritzer et al., 2001; Spritzer et al., 2008). Surprisingly, Gibbs and Johnson (2008) found that testosterone replacement using silastic implants in castrated male rats did not restore spatial working memory performance on the radial arm maze. In contrast, testosterone implants did restore males' performance on a T-maze alternation task and a water radial arm maze (Bimonte-Nelson et al., 2003; Kritzer et al., 2001). Therefore, most current evidence indicates that testosterone enhances spatial working memory among male rats. The effects of testosterone on spatial reference memory remain less clear. Experiments using the radial arm maze suggest that testosterone impairs spatial reference memory (Gibbs and Johnson, 2008; Spritzer et al., 2008), whereas studies employing the Morris water maze to test spatial reference memory have shown improvement of (Khalil et al., 2005) impairment of (Goudsmit et al., 1990; Naghdi et al., 2001) or no effect of testosterone on performance (Hodosky et al., 2010; Naghdi et al., 2005b; Sandstrom et al., 2006; Spritzer et al., 2008). Some of this variation in results is likely due to differences in the doses of testosterone and the method of testosterone replacement. For example, some studies showing that testosterone impairs spatial reference memory involved injections of a high dose of testosterone directly into the CA1 region of the hippocampus (Moradpour et al., 2006; Naghdi et al., 2001, 2005b). It is also important to note that most past studies in this area have failed to assess circulating testosterone levels following an experimental manipulation, making it difficult to compare results among studies and to determine whether subjects experienced physiological or supra-physiological levels of testosterone.

The general goal of the current study was to further clarify the activational effects of testosterone on the spatial ability of males using rats as a model system. More specifically, we conducted a series of experiments designed to determine (1) whether the effects of testosterone replacement depend on the type of maze task (i.e., radial arm maze vs. Morris water maze), (2) the dose–response relationship between testosterone and spatial memory using doses within the physiological range for a male rat, (3) whether duration of exposure influences the effects of testosterone on spatial memory, and (4) whether the effects of testosterone differ between spatial working and reference memory. To achieve these goals, we used a working-reference memory version of the radial arm maze and both reference and working memory versions of the Morris water maze. For the reference memory version of the water maze, we employed a broad dose range and varied the duration of testosterone exposure prior to behavioral testing. We also assessed circulating testosterone levels following hormone replacement for all of our subjects.

Methods

Subjects

Adult male rats (approximately 60 days old) were obtained from Charles River Laboratories (St. Constant, Quebec, Canada). For Experiment 1, Sprague–Dawley rats were used to allow comparison with a previous study that tested this strain using the radial arm maze (Spritzer et al., 2008), while for Experiments 2 and 3 Long-Evans rats were used to facilitate tracking in the water maze. For all experiments, rats were individually housed in opaque polypropylene cages ($21 \times 42 \times 21$ cm) with Tek-Fresh Bedding (Harlan Laboratories, Indianapolis, IN, USA). Animals had free access to water and a soy-protein-free rodent diet (Harlan Teklad Diet 2020X), except during food restriction for rats tested on the radial arm maze. The housing room was temperature controlled (21 ± 1 °C) with a 12:12 h light/dark cycle (lights on at 0700 h). All animal procedures were approved by the Middlebury College Animal Care and Use Committee and were carried out in accordance with ethical guidelines set by the National Institutes of Health.

All subjects were bilaterally castrated 7–14 days after they arrived in the animal facility. Surgeries were performed with aseptic technique and under isofluorane anesthesia (3.5–4.0% in oxygen during induction, 2.0–2.5% in oxygen during maintenance). The analgesic Ketofen was administered before surgery (5 mg/kg body mass, s.c.), and the topical analgesic Fougera (2.5% lidocaine, 2.5% prilocaine) was applied to the incision site immediately after surgery. Each testis was excised through a small incision at the posterior end of the scrotum and ligated with chromic gut suture material (Ethicon, Somerville, NJ, USA). The muscular sheath was closed with chromic gut sutures, and the skin layer was closed with ethilon sutures (Ethicon). For each experiment, the rats were castrated over 2 days with half the rats castrated each day (Table 1). Seven days after surgery, the average mass of the subjects was 350.4 ± 2.7 g.

Apparatus

For Experiment 1, testing was conducted on an eight-arm radial arm maze elevated 53 cm above the floor, with arms (57 cm × 10 cm) projecting at equal angles from a central platform (34 cm diameter). During testing, 45 mg dustless reward pellets (Bio-Serv, Frenchtown, New Jersey) were placed in small plastic cups affixed 1 cm from the end of each arm, which prevented rats from seeing the pellets. The maze remained in the same location in a dimly lit room for the duration of behavioral testing. Each wall of the room was visually distinct from the others to create clear extra-maze cues. Large, high contrast cues were mounted on two of the white walls (a rectangular poster and a large X), one wall had no cues, and the remaining wall consisted of a large black curtain used to divide the testing room. To minimize intra-maze cues, the maze was rotated random 90° increments before beginning testing each day while remaining in the same relative position in the room. Each rat was placed in the same direction in the center of the maze for all trials, and after placing each rat the experimenter sat nearby in the same location for all trials.

For Experiments 2 and 3, the Morris water maze used was a circular white steel pool (180 cm diameter; 60 cm high). The pool was filled to approximately 40 cm with water at equilibrium with room temperature (20 ± 2 °C), which was made opaque with non-toxic white powdered paint (Sargent Art, Hazelton, PA, USA). The pool was divided virtually into four quadrants with four equidistant release points around the edge. The goal platform (10 cm diameter) was submerged 2.5 cm beneath the water surface in the center of one of