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Testosterone modulates spatial recognition memory in male rats

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ABSTRACT

A growing body of research indicates that testosterone influences spatial cognition in male rats; however, the overwhelming majority of studies have been conducted on tasks motivated by either food deprivation or water escape. The hippocampus-dependent version of the Y-maze task, which characterizes spatial recognition memory, capitalizes on the propensity of rats to gravitate toward novel spatial environments and is not contingent upon either appetite or the stress associated with water escape, two factors also affected by testosterone. Accordingly, the aim of the current study was to examine the effects of orchidectomy and subsequent testosterone treatment on spatial recognition memory. Orchidectomy did not impact spatial recognition memory when the delay between the information and retention trials of the Y-maze task was 24 h. Alternatively, on the second Y-maze task, which featured a 48-h delay between trials, orchidectomy reduced, and treatments that produced higher levels of testosterone restored, preference for the arm associated with the novel spatial environment. Importantly, there were no differences in activity levels as a function of orchidectomy or testosterone treatment on either of the two tasks. Consistent with previous findings, orchidectomy attenuated, and testosterone treatment restored, both body weight gain and the relative weight of the androgen-sensitive ischiocavernosus muscle, which confirmed the efficacy of orchidectomy and testosterone treatments on physiological outcomes. Therefore, testosterone influenced spatial cognition on a task that minimized the influence of non-mnemonic factors and took advantage of the innate preference of rodents to seek out novel spatial environments.

Introduction

In rats, spatial learning and memory is a form of hippocampus-dependent cognition in which optimal performance on a particular task is contingent upon successful recognition of the relationships between cues in an extra-maze environment (Eichenbaum, 2002; Olton, 1977). Interestingly, in both sexes, gonadal hormones modulate the morphology (Leranth et al., 2003; Woolley et al., 1990), neurochemistry (Mitsushima et al., 2009) and function (Foy, 2011; Smith et al., 2002) of the hippocampus. Accordingly, although there is fairly robust advantage for male rats on a variety of spatial tasks (Jonasson, 2005; Luine and Dohanich, 2008), the performance of both sexes is influenced by gonadal hormones (Dohanich, 2002). For example, estradiol treatment facilitated performance in ovariectomized rats on spatial tasks, such as the radial-arm maze task (Daniel et al., 1997) and the water maze task (Markham et al., 2002). Higher levels of estradiol in female rats also facilitated performance on both the Y-maze task (Conrad et al., 2004) and the object placement task (Inagaki et al., 2010), which do not require either food reward or water escape as motivating factors, and instead capitalize on the innate propensity of rodents to gravitate toward novel spatial environments and changes in spatial relationships, respectively (Dellu et al., 1992; Ennaceur et al., 1997).

Although a considerable number of studies have examined the effects of gonadal hormones on spatial cognition in female rats, by comparison, fewer studies have been conducted with male rats (Dohanich, 2002). Nevertheless, a growing body of research indicates that testosterone is an important determinant of spatial cognition in male rats. For instance, orchidectomized rats committed a greater number of errors than rats subjected to sham surgeries during the training phases of several different versions of the radial-arm maze task (Daniel et al., 2003; Harrell et al., 1990; Spritzer et al., 2008). In addition, orchidectomized mice and rats also exhibited significantly longer path lengths on the retention trials of delay-dependent versions of the water maze task in which the escape platform was relocated in the maze during pairs of learning and retention trials (Benice and Raber, 2009; Sandstrom et al., 2006). Importantly, the poorer performance of orchidectomized rodents emerged only when the delays between the learning and information trials were relatively longer, which increased the cognitive demands of the task.

Although the effective dose of testosterone treatment varies as a function of the demands of a particular task (Spritzer et al., 2011), deficits in spatial cognition induced by orchidectomy were rescued by treatments that produce somewhat higher levels of testosterone

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relative to gonadally-intact rodents (Gibbs, 2005; Nelson, 2005; Sandstrom et al., 2006; Spritzer et al., 2011). Orchidectomized rats implanted with Silastic capsules that produced levels of testosterone three times greater than the levels found in their gonadally-intact counterparts exhibited enhanced memory on a delayed matching-to-place T-maze task, which was motivated by food reward (Gibbs, 2005). Notably, the memory enhancement on the T-maze task occurred relative to both orchidectomized rats and gonadally-intact rats implanted with cholesterol-filled capsules (Gibbs, 2005). On some water maze tasks (Spritzer et al., 2011), testosterone-induced memory enhancements in orchidectomized rats corresponded with levels of testosterone that were upwards of ten times greater than those typically found in gonadally-intact rats (Nelson, 2005). Conceivably, treatments that produce higher levels of testosterone relative to gonadally-intact rats may be necessary to restore orchidectomy-induced deficits in spatial cognition.

Testosterone exerts a variety of non-mnemonic effects in male rodents that may have contributed to the previously reported deficits in spatial cognition following orchidectomy. For instance, in male rodents, testosterone modulates anxiety-like behavior (Aikey et al., 2002; Fernandez-Guasti and Martinez-Mota, 2005; Frye and Seliga, 2001; Toufexis et al., 2005), depressive-like behavior (Bernardi et al., 1989; Buddenberg et al., 2009; Carrier and Kabbaj, 2012; Frye and Walf, 2009), the reactivity of the hypothalamic–pituitary–adrenal axis (Foilb et al., 2011; Goel et al., 2011; Handa et al., 1994; McCormick et al., 2002; Seale et al., 2004; Viau and Meaney, 1996), food intake (Gentry and Wade, 1976) and body weight gain (Gentry and Wade, 1976; Wainwright et al., 2011). With this in mind, it is rather surprising that the effects of testosterone on spatial tasks that are not motivated by either food reward or water escape have gone almost completely unexplored (McConnell et al., 2012). Given that testosterone modulates spatial cognition on a variety tasks in male rats (McConnell et al., 2012; Sandstrom et al., 2006; Spritzer et al., 2011), we expected orchidectomy to impair, and treatment with testosterone to restore, deficits in spatial recognition memory on a Y-maze task. In addition, because testosterone also modulates appetite and weight gain (Gentry and Wade, 1976), we expected testosterone treatment to restore orchidectomy-induced decreases in body weight gain. Lastly, because higher levels of testosterone may be necessary to restore orchidectomy-induced deficits in cognition on other spatial tasks (Gibbs, 2005; Sandstrom et al., 2006; Spritzer et al., 2011), the secondary aim of the study was to determine whether higher levels of testosterone within physiological range are necessary to restore spatial recognition memory impairments that follow from orchidectomy.

General methods

Animals

Male Long–Evans rats ($N = 48$) were obtained from Harlan, Inc. (Indianapolis, IN) at approximately 70 days of age. Upon arrival, rats were individually housed, provided ad libitum access to food and water, and maintained on a 12:12 h light–dark cycle (lights on at 07:00 h) in animal care facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the Tulane University Institutional Animal Care and Use Committee in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (1996). Rats were acclimated to the vivarium for at least one week upon arrival to the facility and to procedural and behavioral testing rooms for at least 30 min before procedures or testing began. To further minimize the potential for stress associated with surgery and behavioral testing, rats were handled for 1 min daily for approximately two weeks prior to testing, which was conducted during the light cycle.

Surgery and hormone implants

Forty-eight rats were divided into four equal-sized groups ($n = 12$) to examine the effects of testosterone on spatial recognition memory. Consistent with our previous report (Hawley et al., 2012), rats were orchidectomized bilaterally under anesthesia induced by intraperitoneal injections of ketamine (100 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (7 mg/kg, Miles Laboratories, Shawnee, KS). The sac of the scrotum and the underlying tunica were subsequently incised for both orchidectomy and sham surgeries. For rats that received orchidectomy, the vas deferens was ligated bilaterally and the testes were removed. Immediately following orchidectomy or sham surgery, rats received two subcutaneous implants of Silastic capsules constructed of medical tubing (Dow Corning Corporation, Midland, MI) into the dorsal region of the neck. Capsules were filled with either free testosterone (Sigma-Aldrich Co., St. Louis, MO) or remained empty. Each capsule (1.47 mm ID × 1.96 mm OD) was cut 20 mm long and the open ends were sealed with small pieces of beveled dowels covered in waterproof silicone sealant (General Electric Co., Huntersville, NC). Sealed capsules measured approximately 22 mm long with 15 mm of exposed tubing. Rats subjected to sham surgery (SHAM; $n = 12$) and a group of orchidectomized rats (ORX; $n = 12$) were implanted with two empty capsules. The remaining orchidectomized rats were implanted with either two capsules filled with testosterone (ORX-T+; $n = 12$) or one capsule filled with testosterone and one empty capsule (ORX-T; $n = 12$).

Y-maze task

Spatial recognition memory was characterized on the hippocampus-dependent version of the Y-maze task (Conrad et al., 1996). The maze was constructed from grey opaque Plexiglas that formed three identical arms (50 × 10 × 20 cm; Stoelting ANY-maze, Wood Dale, IL), and was surrounded by a variety of two and three dimensional extra-maze cues. Rats were placed into the maze for a 15-min information trial and were allowed to freely explore the start arm and a second arm, but access to the third arm was blocked by an opaque plastic partition. Rats were returned to their home cages and transported back to the vivarium following the information trial for a delay period. After the delay, rats were placed back into the same start arm and allowed to freely explore all three arms for a 5-min retention trial.

Importantly, exposure to novel mazes potentiates the stress response in orchidectomized rats (Handa et al., 1994). Consistent with previous studies that employed similar versions of the Y-maze task, which featured multiple delay periods (McLaughlin et al., 2008), and to minimize the impact of the stress response on cognition, one month after surgery, rats in the current study were first tested on the Y-maze task that featured a 24-h delay between information and retention trials. One week later, rats were tested in the same Y-maze apparatus relocated to a different testing environment that featured a 48-h delay between trials. This design served to both examine the effects of testosterone on spatial recognition memory during a shorter delay period, as well as acclimate rats to the maze prior to the test that featured a longer, more challenging, delay. To minimize the amount of time experimenters spent in proximity to the Y-maze, the start arm for both Y-maze tasks was positioned in the location within the specific testing environment that afforded the most efficient exit for experimenters. All rats were entered into the maze from the same start arm position on both tests. For both tests, the arm associated with the novel environment was randomly assigned between the two remaining arms.

Retention trials were video recorded by an overhead camera for scoring in which entry into an arm was defined as all four paws crossing into the arm proper by an experimenter blind to the conditions. The maze was cleaned thoroughly with 70% ethanol and air-dried after each trial to remove olfactory cues. On the retention trial, spatial recognition memory within hormone conditions was indicated by the percentage of entries into the arm associated with the novel environment