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Assessment of the effects of sex and sex hormones on spatial cognition in adult rats using the Barnes maze

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ABSTRACT

Although sex differences and hormone effects on spatial cognition are observed in humans and animals, consensus has not been reached regarding exact impact on spatial working or reference memory. Recent studies in rats suggest that stress and/or reward, which are often different in tasks used to assess spatial cognition, can contribute to the inconsistencies in the literature. To minimize the impact of these sex- and sex hormone-sensitive factors, we used the Barnes maze to compare spatial working memory, spatial reference memory and spatial learning strategy in adult male, female, gonadectomized (GDX) male, and GDX male rats supplemented with 17β-estradiol (E) or testosterone propionate (TP). Rats received four acquisition trials, four trials 24 h later, and a single retention trial one week after. Males and females acquired the task during the first four trials and retained the task thereafter. In contrast, GDX rats took longer to acquire the task and showed retention deficits at 1 week. All deficits were attenuated similarly by TP and E. Assessment of search patterns also showed that strategies in the males transitioned from random to spatially focused and eventually direct approaches to the goal. However, this transition was faster in control and GDX-TP than in GDX and GDX-E rats. In contrast, the females almost invariantly followed the maze edge in thigmotactic, serial searches. Thus, while Barnes maze reveals activational, in part estrogenic effects on spatial cognition in males, its amenability to animals' use of multiple strategies may limit its ability to resolve mnemonic differences across sex.

Introduction

In humans, sex differences have been identified for a number of cognitive functions, e.g., superiority of adult females in verbal fluency (Herlitz et al., 2013; Mann et al., 1990; Weiss et al., 2003), and superiority of males in spatial working memory (Lejbak et al., 2011; Talarowska et al., 2013). Sex differences have also been documented in the cognitive dysfunction that characterizes neurological and psychiatric disorders such as Parkinson's disease and schizophrenia wherein males are more often and more severely affected than females (Miller and Cronin-Golomb, 2010) (Han et al., 2012; Leung and Chue, 2000; Vaskinn et al., 2011). Particularly robust are the consensus findings for a male advantage in spatial tasks that range from mental object rotation (Hampson, 1990; Kaufman, 2007; Moffat and Hampson, 1996; Parsons et al., 2004) to virtual Morris water maze and radial arm maze tasks (Astur et al., 1998, 2004; Coluccia and Louse, 2004; Moffat et al., 1998; Woolley et al., 2010). Further, positive correlations have been identified between measures of spatial ability and circulating testosterone levels in men and women (Christiansen and Knussmann, 1987; Duff and Hampson, 2000; Gordon and Lee, 1986; Janowsky et al., 1994; Silverman et al., 1999), while negative correlations have been reported between estrogen levels and spatial cognition in women across the menstrual cycle (Hausmann et al., 2000; Simic and Santini, 2012).

While findings from human studies suggest that both organizational and activational hormone actions influence spatial ability, the exact natures of these actions have yet to be fully resolved. However, the numerous studies in animal and especially rodent models that have sought to clarify these issues have yet to reach complete consensus. For example, while a recent meta analysis supports a male over female advantage in spatial working and spatial reference memory in rats (Jonasson, 2005), the extant literature also includes studies finding no sex differences (Faraji et al., 2010; Healy et al., 1999; Juraska et al., 1984; Kolb and Cioe, 1996) or more infrequently, superior spatial performance in females (Gibbs and Johnson, 2008) on spatial tasks. Findings related to activational hormone effects from studies of gonadectomized (GDX) and hormone-supplemented rats span a similar gamut from no effects (Gibbs and Johnson, 2008; Luine et al., 1998; Sandstrom et al., 2006; Singh et al., 1994; Spritzer et al., 2008, 2011; Ziegler and Gallagher, 2005), to those that differentially identify spatial constructs as estrogen vs. androgen sensitive (Gibbs, 2005; Kritzer et al., 2001, 2007; McConnell et al., 2012; Sandstrom et al., 2006; Spritzer et al., 2011).

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Several non-mnemonic factors are known to influence outcome measures in studies of sex and/or sex hormone effects on performance in spatial tasks. These include animals' ages (Bimonte-Nelson et al., 2003; Kanit et al., 2000) and hormone status, including the duration and dose of hormone deprivation and replacement (Bimonte and Denenberg, 1999; Daniel et al., 2006; Galea et al., 2001; Goudsmit et al., 1990; Spritzer et al., 2011, 2013). In addition, evidence for a male preference in utilization of hippocampal-dependent place strategies (Blokland et al., 2006; Hawley et al., 2012) and for high levels of testosterone in males and high levels of estrogen in proestrus females in biasing animals to use place rather than response strategies in solving spatial mazes (Korol et al., 2004; Spritzer et al., 2013) identify animals' approaches as yet another factor likely to influence outcomes, particularly across studies using mazes and testing contingencies where advantage is differentially conferred for place, response, or other strategies (Faraji et al., 2010; Gibbs and Johnson, 2008; Healy et al., 1999; Lund and Lephart, 2001; Ruprecht et al., 2014).

More recently, it has been suggested that task-related variables of stress and/or reward can also impact outcomes in studies of sex and sex hormone effects on spatial cognition (Hawley et al., 2013; McConnell et al., 2012). Both factors are known to differentiate and differentially influence behavior in gonadally intact and castrated male and female rats (Beiko et al., 2004; Belviranli et al., 2012; Conrad et al., 2004; Heinsbroek et al., 1987; Kritzer et al., 2007; Luine, 2007; Osborne et al., 2009). Thus, differences in sensitivity to stress and/or reward contingencies could help explain: the negative impact of GDX in appetitively motivated radial arm maze tasks but not aversely motivated Morris water maze tasks (Spritzer et al., 2008, 2011); the enhancement of male over female sex differences in spatial navigation in the dry-land ziggurat compared to Morris water maze (Faraji et al., 2010); and the dampening effects that pre-training has on the expression of male over female sex differences in the Morris water maze (Bucci et al., 1995; Faraji et al., 2010; Healy et al., 1999; Lukoyanov et al., 1999; Perrot-Sinal et al., 1996).

To minimize potentially confounding sex- and sex hormone-sensitive factors of stress and reward, a recent study compared short term spatial memory in extensively habituated control, GDX, and hormone-replaced male rats using a non-rewarded object in place memory task (McConnell et al., 2012). While findings of GDX-induced spatial working memory deficits were similar to those of previous studies (Gibbs and Johnson, 2008; Kritzer et al., 2001, 2007; Sandstrom et al., 2006; Spritzer et al., 2008), their attenuation by estrogen as well as by testosterone and dihydrotestosterone differs from the estrogen-insensitivity that has been found for GDX-induced spatial working memory deficits in rewarded tasks (Kritzer et al., 2001, 2007). This raises new questions about how activational hormone actions may influence rats' performances on spatial cognitive tasks and underscores the need for utilization of relatively stress- and reward-neutral testing conditions. Accordingly, we used the Barnes maze, a spatial memory paradigm where behavior is motivated by rodents' natural agoraphobia to search among holes to locate a recessed goal chamber (Barnes, 1979). While holding several advantages for the study of sex and sex hormone impact on spatial cognition, the Barnes maze has rarely been used for these purposes (Barrett et al., 2009; Berry et al., 2008; O'Leary et al., 2011; Ryan and Vandenbergh, 2006). Here the Barnes maze was used to compare multiple measures of performance related to task acquisition, spatial working memory, spatial reference memory, and spatial learning strategies in adult male, adult female, GDX males, and GDX male rats supplemented with testosterone propionate (TP) or 17β estradiol (E).

Materials and methods

Animals

A total of 30 male and 8 female Sprague-Dawley rats (Taconic Farms, Germantown, NY) were used. Of the male rats, 8 were gonadectomized (GDX), 8 were GDX and supplemented with testosterone propionate (GDX-TP), 7 were GDX and supplemented with 17β-estradiol (GDX-E), and 7 received sham surgeries (CTRL) 28 days prior to behavioral testing. To allow similar habituation to housing conditions, female rats were housed in the Stony Brook University animal facility for similar lengths of time as the male subjects used in this study prior to the commencement of behavioral testing. All were housed in same sex/same treatment pairs under a 12 h, non-reversed light–dark cycle, in standard-sized cages. Cages and water bottles are purchased from Lab Products, Inc (Seaford, DE) and are made of Zyfone-a bisphenol-free plastic. Ground corncob bedding (Bed O' Cobs, Anderson) was provided and animals had free access to food (Purina PMI LabDiet: ProLab RMH 3000) and water. All animals were roughly 3 months of age and weighed 275–350 g at the time of testing. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Stony Brook University and were designed to minimize animal use and discomfort.

Surgeries

Twenty-eight days prior to maze habituation and behavioral testing, male rats underwent GDX or sham surgery. Both surgical procedures were performed under aseptic conditions using intraperitoneal injections of ketamine (0.9 mg/kg) and xylazine (0.5 mg/kg) as anesthesia. For sham and GDX surgeries, an incision was made into the scrotum. For GDX, the vas deferens was bilaterally ligated (sterile non-absorbable, 6-0 silk sutures) and both testes were removed. For hormone-supplemented animals, slow-release pellets containing either testosterone propionate or 17β-estradiol (Innovative Research of America, Sarasota, FL) were implanted within the tunica. All incisions were closed with wound clips, which were removed after 10 days. Rats were given subcutaneous injections of buprenorphine (0.03 mg/kg) post operatively before being returned to home cages.

Hormone replacement and estrous cycle determination

Male rats were implanted with slow release pellets at the time of GDX. The testosterone propionate (TP) pellets used released 3–4 ng of TP per milliliter of blood per day and the 17β-estradiol (E) pellets used released 25 pg of E per milliliter of blood per day; both have been used previously in this and other labs and have been shown to produce sustained plasma hormone levels falling within physiological ranges (Adler et al., 1999; Collins et al., 1992; Kritzer, 2000). The efficacies of GDX and hormone replacement were verified in quantitative analyses of the weights of animals' androgen-sensitive bulbospongiosus muscles (BSMs) (Wainman and Shipounoff, 1941). The estrous cycle stage of female rats was assessed each day following maze testing via vaginal lavage and vaginal cytology (Goldman et al., 2007; Marcondes et al., 2002). Lavage samples were collected in saline using a fire-polished, sterile glass pipette. The sampled fluid was immediately placed on a slide, a coverslip was gently placed over it, and cytology was evaluated using light microscopy, differential interference contrast (DIC) optics, and a 20× objective. Estrous smears were identified by the predominance of cornified epithelial cells; proestrus smears were identified by the abundance of nucleated, non-cornified epithelial cells; and diestrus smears were identified by the predominance of leukocytes along with cornified (diestrus I) or nucleated epithelial cells (diestrus II). On testing Day 1 four rats were in diestrus and four were in proestrus. Twenty-four hours later on testing Day 2, one rat transitioned from proestrus to estrus, two rats transitioned from diestrus to proestrus, and two rats transitioned from proestrus to diestrus (Table 1). Two rats remained in diestrus and one in proestrus from Day 1 to Day 2 (Table 1). On testing Day 7, one week later, five rats were in diestrus and three were in proestrus (Table 1).