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Pre-pubertal castration improves spatial learning during mid-adolescence in rats☆

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ABSTRACT

Hippocampus functions, including spatial cognition and stress responses, mature during adolescence. In addition, hippocampus neuronal structures are modified by circulating sex steroids, which dramatically increase during adolescence. Therefore, the effects of castration and the circulating levels of the main sex steroid testosterone on spatial learning and memory were examined across postnatal ages to test whether pre-pubertal castration affected rats' spatial ability in the Morris Water maze (MWM). Male rats were either castrated or sham-castrated at 22d (days of age), or left gonadally intact. They were then trained and tested in the MWM beginning at 28d, 35d, 45d or 60d. We found that all of the intact rats learned the spatial task; however, the males at 22d and 28d required more trials to acquire the task than the males at older ages. The males castrated at 22d and tested at 35d had significantly lower escape latency and traveled distance during training than the sham-castrated males trained at the same age. No differences were observed in mean values of escape latency and traveled distance at 45d even though they had comparable levels of testosterone. We conclude that adult-typical performance for male spatial memory emerges during mid-adolescence and that pre-pubertal castration appears to improve spatial learning during this time.

1. Introduction

The relative importance of early organizational and later activational effects of sex steroids upon cognition is a fundamental question in the field of behavioral neuroendocrinology. The research to date has focused on early postnatal rather than later life periods to address the role of androgens in the development of male spatial memory (Feeser and Raskin, 1987; Isgor and Sengelaub, 1998; Isgor and Sengelaub, 2003). Exposure to high levels of androgens in early life can produce enduring changes through substantial remodeling of the developing nervous system (Isgor and Sengelaub, 2003). Upon gonadal development during puberty, testicular and ovarian hormones may act on formerly sexually differentiated circuits to enable expression of sex-typical behaviors in particular cognition function (Gladwin et al., 2011; Schulz et al., 2009; Sisk and Zehr, 2005). It is very worthwhile to examine the role of gonadal hormones during adolescence on spatial memory. Additionally, it appears likely that secretion of androgens during adolescence can exert programming on neural circuits that are involved in learning and memory (Kanit et al., 2000; McCarthy and Konkle, 2005). The ability for spatial learning has been studied extensively, and it is understood that male individuals perform better in spatial tasks and solve spatial problems more quickly (Jonasson, 2005; Mitsushima et al., 2009; Sisk and Zehr, 2005). Previous studies have reported that sex differences in spatial performance have not been observed before puberty and male superiority in spatial performance emerges around 45 days of age, when the circulating sex hormones have risen to adult levels. This indicates that the devel development of spatial task performance could be induced by the main male sex hormone, testosterone, during adolescence (Isgor and Sengelaub, 2003; Sisk and Foster, 2004; Sisk and Zehr, 2005). Conversely, Frankola et al. (2010) and Grissom et al. (2012a, 2012b) reported sex differences on cognitive measures in rats prior to puberty. Therefore, a developmental strategy was used to test whether pre-pubertal castration affected the ability to acquire spatial learning across postnatal ages. Changes in the circulating levels of the main sex steroid testosterone were also traced.

Abbreviations: d, days; MWM, Morris water maze; fEPSP, field excitatory postsynaptic potential; PS, population spike; HFS, high frequency stimulation; NMDA, *N*-methy D-aspartic acid; LTP, long term potentiation.

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2. Materials and methods

2.1. Subjects

The subjects were 136 male Wistar rats (Pasteur Institute, Tehran, Iran), which were divided into intact, sham-castrated and castrated groups. Rats were housed in polycarbonate cages (five rats per cage) at 20–23 °C on a 12:12 h light–dark cycle with light beginning at 7:30 a.m., with free access to food pellets which were free of steroidal components (metabolic energy 3160, digestive energy 3560, Raw protein 13.5%, Raw fiber 6%, total phosphor 0.62% and calcium 0.56%) and tap water.

2.1.1. Intact rats

Forty-five rats were gonadally intact. Intact rats were aged postnatal day 22 (n = 8), 28 (n = 7), 35 (n = 10), 45 (n = 10) and 60 (n = 10).

2.1.2. Sham-operated and castrated rats

Thirty-five rats were castrated and 37 rats were sham-castrated. Three rats died during surgery. Male rats were castrated or sham-castrated at 22d and trained on the MWM beginning at 28d, 35d, 45d and 60d. No biochemical or behavioral tests were performed on rats castrated or sham-castrated 22d. Sham-castrated rats were ages 28d (n = 10), 35d (n = 10), 45d (n = 10) and 60d (n = 7). Castrated rats were ages 28d (n = 8), 35d (n = 10), 45d (n = 10) and 60d (n = 7).

2.1.3. Testosterone therapy subjects

Sixteen rats at 35d were divided into sham-castrated (n = 9) and castrated (n = 7) and were treated with vehicle or testosterone respectively. All of the experiments were performed between 12:00 p.m. and 3:00 p.m. in accordance with internationally accepted principles for the use of experimental animals.

2.2. Surgical procedures

Surgical procedures were performed under ketamine-xylazine (80 mg/kg ketamine, 25 mg/kg xylazine) anesthesia. To castrate the animals, the sac of the scrotum along with underlying tunica were incised, the vas deferens were bilaterally tied off with a sterile 3/0 chromic (HUR TEB, Iran) ligature, and both testes were removed; thereafter, incisions on the scrotum were sutured. For the sham-castration, incisions into the skin and muscle layers of the scrotum were made and then sutured without removing the testes (Spritzer et al., 2008). The topical analgesic Fougera (2.5% lidocaine, 2.5 % prilocaine) was applied to the incision sites immediately after surgery.

2.3. Hormone replacement therapy

Testosterone (0.032 mg/rat dissolved in 0.1 mL sesame oil) or vehicle (sesame oil) was subcutaneously injected into castrated or sham-operated rats, respectively, from 30d to 37d in 35d groups. Animals in the 60d group received subcutaneous injections of testosterone, or vehicle, in the following manner: 0.032 mg from 30d to 40d, 0.062 mg from 40d to 50d, and 0.25 mg from 50d to 64d. Testosterone therapy was done from 30d to the end of the behavioral assessment day.

2.4. Morris water maze

The water maze is a black circular tank that is 136 cm in diameter and 60 cm in height. The tank was filled with tap water (21 ± 1 °C) to a depth of 25 cm. The maze was located in a room with external maze cues (e.g. bookshelves and posters). The maze was divided geographically into four quadrants (Northeast (NE), Northwest (NW), Southeast (SE), Southwest (SW)), and starting positions (North (N), South (S), West (W), East (E)) were equally spaced around the perimeter of the tank. A hidden circular platform (diameter: 10 cm) was located in the center of the NW quadrant and was submerged 1 cm below the surface of the water. A video camera was mounted directly above the maze to record the rats' swim paths. Data was recorded using an automated tracking system (Noldus Information Technology, The Netherlands).

2.5. Spatial learning procedure

To train animals on the MWM, each rat was given two blocks per day for two consecutive days. Each block had four trials so that each rat performed a total of 16 trials. During the trials the rats were placed in the pool from different, random starting points. They learned to find the hidden platform. The escape latency and the time required by the rat to find and climb onto the platform was recorded for up to 90 s. If a rat did not find the platform within 90 s, it was manually placed on the platform. The inter-trial interval for each rat was 30 s, and the inter-block interval was five min. On the third day the platform was removed from the pool and each rat was tested by a probe trial for 60 s. Then, the platform with a bright colored stick was placed in a quadrant that was different from the acquisition training quadrants and was raised two cm above the water to make it visible (visible platform). The visible platform learning was performed in one block with four trials, immediately following the probe trial. The time to reach the visible platform was considered to be an indication of alternations in the visual acuity, motor or motivational attributes of animals. The experiments were started at stated ages; for example, the 22d male rats were tested at 22d, 23d and 24d, with hidden platform testing was done at 22d and 23d, and probe trial or visible platform testing was done at 24 d of age.

2.6. Hormone assay

After behavioral tests, all of the rats were sacrificed by intraperitoneal ketamine-xylazine injection. Immediately prior to sacrificing, blood samples (3–5 mL) were collected via cardiac puncture and samples were centrifuged at 10,000 rpm for 20 min. After centrifugation, serum was decanted and stored at −80 °C. The testosterone level was measured using the ELISA kit (DRG Instruments, Germany). The sensitivity, the intra-assay coefficient of variance and the inter-assay coefficient of variance for the ELISA kit were 0.083 ng/ml, 4.16% and 8.94%, respectively. The testosterone antibody has some cross reactivity with 5α-dihydrotestosterone (0.8%), androstenedione (0.9%), 11β-hydroxyestostosterone (3.3%), 19-nortestosterone (3.3%), 17α-methyltestosterone (0.1%), epitestosterone, estradiol, progesterone, cortisol, estrone and danazol (all 0.1%). Q3 Corticosterone was also measured using the ELISA kit. The sensitivity, the intra-assay coefficient of variance and the inter-assay coefficient of variance for the ELISA kit were 0.5 ng/ml, 4.6% and 6.9%, respectively. All blood samples were run in duplicate.

2.7. Statistical analysis

The data of learning curves were analyzed using repeated measures ANOVA to evaluate the learning curve for groups across blocks. The blocks were run as within-subject factors, age and treatment (castration) as between-subject factors and speed as a covariate. Because traveled distance and escape latency of rats to reach hidden platform could be affected by speed changes among groups, analysis of covariance (ANCOVA) was used on speed as covariate. Statistical analysis was followed by tests for simple main effects using syntax. For probe tests, percentage of time spent in the target quadrant and visible platform escape latency for each rat was analyzed using two-way ANOVA. Age and treatment were considered as between-subject factors. To analyze the data from hormone assays, age and treatment were considered as between-subject factors in two-way ANOVA. When Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, the Greenhouse–Geisser correction was used to combat the violation of the assumption of sphericity. Fisher's least significant difference (LSD)

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