

Original Article

No Significant Difference Between Intact and Testosterone Depleted or Administrated Male Rats in Spatial Learning and Memory

Nasser Naghdi^a, Gisso Mohaddess^b, Saeid Khamnei^b and Mohammad Arjomand^c

^aDepartment of Physiology & Pharmacology, Pasteur Institute, Tehran, Iran. ^bDepartment of Physiology, Tabriz University of Medical Sciences, Iran. ^cDepartment of Biochemistry, Pasteur Institute of Iran, Tehran, Iran.

Abstract

Androgens have been shown to affect cognitive aspects of spatial memory. Testosterone which is the most important androgen, plays a role in the organization of behavior during development. Also, it has been shown that androgens cause sex related differences in learning and memory especially during neonatal period. In the current study, we assessed the effects of castration and testosterone enanthate (TE) administration on spatial cognition. Multiple doses of testosterone enanthate (20, 40, 80 and 120 mg/Kg) were examined on different groups using Morris water maze. Spatial memory was preserved in castrated rats. There was also no difference among multiple doses and control groups. For control of the level of testosterone in the blood of castrated rats and intact rats, blood samples were collected from intact group and 7, 10, 12, 14, 21 days after castration. Testosterone levels were measured by Radio-immuno assay (RIA) technique and compared among all groups. The level of testosterone after 7 days in castrated rats were 0 nmol/L and after 21 days were 0.02 ± 0.02 nmol/L while in intact rats were 2.69 ± 0.88 nmol/L. These data suggests that changes in the level of androgen in circulation have no effect on spatial localization, at least after puberty in male rats.

Keywords: Castration; Spatial memory; Morris water maze; RIA; Rat.

Introduction

Hippocampus, which is involved essentially in learning and memory processes, is known to be a target for the neuromodulatory action of the steroid hormones produced in the adrenal glands and gonads (1). Androgens have been shown to affect many brain functions including cognitive and mnemonic aspects of spatial processing. Studies have demonstrated that male rats have better spatial abilities than females (2). Testosterone, which is the most important androgen, plays a role in the organization of

behavior during development (3). Female rats which had been treated with testosterone during development, improved spatial performance that was similar to that of intact males (2) and better than non-androgenized control females (4). Findings from animal models suggest that androgens can improve cognitive performance. For example, testosterone replacement to gonadectomized rodents increases acquisition of T-maze (5). In contrast, another studies suggested that high levels of androgens may adversely affect memory in human (6) and laboratory animals (7).

Aging males experience hormonal changes such as decline in testosterone that may lead to the loss of cognitive function including

* Corresponding author:

E-mail: naghdi@institute.pasteur.ac.ir

memory and visual-spatial loss (8). Reports from human studies suggest that administration of pharmacological doses of exogenous testosterone by patch or intravenous infusion is associated with improved spatial memory in healthy older men (3), but an animal model revealed that testosterone administration did not reverse age-related spatial memory deficits in rats and impaired retention in middle-aged rats (7).

It has been shown that neonatal castration of male rats results in maze learning deficiency in adulthood which resembles that of the opposite sex (9). In addition, some studies of hormone manipulation during adulthood, gonadectomy in adult male rats was associated with acquisition deficits in a spatial learning task as compared to controls (2). Since the results of systemic testosterone is so controversial, we decided to examine the effects of castration and testosterone administration on spatial cognition in male rats.

Experimental

Subjects

Adult male Wistar rats (220-250 gr) were individually housed in a temperature- and humidity-controlled room with food and water available ad libitum. They were maintained on a reverse light cycle, with lights off at 7:00. Fifty eight of animals (200-250 g) divided into 7 groups: (I) intact (n=8), (II) castrated (n=8), (III) DMSO (n=14) which received dimethylsulfoxide, the solvent of testosterone, (IV) to (VII) testosterone administered rats (i.p.) which received testosterone enanthate (TE) 35 minutes before each day training as following doses: 20 (n=7), 40 (n=7), 80 (n=7) and 120 (n=7) mg/Kg. Forty two other animals divided into 6 groups: one intact or control (n=7) group and 5 castrated groups. Blood samples were collected from intact group and 7, 10, 12, 14, 21 days after castration. Testosterone levels were measured by RIA technique and compared

among all groups. All experimental procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Drugs

Testosterone enanthate (Aburaihan Pharmaceutical Co., Iran) was dissolved in DMSO (10,11). Four groups of animals received different doses of testosterone (20, 40, 80 and 120 mg/kg). The vehicle group just received the solvent, dimethylsulfoxide (Merck, Germany). The intraperitoneal injection was performed 35 min prior to the first trial daily.

Behavioral Assessment

The Morris task was performed in an water tank which was a circular black tub (Diameter: 136 cm, depth: 60 cm), filled with water (25 cm in depth) at a temperature of 20±1°C. The platform, made of plexyglass, was submerged 1.5 cm beneath the surface of water. An infrared camera was mounted in the center above the circular pool. An infrared LED was attached to each rat as a probe so that the animal motion can be recorded and sent to the computer. Each animal was tested for 5 days. Four trials were applied daily and in each trial, the animal was placed at a different position in maze (north, south, east or west). The platform was hidden and submerged on the first four days but was on the surface and marked by aluminium foil on 5th day.

Castration

All animals were anaesthetized with diethyl ether (Merck, Germany). A horizontal incision was performed in scrotum and the testes were tied off and removed with a cut distal to the ligature. Then, the incision was sutured and disinfected with povidine- iodine.

Testosterone Measurement

Animals were deeply anaesthetized with diethyl ether and trunk blood was collected immediately after decapitation of the animal. All Samples were collected in the morning at 8:00. The serum obtained, was stored at -20°C till

Table1. Serum testosterone level in castrated and intact animals (nmol/L).

Group	Intact	Castrated (7 th Day)	Castrated (12 th Day)
Serum Testosterone Level (Nmol/L) Mean± SEM	2.69±0.88	0±0	0.02±0.02

hormonal assay by RIA technique. Testosterone RIA kit purchased from Spectria (Finland).

Statistical Analysis

All data were initially subjected to a two-way ANOVA (dose-day interaction) followed by post-hoc analysis, using Tukey's honestly significant difference for assessing differences between specific groups. Student's t-test was performed for comparing serum testosterone between two groups (intact and castrated). In all comparisons, $P<0.05$ was considered significant.

Results and Discussion

Figure 1 is a dose-response curve of our results; in which response is average time spent to find the hidden platform on the first four days. Comparison of intact, castrated, DMSO and multiple dose groups on the first four days, in Fig.2. is represented. The results indicate that neither castration, nor multiple testosterone doses affected time latency (Fig. 2-A) or traveled distance (Fig. 2-B) to reach the hidden platform within first four days or daily. Moreover, the speed of different groups shows no significant differences (Fig. 2-C). Also, there are not any effects of either multiple doses of testosterone or testosterone depletion (castration) on motivation, motor activity and visual ability of animals related to the 5th day of study (data not shown).

Table 1 shows the level of serum testosterone measured for admission of castration and permanent deficiency of hormone after surgery. Our results indicate that there is no significant difference between intact and DMSO groups; so DMSO proves as a suitable vehicle for the present study. DMSO was also used as vehicle

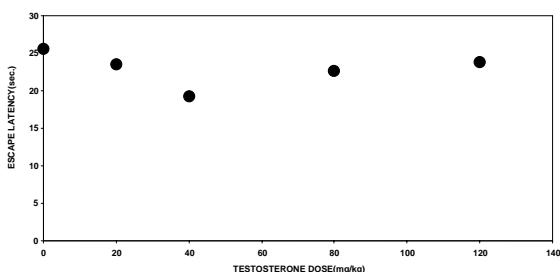


Figure 1. Dose-response curve related to multiple doses of testosterone enanthate administration on escape latency (in seconds) within the first four days of trial. No statistically significant effects was observed with several doses.

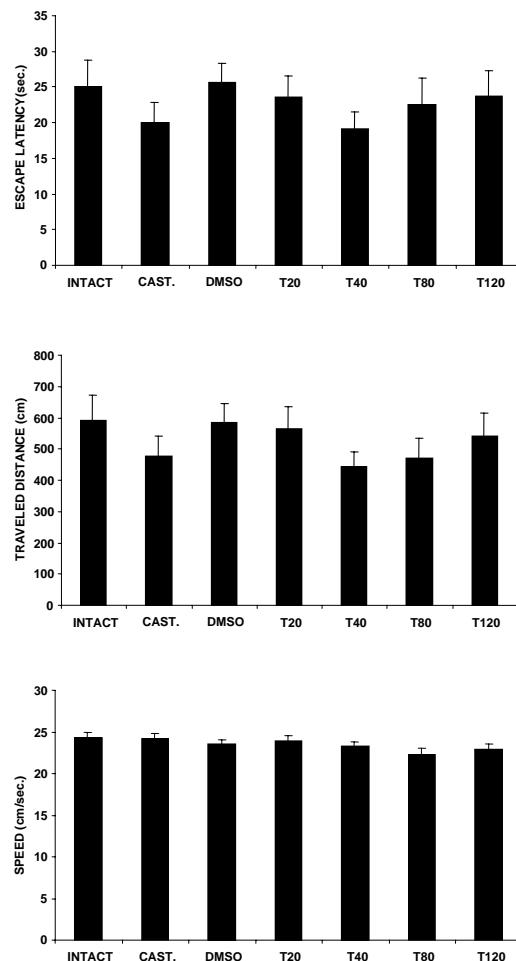


Figure 2. Escape latency (A), traveled distance (B) and speed (C) within the first four days of trial in intact, castrated, control (DMSO) and multiple dose groups. There was no significant difference among all groups.

in other investigations (10,11). There are also no differences between sham operated group and castrated or testosterone administrated groups. The literature of androgen effects on spatial memory in adult animals and human is complex and contradictory. Some evidence suggests a positive correlation between testosterone and spatial ability (3, 12, 13, 14). In contrast, several reports indicates that chronic treatment with androgenic compounds has impaired spatial learning and retention of spatial information in young and middle-aged animals (7, 15) and humans (6, 16). At the same time, many investigators observed no association between visuospatial ability with either endogenous or exogenous testosterone in adult male mammals (15, 17, 18). The results of our experiments are consistent with studies

performed by Smith et al. (1996) and Galea et al. (1995). It seems that androgen is not effective on spatial memory of adult rats when administered systemically; moreover, androgen depletion due to castration of adult rats appears not to affect spatial memory. As it has been proved that intrahippocampal microinjection of testosterone impairs spatial memory in male rats (10), so it is logical to conclude that modulatory action of many steroids like testosterone in CNS areas involved in spatial memory, may be independent of circulation steroids, at least after puberty in rats or there may exist an optimal level of sex hormones for some cognitive functions(19). On the other hand, testosterone can influence cognitive performance after being converted to estradiol in the CNS (20). Thus similar studies on adult female rats would clarify the role of steroids in spatial memory.

References

- (1) Kimoto T, Tsurugizawa T, Ohta Y, Makino J, Tamura H, Hojo Y, Takata N and Kawato S. Neurosteroid synthesis by cytochrome P450-containing systems localized in the rat brain hippocampal neurons:N-Methyl-D-Aspartate and calcium-dependent synthesis. *Endocrinology* (2001) 142: 3578-3589
- (2) Nakamura N, Fujita H and Kawata M. Effects of gonadectomy on immunoreactivity for choline acetyltransferase in the cortex, hippocampus, and basal forebrain of adult male rats. *Neuroscience*. (2002) 109: 473-485
- (3) Janowsky JS, Oviatt SK and Orwoll ES. Testosterone influences spatial cognition in older men. *Behav. Neurosci.* (1994) 108: 325-332
- (4) Frye CA and Sturgis JD. Neurosteroids affect spatial/reference, working and long-term memory of female rats. *Neurobiology of Learning and Memory* (1995) 64: 83-96
- (5) Frye CA, Edinger KL, Selige AM and Wawrzynski JM. 5 α -reduced androgens may have actions in the hippocampus to enhance cognitive performance of male rats. *Psychoneuroendocrinology* (2004) 29: 1019-1027
- (6) Hampson E. Spatial cognition in humans: possible modulation by androgens and estrogens. *J. Psychol. Neurosci.* (1995) 20: 397-404
- (7) Goudsmith E, Van de Poll NE, Swaab DF. Testosterone fails to reverse spatial memory decline in aged rats and impairs retention in young and middle-aged animals. *Behavioral & Neural Bio.* (1990) 53: 6-20
- (8) Tan RS. Memory loss as a reported symptom of andropause. *Arch. Andrology* (2001) 47:185-189
- (9) Dawson JLM, Cheung YM and Lau RTS. Developmental effects of neonatal sex hormones on spatial and activity skills in the white rat. *Biol. Psychol.* (1975) 3: 213-229
- (10) Naghdi N, Nafisy N and Majlessi N. The effects of intrahippocampal testosterone and flutamide on spatial localization in the Morris water maze. *Brain Res.* (2001) 897: 44-51
- (11) Wehling M. Specific, non-genomic actions of steroid hormones. *Annu. Rev. Physiol.* (1997) 59: 365-393
- (12) Alexander GM, Packard MG and Hines M. Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. *Behav. Neurosci.* (1994) 108: 424-428
- (13) Flood JF and Roberts E. Dihydroepiandrosterone sulfate improves memory in aging mice. *Brain Res.* (1988) 118: 178-181
- (14) Vazquez-Pereyra F, Rivas-Arancibia S, Castillo ALD. Modulation of short-term and long-term memory by steroid sexual hormones. *Life Sci.* (1995) 56: 255-260
- (15) Galea LA, Kavaliers M, Ossenkopp KP and Hampson E. Gonadal hormone levels and spatial learning performance in the Morris water maze in male and female meadow voles, *Microtus pennsylvanicus*, *Horm. Behav.* (1995) 29: 106-125
- (16) Gouchie C and Kimura D. The relationship between testosterone levels and cognitive ability patterns. *Psychoneuroendocrinology* (1991) 16: 323-334
- (17) Kampen DL and Sherwin BB. Estradiol related to visual memory in healthy young men. *Behav. Neurosci.* (1996) 110: 613-617
- (18) Smith ST, Stackman RW, Clark AS. Spatial working memory is preserved in rats treated with anabolic-androgenic steroids. *Brain Res.* (1996) 737: 313-316
- (19) Barret-Connor E, Goodman-Gruen D and Patay B. Endogenous sex hormones and cognitive function in older men. *Clinical Endocrinology and metabolism* (1999) 48: 3681-3685
- (20) Wolf PT and Kirschbaum C. Endogenous estradiol and testosterone levels are associated with cognitive performance in older women and men. *Hormones and Behavior* (2002) 41: 259-266

This article is available online at <http://www.ijpr-online.com>