The Effects Of Testosterone Administration Or Depletion On Spatial Learning In Adult Male Rats

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Abstract

The hippocampus, which is involved in spatial mapping and place learning, is known to be a target for neuromodulatory action of the steroid hormones produced in adrenal glands and gonads. Androgens have been shown to affect cognitive aspects of spatial memory. It has been shown that androgens cause sex related differences in learning and memory. We assessed the effects of castration and testosterone administration on spatial cognition using Morris water maze. Multiple doses of testosterone enanthate (TE) (20, 40, 80, and 120 mg/Kg) were examined on different groups of male adult rats.

Spatial memory was preserved in castrated rats and there was also no difference among control and multiple doses groups. These data suggest that a change in circulation levels of testosterone has no effect on spatial localization at least after puberty in male rats.

INTRODUCTION

The hippocampus, which is involved in many learning and memory functions ([13], [16]) including spatial mapping, working memory, place learning and reversal training ([20]), is known to be a target for the neuromodulatory action of the steroid hormones produced in the adrenal glands and gonads ([16]).

Androgens have been shown to affect many brain functions including cognitive and mnemonic aspects of spatial processing ([14]). Studies have demonstrated that male rats have better spatial abilities than female. Female rats, which have been treated with testosterone during development, have improved spatial performance that was similar to that of intact males ([23]) and better than nonandrogenized control females ([8]).

It is suggested that in animal models androgens can improve cognitive performance, for example, testosterone replacement to gonadectomized rodents increases acquisition of T-maze ([7]). In contrast, other studies suggested that high levels of androgens might adversely affect memory in humans ([12]) and laboratory animals ([11]). Aging males experience hormonal changes such as decline in testosterone that may lead to loss of cognitive function including memory and visual-spatial loss ([24]). SAMP8 (P8) mice show an age-related loss of learning and memory for a variety of tasks ([6]).

Reports from studies on healthy aged men suggest that administration of pharmacological doses of exogenous testosterone by patch or intravenous infusion an animal associated with improved spatial memory ([14]). On the other hand, testosterone administration did not reverse age-related spatial memory deficits and impaired retention in middle-aged rats ([11]).

Moreover, it has been shown that neonatal castration of male rats results in maze learning deficiency in adulthood which resembles that of the opposite sex ([3]) and gonadectomy in adult male rats was associated with acquisition deficits in a spatial learning task as compared to controls ([23]). Flood et al ([6]) has shown that castration of 4-month-old P8 mice did not produce deterioration in learning and memory, indicating that low levels of testosterone per se are not responsible for the impairment seen in 12-month-old P8 mice. In contrast, results obtained by Kritzer et al ([17]) from gonadectomized placebo-treated and estradiol treated rats took significantly longer to acquire the T-maze rule than controls and gonadectomized, testosteone-treated rats acquired the task within the same time frame as control.

Since the human and animal studies on the effect of systemic testosterone are so controversial, we decided to examine the effects of castration and testosterone administration on spatial cognition in male rats.

MATERIALS AND METHODS

SUBJECTS

Adult male albino wistar rats (220-250 gr) were obtained from Pasteur Institute of Iran. They (five per cage) were housed in a temperature (25±2°C) and humidity-controlled room. The animals were maintained on a reversed light cycle, with lights off at 7:00. Food and water provided ad libitum except for the periods of behavioral testing in Morris Water Maze (MWM).

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

CASTRATION SURGERY

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.

TESTOSTERONE MEASUREMENT

To measure serum testosterone level, animals were deeply anaesthetized with Diethyl ether and trunk blood was collected immediately after decapitation. All Samples were collected at 8.00 a.m.

The obtained serum was stored at -20° C till hormonal assay by RIA technique. Testosterone RIA kit was purchased from Spectria (Finland).

42 animals divided into 6 groups: one Intact or Control group (n=7) and 5 Castrated groups (n=7), which blood samples were obtained from them 7, 10,12,14,21 days after castration.

BEHAVIORAL ASSESSMENT

APPARATUS

The water maze was a black circular pool with a diameter of 136 cm and a height of 60 cm, filled with 20 ±1°C water to a depth of 20 cm. The maze was divided geographically into four equal quadrants and release points that were designed at each quadrant as N, E, S, and W.

A hidden circular platform (10 cm in diameter), made of Plexiglas, was located in the center of the southwest quadrant, submerged 1.5 cm beneath the surface of the water.

Fixed, extra maze visual cues were present at various locations around the maze (i.e. computer, MWM hard wares, posters). An infrared camera was mounted above the center of the maze. An infrared LED was attached to each rat as a probe so that the animal motion can be recorded and sent to the computer. A tracking system was used to measure the escape latency, traveled path and swimming speed.

PROCEDURE

The animals were tested for 5 days and all runs began at 9:00 a.m. Each animal received four trials during four daily acquisition sessions. Each rat was placed in the water facing the wall of the pool at one of the four designated starting points (north, east, south, and west) and allowed to swim and find the hidden platform. Each of four starting positions was used once in a series of four trials; their order was randomized. If a rat found the platform it was allowed to remain on it for 30 s. If the rat failed to find the platform within 90 s, it was placed on it for 30 s.

During the first 4 days the platform position remained constant, in the SW quadrant. On day 5, the platform was elevated above water surface, marked by aluminum foil and placed in the SE quadrant.

58 animals divided into 7 groups: (I) Intact or Control group (n=8), (II) Castrated group (n=8), (III) DMSO or Vehicle group (n=14) which received Dimethylsulfoxide, (a testosterone solvent) (Merck, Germany), (IV) to (VII) Testosterone Enanthate (TE) groups (n=7) (Aburihan pharmaceutical co., Iran), which received testosterone as following doses: 20, 40, 80 and 120 mg/Kg. All injections were performed intraperitoneally (i.p.), 35 minutes prior the first trial everyday.

STATISTICAL ANALYSIS

Statistical analysis was performed using Student's t-test for comparing serum testosterone level between intact and castrated groups.

All spatial learning data over training days from hidden and visible platform tests were analyzed by two-way ANOVA, followed by post-hoc analysis using Turkey honestly significant difference for assessing differences between specific groups.

All results were shown as means± S.E.M. In all comparisons, $P<0.05$ was considered significant.

RESULTS

SERUM TESTOSTERONE MEASUREMENT

In intact group, the mean serum level of testosterone was 2.96±0.88 ng/ml. After castration serum testosterone level

dropped to undetectable values and did not rise even 21 days after castration.

HIDDEN PLATFORM TRIALS (DAYS 1-4)

The group mean escape latencies to reach the hidden platform in the water maze by intact, castrated and groups treated with different doses of testosterone is shown in figure 1.

Neither castration nor multiple testosterone doses affected time latency.

Traveled distance (Fig.1-B) and swim speed (Fig.1-C) to reach the hidden platform were also not significantly different between groups within first four days of training.

Figure 2 represents the effect of depletion (castration) or administration of different doses of testosterone on spatial memory; in which escape latency is compared among groups during four days of training.

VISIBLE PLATFORM TRIALS (DAY 5)

There was no significant difference in performance among the groups for latency, distance and speed on 5 th day of study (data not shown).

Figure 1

Table 1: Serum testosterone level in castrated and intact animals (ng/ml). UD, Undetectable.

GROUP	INTACT	CASTRATED (7th day)	CASTRATED (12th day)
SERUM TESTOSTERONE LEVEL (ng/ml) Means ± SEM	2.69±0.88	UD	UD

RAT No.	1	2	3	4	5	6	7
INTACT	5.2	6.0	0.7	2.9	0.5	3.3	0.2
CASTRATED (7th day)	UD	UD	UD	UD	UD	UD	UD

Figure 2

Figure 1: Escape latency (A), traveled distance (B) and speed (C) within first four days of trial in intact, castrated, control (DMSO) and multiple dose groups of testosterone (T).

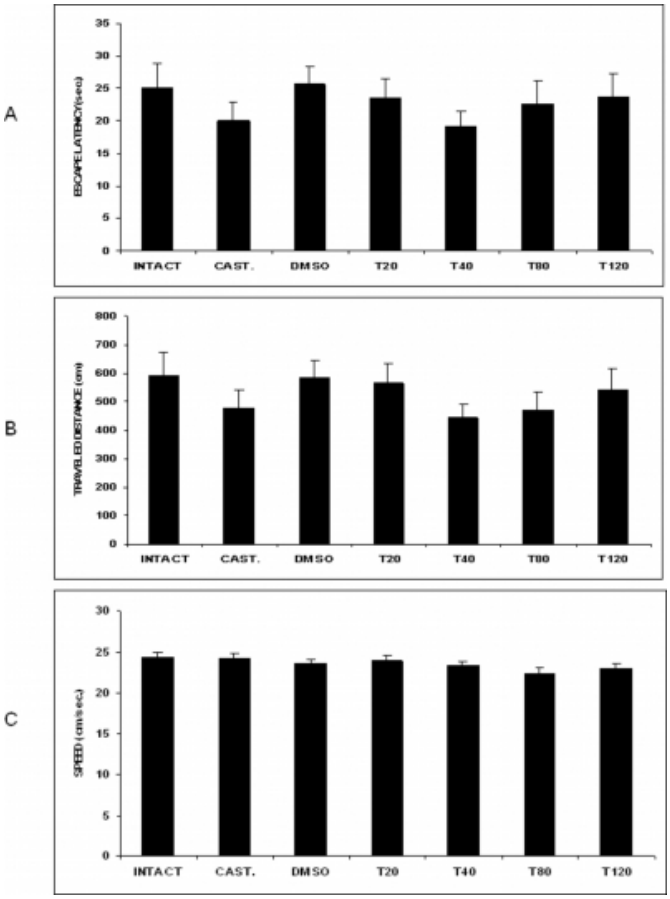
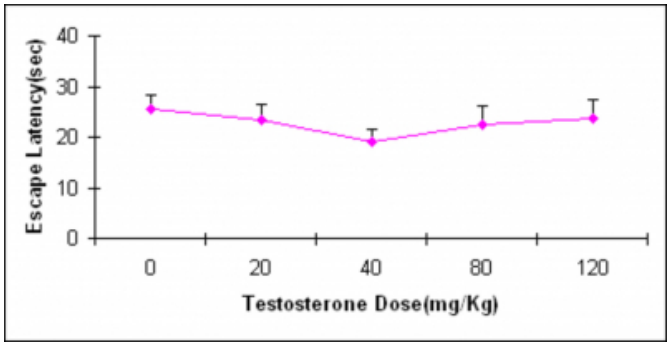


Figure 3

Figure 2: Dose-response curve related to multiple doses of testosterone enanthate administration on escape latency (in seconds) within first four days of trial.



DISCUSSION

Initially, it is worth the mention that according to our results 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 in other

investigations ([20], [30]).

Since there was no statistical difference between control and castrated or testosterone administered groups on the fifth day of study in which the platform was elevated above the water level, it can be inferred that there were no motivational, motor activity and sensory processes deficits induced by treatment in animals.

The literature of androgen effects on spatial memory in adult animals and humans is complex and contradictory. Some evidence suggests a positive correlation between testosterone and spatial ability ([1], [5], [14], [29]). In contrast, several reports indicate that chronic treatment with androgenic compounds has impaired spatial learning and retention of spatial information in young and middle-aged animals ([9],[11]) and humans ([10], [12]). At the same time, many investigators have reported no association between visiospatial ability and either endogenous or exogenous testosterone in adult male mammals ([9], [15]).

The results of our experiments are consistent with studies performed by Smith et al. and Galea et al., who report that testosterone, when administered systemically, is not effective on spatial memory of adult rats ([9], [25]). Moreover, androgen depletion due to castration of adult male rats also appears not to affect spatial memory ([28]), which is again confirmed by the present study.

On the other hand, it has been shown that intrahippocampal ([20]) and intrabasolateral nucleus of amygdale ([21]) microinjection of testosterone enanthate impairs spatial memory in male rats. Therefore it seems logical to conclude that in rats, at least after puberty, modulatory action of testosterone in CNS areas involved in spatial memory may be independent of circulating hormones or there may exist an optimal level of sex hormones for some cognitive functions ([2]).

Moreover, testosterone can influence cognitive performance after being converted to its metabolites like estradiol ([15], [19], [31]) or dihydrotestosterone ([18]) in CNS. Intrahippocampal injections of estradiol enhance memory in male adult rats ([23]). Electrophysiological evidence indicates that estradiol increases the amplitude of hippocampal CA1 cell field population spikes invitro in adult male rat brain slices ([27]). Dihydrotestosterone also may have mnemonic effects in hippocampally-mediated learning ([4])

In addition, brain is capable of de novo biosynthesis of neurosteroids independent of gonads, adrenals, and other peripheral steroidogenic organs ([32]). Testosterone and estradiol may be produced endogenously in the adult brain, where they play an essential role in the plasticity of neurons ([13]).

In conclusion, it seems that circulating testosterone with doses utilized in the present study has no effect spatial memory.

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