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The effects of exogenous testosterone on spatial memory in rats

Research Article

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Abstract: Testosterone (T) is known to affect spatial abilities in men and women. Studies focusing on this relationship showed that both endogenous variability of T and administration of exogenous T, altered mental rotation and spatial visualization. Organizational and activational effects of T can be separately identified. The aim of our study was to evaluate the activational effects of exogenous T on spatial memory in male and female rats. T was administered 3 times a week over a two week period in either 1 mg/kg for low testosterone group or 10 mg/kg for high testosterone group. The Morris water maze was performed to assess the rat's working and reference spatial memory. T and estradiol levels were measured in plasma. Increase in plasma T levels was confirmed in the experimental groups in comparison to the control groups (receiving sterile oil, 3 times a week over a two week period). Low dose T impaired working, but improved reference memory in female rats. In male rats the negative effects of T (both doses) on reference memory were shown. This experiment showed that the activational effects of exogenous testosterone on spatial memory of rats were gender and dose-dependent.

Keywords: *Testosterone • Androgens • Spatial abilities • Sex differences • Water maze*

1. Introduction

Sex differences have been shown in several cognitive functions including spatial abilities like mental rotation (ability to rotate a 3D object virtually) and spatial visualization (ability to fold the 2D pattern into 3D object) [1,2]. To uncover the mechanism behind these differences several approaches have been used. Functional MRI revealed that while solving the same 3D task, men were predominantly activating parietal areas, while women appeared to use the inferior frontal areas [3]. These functional differences are linked to subtle morphological differences [4]. Interestingly, sex differences have only been seen in pencil and paper tests on mental rotation, but not in the virtual environment [5].

Sex differences are associated with sex steroids. Sex differences in spatial abilities are already present in prepubertal children [6,7]. These findings might be explained by the effects of prenatal testosterone (T)

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affecting the very early development of brain tissues. The importance of prenatal testosterone on later spatial abilities was shown for humans [8], as well as for rats [9]. The long-term effects of T on brain development are called organizational effects [10-12].

In addition, spatial abilities vary during the menstrual cycle in women as reported recently [13]. Our unpublished data indicate that similar intra-individual variability in relation to T dynamics is present in men. The short-term effects of T on brain function including spatial abilities are called activational effects [14]. However, these short-lasting effects are not generally accepted and are described as inconsistent in a recent systematic review [15].

The aim of our study was to evaluate the activational effects of exogenous T on spatial memory in male and female rats and support the hypothesis that the effects are dose-dependent.

2. Experimental Procedures

2.1 Animals

After one week of acclimatization to conditions in the animal house and handling by the investigators, fifteen male and fourteen female adult Wistar rats sourced from Slovak Academy of Sciences, Dobrá Voda, Slovakia (5 months old, weight 200-300g) were randomly divided into 3 groups (4-5 animals per group and gender). The control group (CTRL) that received no exogenous testosterone; the low dose testosterone group 'T', and the high dose testosterone group 'TT'. Rats were housed in polycarbonate cages with *ad libitum* access to a standard diet and tap water in a room with controlled temperature and humidity (temperature 18-22°C, humidity 50-70%).

2.2 Interventions

Rats were treated with testosterone (T and TT groups) or the same volume of olive oil (CTRL group) intramuscularly 3 times a week for one week before maze tests and then during maze tests always at the beginning of the dark phase of the light-dark cycle. In the T group the dose of T was low – 1 mg/kg bodyweight per injection, in the TT group the dose of T was high – 10 mg/kg bodyweight per injection (testosterone propionate, Sigma, St. Louis, USA).

2.3 Spatial memory

To evaluate spatial memory the standard Morris water maze was used. A circular tank (diameter 1.5 m, height 1 m) half filled with tap water (room temperature) was placed in a dark room. A transparent platform was placed into one of four quadrants. Intra-maze and extra-maze cues were present to enable spatial learning. Rats underwent 3 swimming/learning sessions a day always starting from a different quadrant. Maximum time for one session was 60 seconds. If the escape platform was not found, the animal was navigated to the platform by the investigator. The procedure was repeated for four days. On day 4 the latency times to find the platform from the first and the last sessions were used for the calculation of the coefficient of working memory ((latency time from the first session – latency time from the last session)/latency time from the first session). An additional session was done on day 5 to assess reference memory ((average latency time on day 1 – average latency time on day 5)/average latency time on day 1).

2.4 Biochemical analysis

One day after the last session rats were sacrificed by exsanguination in deep anesthesia. Blood was collected into heparin tubes and centrifuged at 5000 g for 5 minutes. Plasma was stored frozen until analysis. T and estradiol were measured in plasma samples using commercially available ELISA kits according to the protocol of the manufacturer (DRG Diagnostics, Marburg, Germany). The reported technical variability was very low with an intra-assay coefficient variation of 3% and inter-assay coefficient variation of 5%.

2.5 Statistical analysis

Data were analyzed using one way ANOVA with the 3 groups being the tested factor. Least significant difference (LSD) *post hoc* test was used for the comparison between pairs of groups. P-values less than 0.05 were considered significant. XLStatistics 5 and GraphPad Prism 5 were used for all calculations and tests. Data were presented as mean + standard deviation.

3. Results

Results from the analysis of hormonal levels are shown on Figure 1. Female rats treated with T had approximately a 4-fold higher T concentrations in the T group and 15.5-fold in the TT group in comparison to the CTRL group at the end of the experiment (both $P<0.001$). Male rats in the T groups had similar T concentrations to the control rats. Rats in the TT group treated with a higher dose of T had approximately 5-fold higher T concentrations than male rats in the CTRL group ($P<0.001$). There were no significant differences between the groups in estradiol levels, although female

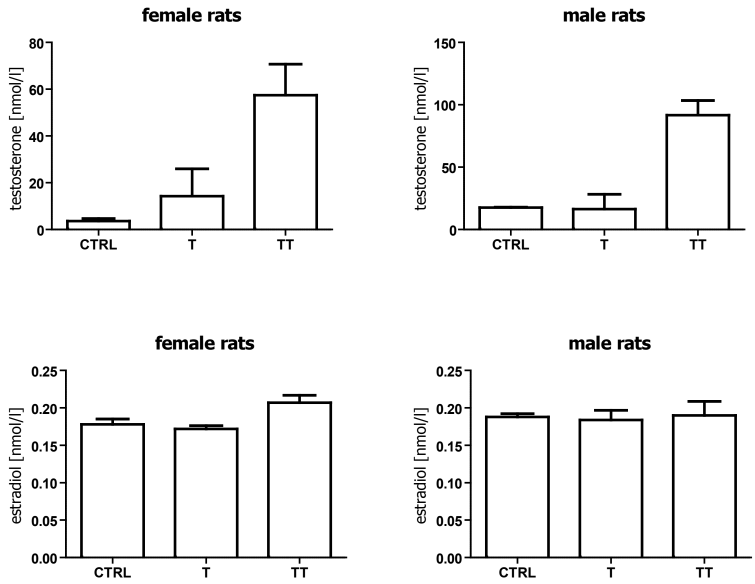

Figure 1. Plasma levels of testosterone and estradiol in male and female rats. The increase of testosterone in rats receiving testosterone was dose-dependent.

rats in the TT group had slightly higher estradiol concentrations on average (approximately by 16%).

Average latency times to find the platform on day 4 and the corresponding coefficients of working memory are shown on Figure 2. The latency times were higher in the T group (by 70%) in comparison to the CTRL group in female rats. The TT group had similar latency time to the CTRL group. In male rats the latency times gradually increased with the administered dose of T. On average in the T group the latency times were 1.6-fold higher than in the CTRL group, in the TT group the difference was 2.3-fold. Coefficients of working memory in female rats treated with T were lower than in the CTRL group. A similar but non-significant trend was found in male rats.

Coefficient of reference memory in this experiment was higher in the T group than in the CTRL, but there

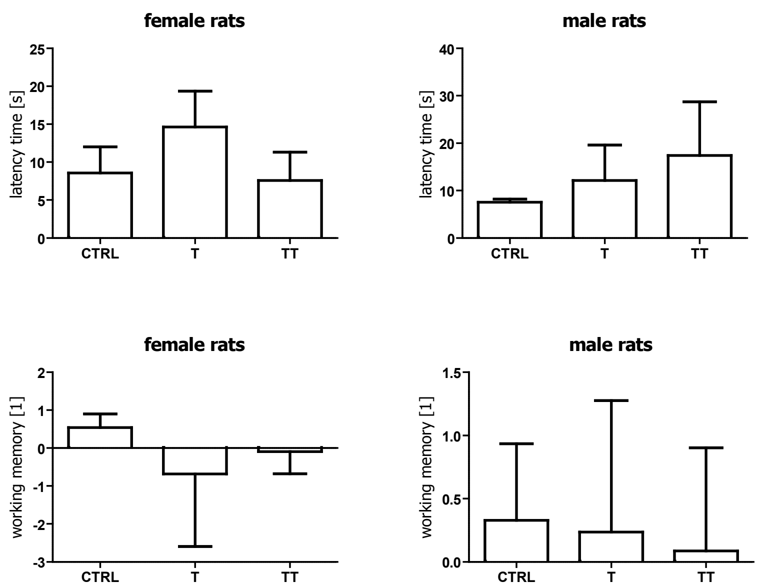

Figure 2. Average latency times in Morris water maze on day 4 and corresponding coefficients of working memory in male and female rats.

was no difference between the CTRL and TT group in female rats. On the contrary, in male rats the higher coefficient of working memory was achieved by rats in the CTRL group, while rats in the T group achieved lower scores (by 29%) and the lowest scores were found in the TT group (by 73%). Graphs showing the results of the analysis of the reference memory are presented in Figure 3.

4. Discussion

Our results show that the relationship between plasma T in female rats is dependent on the dose administered. In male rats the administration of low dose T did not change the plasma T levels considerably. This might be explained by high intra-individual variability of T levels found in our preliminary experiments. To increase T levels significantly, in male rats, the dose of administered T needs to be at least 10 mg/kg. Subtle changes to T levels might have behavioral effects, however repeated monitoring of T levels might be needed to detect these changes on the background noise of physiological variance. Interestingly, such a subtle difference was found in estradiol levels of female rats in the TT group. As T is a precursor of estradiol, a high dose of T might represent an increase of availability of substrates for the enzyme aromatase that produces estradiol.

Endogenous T levels are associated with spatial abilities [16] however, the association is far from being clear. A cross-sectional study for example showed a positive correlation with T levels in men [17]. Similarly, T has been shown to improve spatial abilities in hypogonadal men or patients with Alzheimer's disease [18-20]. Conversely, other studies in hypogonadal men supplemented with T showed no effects on cognitive abilities [21]. Our previous research indicate a negative correlation between T levels and spatial abilities [22]. In women, most studies showed an association of higher T levels with better spatial cognition [23]. This has also been shown for exogenous administered T [24] and for intra-individual variability of T levels [25]. Recently, even antioxidants have been shown to improve castration-induced decrease in spatial abilities of rats [26].

In this experiment, there were no statistically significant differences between the groups in the results from the Morris water maze, mainly due to high inter-individual differences. However, slight changes in the monitored parameters indicated effects of T administration in both sexes. Administration of low dose T to female rats improved the reference memory, but worsened slightly the working memory. Interestingly, high dose T administered to female rats did not affect working or reference spatial memory. In male rats the effects of T were consistently negative. Gradual increase in latency times and decrease in reference memory were found.

Castration of male rats impairs spatial memory, while supplementation with T improves spatial memory [27]. Another study on castrated male rats showed impaired working memory, but an improved reference memory [28]. Others who investigated intact animals, found T reduced spatial memory similar to estradiol, while anastrozol – an inhibitor of aromatase, decreased latency times in the Morris water maze [29]. This is in contrast to our previously published results that demonstrated negative effects of anastrozol on performance in the Morris water maze [30]. On contrary, the anti-anxiety effects of testosterone seem to be clearly mediated by aromatase and estrogen receptors [31].

One research group has highlighted the difference between systemic and local cerebral concentrations of T. Their research showed that local injections of T, rather than systemic T manipulation, affected cognitive abilities [32]. A dose-dependent increase in latency times was shown for male rats when T was injected into the amygdala [33] and into the hippocampus [34]. Interestingly, in the same experiment similar effects were shown for flutamide – an androgen receptor antagonist. However similar results were also achieved in an experiment with administration of T and anisomycin – a protein synthesis inhibitor [35]. Currently, it is not clear which areas and regions of the brain are involved in the

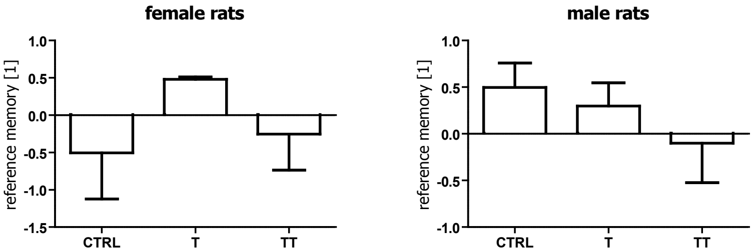

Figure 3. Coefficients of reference memory in male and female rats in the Morris water maze.

processing of spatial information. However, induced experimental damage and injections of neurotransmitters to some areas clearly affect spatial memory [36,37].

In conclusion, our experiment shows that exogenous T affects the spatial memory of intact adult male and female rats in a sex and dose-dependent manner. Molecular mechanisms of T on neurons in different areas of the brain that are currently beginning to be elucidated might shed light on the inconsistencies in the published data regarding the activational effects of T on cognitive abilities.

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