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Research report

Behavioral performance of *tfm* mice supports the beneficial role of androgen receptors in spatial learning and memory

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Abstract

In adulthood, androgens and androgen receptors might contribute to the sexually dimorphic performance in spatial learning and memory, but their roles seem complex. To study the potential role of androgen receptors in spatial learning and memory, we tested adult 6–8-month-old mutant mice with a naturally occurring defect in the androgen receptor gene (testicular feminization mutant or *tfm*) and C57Bl/6J wild-type mice. Because the trait is X-linked, only *tfm* males are completely androgen insensitive while female *tfm* mice are heterozygous, carrying one wild-type and one *tfm* copy of the androgen receptor. Here we show that female *tfm* carrier mice outperform *tfm* male mice in the water maze, while there are no gender differences in water maze performance in wild-type mice. In *tfm* mice, there were no gender differences in measures of anxiety in the open field or plus maze or sensorimotor function, indicating that potential differences in these measures did not contribute to the differences observed in the water maze. There were no differences in *tfm* and wild-type female and male mice in emotional learning and memory in the passive avoidance test. These findings support a beneficial role for androgen receptors in spatial learning and memory.

Theme: Neural basis of behavior
Topic: Learning and memory: physiology

Keywords: Androgen receptor; Mice; Water maze; *tfm*; Spatial learning and memory; Gender

1. Introduction

Many nonreproductive behaviors, including learning and memory [4,40], are sexually dimorphic in both humans [38] and rodents [3,42]. Spatial learning and memory have been attributed to the hippocampus [24,33]. Some studies of spatial learning and memory in rodents suggest that males learn more quickly than females and exhibit superior performance in a variety of mazes [8,12,23,25,29–31,39], which may relate to sex differences in hippocampal structure and the sexual dimorphic cognitive performance in tests of verbal and spatial learning and memory in humans [2,44]. Other studies, however, have not shown such differences between the sexes [6].

In adulthood, androgens and androgen receptors might contribute to the sexually dimorphic performance in spatial learning and memory, but their roles seem complex. Androgens have been shown to enhance spatial learning and memory [1,11,22,36] and both short-term and long-term emotional learning and memory [43]. In addition, posttraining administration of androgens to ovariectomized rats enhanced spatial and emotional learning and memory temporally distinct from anxiety-reducing effects of androgens [13]. However, in some studies, androgens impaired spatial learning and memory [14,17–19], and administration

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in the CA1 of the hippocampus, a brain area with a high concentration of androgen receptors, of either testosterone or the androgen receptor antagonist, flutamide impaired spatial learning and memory [32].

To study the potential role of androgen receptors in spatial learning and memory, we behaviorally analyzed mutant mice with a naturally occurring defect in the androgen receptor gene (testicular feminization mutant or *tfm*) [27], which are available on the C57Bl/6J background [7,41], and C57Bl/6J wild-type mice. A single point mutation in the N-terminal region of the androgen receptor gene results in a premature stop codon [15] and the expression of nonfunctional truncated androgen receptors [46] in *tfm* mice. Because the trait is X-linked, only males are androgen insensitive. The lack of functional androgen receptors results in complete infertility of the *tfm* male mice. Female *tfm* mice are heterozygous, carrying one wild-type and one *tfm* copy of the androgen receptor. Here we show that female *tfm* carrier mice outperform *tfm* male mice in their ability to locate the hidden platform location in the water maze, supporting an important beneficial role for androgen receptors in spatial learning and memory.

2. Materials and methods

2.1. Mice

Tfm and wild-type female and male C57Bl/6J mice were provided by Jackson labs and bred to obtain the cohort of mice for behavioral testing. A gender determination PCR for the DNA of the *tfm* pups that lack male genitalia was performed to distinguish *tfm* female from *tfm* male mice. For the behavioral studies, 12 female and 13 male *tfm* mice and 8 female and 8 male wild-type mice were used. As one *tfm* female mouse fell asleep on the open arms of the plus maze (total rest time on open arms: 277.7 s), only 11 *tfm* female mice were used for the plus maze analysis. As one *tfm* male mouse died prior to water maze testing, 12 *tfm* male mice were tested in the water maze. As two other *tfm* male mice died prior to rotorod testing, 10 *tfm* male mice were used for the rotorod and passive avoidance tests. To minimize the effects of social influences on behavior, all mice were housed singly starting 1 week before behavioral testing under conditions of constant temperature (18 °C), and light from 6:00 a.m. to 6:00 p.m. All mice had free access to food (PicoLab Rodent Diet 20, #5053, PMI Nutrition International, St. Louis, MO) and water. The mice were tested at 6–8 months of age.

2.2. Behavioral testing

The sequence of behavioral testing was open field activity and elevated plus maze (week 1, the plus maze test was performed on the day following the open field test), water maze (week 2, the water maze started 1 week after the open field test), rotorod test (week 3, the rotorod was performed 1 day after the water maze test), and passive avoidance (week 4, the passive avoidance was performed 1 week after the rotorod test). The experimenter was blinded to the genotype of the mice and the gender of the *tfm* mice.

2.2.1. Open field

Since different levels of exploratory drive and/or anxiety can affect motivation and performance in cognitive tests, these functions were evaluated first. Exploratory behavior was assessed in the open field as described [34]. Mice were placed singly in a brightly lit, automated infrared photocell activity arenas (40.64 × 40.64 cm with 16 × 16 photocells for measuring horizontal movements, 8 photocells for measuring rearing) interfaced with a computer (Hamilton-Kinder, Poway, CA). The following parameters were calculated: active times (defined as time, within 1 s, in which a new beam was broken), distance moved, and rearing events. Open field activity was recorded after a 1-min adaptation period for 10 min.

2.2.2. Elevated plus maze

The elevated plus-shaped maze consisted of two open arms and two closed arms [28] equipped with rows of infrared photocells interfaced with a computer (Hamilton-Kinder, Poway, CA) [35]. Rodents avoid the open arms of the plus maze so that decreased time spent in and decreased entries into the open arms are thought to reflect enhanced measures of anxiety. Mice were placed individually in the center of the maze and allowed free access for 10 min. Animals spent time either in a closed, safe area (closed arms), in an open area (open arms), or in the middle, intermediate zone. Recorded beam breaks were used to calculate the time spent and the distance moved in the open arms, the ratio of entries into the open arms (entries into the open arms/(entries into open + closed arms)), and the number of times the mice reached over the edges of the arms.

2.2.3. Water maze

To assess spatial learning and memory, mice were tested in the water maze. A pool (diameter 140 cm) was filled with opaque water (24 °C) and mice were first trained to locate a visible platform (wild-type mice: days 1,2; *tfm* mice: days 1–3) and then a submerged hidden platform (wild-type mice: days 3–5; *tfm* mice: days 4–7) in two daily sessions 3.5 h apart, each consisting of three 60-s trials (10-min inter-trial intervals). Mice that failed to find the hidden platform within 60 s were put on it for 15 s. A 60-s probe trial (platform removed) was carried out 1 h after the last hidden platform session. For analysis of data, the pool was divided into four quadrants. During the visible platform training, the platform was moved to a different quadrant for each session. During the hidden platform training, the platform location was kept constant for each mouse (in the center of the target quadrant). The starting point at which the mouse was placed