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Physiology & Behavior 85 (2005) 135 – 141

PHYSIOLOGY & BEHAVIOR

Spatial memory performance in androgen insensitive male rats

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Received 5 August 2004; received in revised form 25 February 2005; accepted 25 March 2005

Abstract

Masculinization of the developing rodent brain critically depends on the process of aromatization of circulating testosterone (T) to its estrogenic metabolite 17β-estradiol, which subsequently interacts with estrogen receptors to permanently masculinize the brain. However, it remains unclear what role other androgenic mechanisms may play in the process of masculinization. A novel way of examining this is through the study of male rats that express the *tfm* mutation of the androgen receptor (AR) gene; such males are fully androgen insensitive and manifest a female phenotype due to a failure of AR-mediated masculinization of peripheral structures. Because *tfm*-affected males develop secretory testes and have near-normal T titers during development, aromatization would be expected to proceed normally, and brain mechanisms may be developmentally masculinized despite the feminized periphery. We compared *tfm*-affected males ($X^{tfm}Y$) with normal males and females in the Morris Water Maze, a task in which males typically perform better than females. Performance of *tfm*-affected males was intermediate between that of normal males and females. While an overall male superiority was found in the task, the $X^{tfm}Y$ group reached male-typical escape latencies faster than females. Furthermore, in the $X^{tfm}Y$ group, the granule cell layer of the dentate gyrus was significantly larger than in females. These results support the suggestion that that AR mediated mechanisms contribute to the masculinization of spatial behaviours and hippocampal morphology, and this may be independent of estrogenic processes.

Keywords: Androgen; Androgen insensitivity; Aromatization hypothesis; Dentate gyrus; Estrogen; Hippocampus; Morris water maze; Sex differences; Spatial memory; Testicular feminization mutation; Volume estimation

1. Introduction

The aromatization hypothesis [1] posits that the brain of the developing male rodent is masculinized by the activity of estrogen receptors, following the conversion of testosterone (T) into 17-β estradiol (E_2) by the enzyme aromatase. While most of the support for this hypothesis has come from examinations of sexual behaviour and morphological changes in the hypothalamus following hormone application neonatally [2–6], other work examining non-reproductive behaviours has also been found to fit within this framework [7,8].

Spatial reference memory is a sexually dimorphic cognitive ability in which males are often found to outperform females [9–14] on a variety of different tasks, including the Morris Water Maze (MWM). Neonatal castration of males leads to a female-typical pattern of performance in several different MWM paradigms measuring this ability, while the application of testosterone to females early in development leads to a male-typical performance in adulthood [10,15]. Similarly, work using the Radial Arm Maze (RAM) has shown that the neonatal application of estrogen to females results in a male typical pattern of performance in adulthood [7]. Given that the neonatal administration of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) seems to diminish adult male ability in adulthood [8], and that the castration of normal males in adulthood does not affect their performance [13,14,16], it would seem that the aromatization of T is responsible for the organization of this ability during some critical period immediately after birth.

Challenges to this hypothesis have emerged, however. A pair of experiments using the MWM have found that E_2 administration to females does not have any masculinizing

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doi:10.1016/j.physbeh.2005.03.023

effect on spatial reference memory; these authors suggest, instead, that the organization of this behaviour is accomplished by dihydrotestosterone (DHT), a non-aromatizable androgen that is likely acting through the androgen receptor (AR) [13,14]. Convergent evidence comes from the findings that males administered the AR antagonists flutamide [13] or cyproterone [16] prenatally perform at a female-typical level, and worse than normal males, on spatial reference memory tasks, as do males administered flutamide in adulthood [17]. Taken together, this work suggests that the AR may play more of a role in the organization (and activation) of spatial reference memory than was previously believed.

A novel way of approaching this issue is through the use of animals which lack functional AR. The testicular feminization mutation (*tfm*) is a single base-pair mutation of the gene encoding the AR [18], rendering the AR protein non-functional. As a result, males carrying this X-linked gene are fully affected, and develop a female phenotype. However, *tfm*-affected males develop secretory testes [19], and serum T levels are consistent with those of normal male littermates [19,20], so the aromatization hypothesis would suggest that the conversion of T to E_2 should be sufficient to masculinize the brains of *tfm*-affected males.

This report is the first to examine the non-reproductive behaviours resulting from the testicular feminization mutation, and we are looking to determine if this is an appropriate model for clarifying the effects of AR activity on physiology and behaviour. In this study, we compared the performance of wild-type (WT) males and females with *tfm*-affected males and heterozygous female carriers of the mutation, using a hidden platform version of the water maze. We also performed a morphological analysis of the granule cell layer of the dentate gyrus (GCL-DG) in the hippocampus, a structure that is heavily implicated in spatial processing. Previous work has found that while two-dimensional measures in the dentate yield a sexual dimorphism [21,22], a volumetric analysis failed to find any difference [13]. We wanted to see if we could replicate that lack of a difference, as well as begin to characterize the GCL-DG of *tfm*-affected males.

2. Methods

2.1. Animals

A total of 56 Sprague–Dawley (SD) rats between 70 and 80 days old were used for the water maze testing; 22 females, 22 males (XY), and 12 tfm's ($X^{tfm}Y$). Of the females, 8 were heterozygous for the *tfm* mutation ($X^{tfm}X$), and 14 were WT, not carrying the mutation (XX). All animals used in this study were littermates bred in our *tfm* colony, group housed at the Simon Fraser University (SFU) Animal Care Facility (ACF), with access to rat chow and tap water ad libitum. The room temperature was held constant at 21 °C, and a 14:10 LD cycle was maintained, with lights on at 12:00 pm, noon. All behavioural testing was carried out at the ACF, and was approved by the SFU University Animal Care Committee, meeting the standards set forth by the Canadian Council on Animal Care.

2.2. Handling

All animals were handled for a total of 5 min per day for the 7 days preceding water maze testing, to reduce stress reactions resulting from handling during the testing period. During this time, animals were removed from their home cages, and placed on an elevated holding area [12], similar to the one on which they would be placed during testing. This procedure was used to allow the animals to habituate to daily handling by the investigator and to being on a similar type of the open, raised platform upon which they would be placed in between water-maze trials.

2.3. Testing room and apparatus

Testing occurred in a 1.5 m diameter pool centered within a rectangular room measuring 4.7×4.0 m. An overhead camera was connected to a video monitor and a computer. Software (Chromotrack, San Diego Instruments, San Diego, CA) was used to track the animals' progress, and to calculate the time spent in each area of the pool. The water in the pool was made opaque by the addition of a non-toxic acrylic white paint. Water levels were 2.5 cm above the hidden platform, and maintained at 23 ± 1 °C. A white, opaque curtain surrounded the pool, blocking access to major visuo-spatial cues and prominent landmarks within the testing room, and thereby increasing the difficulty of the spatial processing task. The use of a curtain has been described previously [12] and was used to here as it has been previously found to provide a male-typical sex difference in water-maze testing.

2.4. Water maze protocol

Each animal was tested four times a day for five consecutive days [12]. For scoring purposes, the pool was broken up into 4 equal sections, arbitrarily identified as northeast (NE), southeast (SE), northwest (NW), and southwest (SW). The platform was placed in the middle of the NE quadrant, for all testing. For some scoring procedures, the pool was further divided into three equal-width rings: an outer ring (OR) immediately adjacent to the wall, an inner ring (IR) at the center area of the pool, and a middle ring (MR) lying between the OR and the IR. The animals were released into the pool from each of 4 starting locations daily, in a pattern that was randomly determined prior to testing. The starting points corresponded to north, east, west, and south, based on the position of the arbitrary quadrants discussed above.

Prior to each day of testing, the video recording device was calibrated and recording was started. For every trial, the