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Research report

Androgen insensitive male rats display increased anxiety-like behavior on the elevated plus maze

Dwayne K. Hamson*, Bryan A. Jones, Attila S. Csupity, Faezah M. Ali, Neil V. Watson

Hormones and Behaviour Lab, Department of Psychology, Simon Fraser University, Canada

HIGHLIGHTS

- Wild type females displayed increased percentage of time on the open arms compared to both wild type and TFM-affected males.
- Percentage of open arm entries was comparable between all genotypes.
- Percentage of time spent on the open arms was lowest in the TFM-affected males.
- Results indicate the androgen receptor normally masculinizes adult anxiety like behavior.

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ABSTRACT

Male rats carrying the testicular feminization mutation (Tfm-affected males) are insensitive to androgens, resulting in a female-typical peripheral phenotype despite possession of inguinal testes that are androgen secretory. Androgen-dependent neural and behavioral processes may likewise show atypical sexual differentiation. Interestingly, these mutant rats display elevated serum corticosterone, suggesting a chronic anxiety phenotype and dysregulated hypothalamic–pituitary–adrenal axis. In order to understand if elevated anxiety-like behavior is a possible mediating variable affecting the display of certain androgen-dependent behaviors, we compared the performance of Tfm-affected males to wild type males and females in the elevated plus maze (EPM). Two well-established indicators of anxiety-like behavior in the EPM were analyzed: total percentage of time spent on the open arms, and the percentage of open arm entries. We also analyzed the total number of open arm entries. Interestingly, Tfm-affected males spent less percentage of time on the open arms than both males and females, suggesting increased anxiety-like behavior. Percentage of open arm entries and the total number of arm entries was comparable between the groups, indicating that the observed decrease in the percentage of time spent on the open arms was not due to a global reduction in exploratory behavior. These data, in contrast to earlier reports, thus implicate androgen receptor-mediated functions in the expression of anxiety behaviors in male rats. Given that anxiety is widely reported as a precipitating factor in depression, studying the role of the androgen receptor in anxiety may give insights into the pathogenesis of major depressive disorder.

1. Introduction

The testicular feminization mutation (Tfm) is a naturally occurring mutation in the gene encoding the androgen receptor (AR) [1]. In rats, sequence analysis reveals that the mutation consists of a single base substitution at amino acid 734, in which a glutamine is substituted for an arginine (R734Q) in a region that encodes the steroid-binding domain [1]. Interestingly, a homologous mutation in humans (R752Q) results in complete androgen insensitivity syndrome (CAIS; [2]). *In vitro* analysis of ARs harboring the R734Q and R752Q mutations indicate that AR-mediated transcription of a reporter vector requires a 10,000-fold higher concentration of dihydrotestosterone at mutant AR than wild type AR; this implies that Tfm-affected male rats are completely insensitive to androgens at physiological or even elevated concentrations [3]. Consequently, Tfm-affected male rats are under virilized and phenotypically feminine, with a patent but underdeveloped vagina, short anogenital distance, and an evident nipple line [4]. Nevertheless, because they posses a Y chromosome and thus express

* Corresponding author. Current address: Laboratory of Behavioural Neuroendocrinology, Department of Psychology, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada. Tel.: +1 604 822 3941.
E-mail addresses: dhamson@psych.ubc.ca, dkhamson29@gmail.com (D.K. Hamson).

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testis determining factor, Tfm-affected males develop inguinal testes that are androgen secretory, resulting in normal to elevated levels of serum testosterone, estradiol, and dihydrotestosterone [5].

The mutation in the androgen receptor gene results in a number of neuronal and behavioral abnormalities in Tfm-affected males. Previous reports indicated sexual performance (intromissions and ejaculations) is severely compromised in gonadally intact Tfm-affected male rats, with the majority only displaying mounts [6–8]. The Tfm mutation partially feminizes spatial navigation performance in the Morris Water Maze in both rats [9] and mice [10], and visuospatial performance is significantly impaired in humans with CAIS compared to unaffected male and female siblings [11]. Furthermore, adult neurogenesis in the sub granular zone of the dentate gyrus, a region of the hippocampus important for spatial navigation, is significantly decreased in Tfm-affected males compared to wild type males [12]. In addition to being involved in spatial navigation, evidence indicates that adult hippocampal neurogenesis is important in the regulation of the hypothalamic–pituitary–adrenal axis (HPA) and the stress response [13,14]. Revest et al. [15] ablated neurogenesis in the adult dentate gyrus of male mice and found increased anxiety-like behavior compared to controls in the elevated plus maze (EPM). Neurogenesis-ablated mice displayed decreased percentage of time on the open arms, an effect that was reversed with benzodiazepine treatment [15]. In Tfm-affected males, mild stress elicited from the open field task induced significantly greater accumulation of corticosterone [16,45]. Baseline levels of corticosterone are not observed to be different in Tfm-affected males compared to wild type males (Purvis et al., 1977) [16], however, Tfm-affected males display increased serum levels of corticosterone binding globulin and adrenal hyperplasia, which suggests increased corticosterone secretion.

Together these studies seem to suggest that the behavioral deficits observed in Tfm-affected males may be due, at least in part, to elevated levels of anxiety. Anxiety-like behavior has previously been assessed in Tfm-affected male mice and rats using the EPM. Rizk et al. [10] observed increased anxiety-like behavior in mice, however, Zuloaga et al. reported comparable anxiety-like behavior between Tfm-affected and wild type male mice [17] and rats [18] in the EPM. However, Zuloaga et al. [16–18] collected their behavioral data under illuminated conditions, an important factor known to contribute to the display of anxiety behaviors [19]. This raises the possibility that any genotypic differences in EPM performance may have been masked by a ceiling effect in the Zuloaga et al. studies [16–18], with restricted range resulting from the elevated anxiety of all animals. In the current study, all animals were tested under red light illumination during the dark (activity) phase of the light-dark cycle, and removing the potential confound of light. In addition, in the Zuloaga et al. studies [16–18], WT males and Tfm-affected males were exposed to multiple anxiety tests, 72 h apart. A previous report indicated the optimal time between tests to be 7–8 days without any carryover effects [19] so again, differences in the testing paradigm employed may account for the results in [16–18]. A role for the androgen receptor in regulating anxiety is supported by studies showing increased anxiety-like behavior in castrated male rats exposed to the open field and elevated plus maze [20], an effect that was reversed by DHT administration [21]. Intrahippocampal administration of flutamide blocked the anxiolytic actions of systemic DHT treatment, suggesting androgens act upon androgen receptors located in the hippocampus to regulate anxiety-like behavior [21].

We attempted to minimize external stressors in the current study, and report that in our elevated plus–maze protocol Tfm-affected males display increased anxiety-like behavior compared to both wild type male and female rats.

2. Results

2.1. Percentage of time spent on the open arms

A repeated measures ANOVA was used to assess the impact of genotype (wild type male, wild type female, or Tfm-affected male) on the percentage of time spent on the open arms across two different time periods (day 1 *versus* day 2). There was a statistically significant interaction between genotype and day ($F_{2,41} = 8.921$, $p = 0.001$, partial eta squared = 0.303), with Tfm-affected males spending a significantly reduced percentage of their time on the open arms compared to either the wild type females ($p < 0.001$) or wild type males ($p < 0.002$), on Day 1. These differences were not evident on Day 2 (all p's > 0.08) (see Fig. 1, panel A and B).

A significant main effect was also observed for test day ($F_{1,41} = 23.613$, $p = 0.00017$, partial eta squared = 0.365), with both wild type males and wild type females decreasing the percentage of time spent on the open arms on day two compared to day one. In contrast, the Tfm-affected males did not display a change in the percentage of time spent on the open arms from test day one to day two.

2.2. Percentage of open arm entries

A repeated measures ANOVA was used to assess the impact of genotype (wild type male, wild type female, Tfm-affected male) on the percentage of open arm entries across the two different test days. There was no significant interaction of genotype and test day ($F_{2,45} = 1.797$, $p = 0.177$). However, there was a main effect of test day ($F_{1,45} = 31.947$, $p < 0.0005$, partial eta squared = 0.415), with the *post hoc* analysis indicating a decreased percentage of open arm entries made on day two compared to day one ($p < 0.0001$) (see Fig. 1, Panels C and D).

2.3. Total arm entries

A repeated measures ANOVA was used to assess the impact of genotype on the total number of arm entries across the two testing days. There was no significant interaction of genotype and test day ($F_{2,45} = 0.671$, $p = 0.516$) or a main effect of day ($F_{1,45} = 2.54$, $p = 0.118$) (data not shown).

2.4. Closed arm entries

A repeated measures ANOVA was used to assess the impact of genotype on the number of closed arm entries across the two testing days. There was no significant interaction of genotype and day ($F_{2,45} = 0.655$, $p = 0.524$), however, there was a main effect of day ($F_{1,45} = 5.179$, $p = 0.028$, partial eta squared = 0.103) with all three genotypes increasing the number of closed arm entries from day one to day two, suggesting no habituation to the testing apparatus (see Fig. 1, Panels E and F).

3. Discussion

We assessed anxiety levels in chromosomal male rats carrying the testicular feminization mutation (Tfm-affected males) using the EPM and compared their performance to gonadally-intact wild type males and females. Females displayed the greatest percentage of time on the open arms compared to both the males and Tfm-affected males, replicating a previously reported sex difference in which females display less anxiety-like behavior than males in the EPM [38]. This sex difference cannot be reduced to differences in the overall number of open arm entries as all three genotypes ventured on to this part of the EPM with similar frequency. Interestingly, Tfm-affected males spent the least percent of their total time on the open