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Sex differences and sex hormones in anxiety-like behavior of aging rats

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ABSTRACT

Sex differences in the prevalence of affective disorders might be attributable to different sex hormone milieu. The effects of short-term sex hormone deficiency on behavior, especially on anxiety have been studied in numerous animal experiments, mainly on young adult rats and mice. However, sex differences in aged animals and the effects of long-term hypogonadism are understudied. The aim of our study was to analyze sex differences in anxiety-like behavior in aged rats and to prove whether they can be attributed to endogenous sex hormone production in males. A battery of tests was performed to assess anxiety-like behavior in aged female, male and gonadectomized male rats castrated before puberty. In addition, the aged gonadectomized male rats were treated with a single injection of estradiol or testosterone or supplemented with estradiol for two-weeks. Female rats displayed a less anxious behavior than male rats in most of the conducted behavioral tests except the light-dark box. Long-term androgen deficiency decreased the sex difference in anxiety either partially (open field, PhenoTyper cage) or completely (elevated plus maze). Neither single injection of sex hormones, nor two-week supplementation of estradiol in gonadectomized aged male rats significantly affected their anxiety-like behavior in the elevated plus maze. In conclusion, our results confirm sex differences in anxiety in aged rats likely mediated by endogenous testosterone production in males. Whether long-term supplementation with exogenous sex hormones could affect anxiety-like behavior in elderly individuals remains to be elucidated.

1. Introduction

The prevalence of affective disorders, including anxiety, is twice as high in women as in men. The cause of these sex differences has not yet been clarified, but a role of sex hormones is assumed (Altemus, 2006; Maeng and Milad, 2015; McHenry et al., 2014; Solomon and Herman, 2009). In contrast to humans, adult female rodents behave less anxious than males in many behavioral tests of anxiety, such as in the open field (Brand and Slob, 1988), elevated plus maze or Vogel conflict test (Johnston and File, 1991). Both, interindividual and intraindividual variability of anxiety is associated with the variability of endogenous sex steroid hormone production (Frye et al., 2000; Mercondes et al., 2001; Mora et al., 1997; Toufexis et al., 2006). Despite the observed sex difference in anxiety, exogenous testosterone is frequently reported to have anxiolytic effects in males (Aikey et al., 2002; Fernandez-Guasti and Martinez-Mota, 2005; Frye et al., 2008a; Frye et al., 2010; Hodosy et al., 2012; Osborne et al., 2009). It has been hypothesized that this effect could be limited to young adult males (Carrier and Kabbaj, 2012). Whether sex differences in anxiety are affected by aged-related testosterone decline in males is unknown.

To mirror human androgen deficiency symptoms in animal models the surgical removal of the main endogenous androgen source - the testes - is often used. It has been reported that gonadectomy in adult male rats is associated with increased anxiety (Edinger and Frye, 2006; Frye and Seliga, 2001), which can be reversed by testosterone supplementation (Carrier et al., 2015; Edinger and Frye, 2006; Khakpai, 2014). However, other experiments have brought contradictory results (Filova et al., 2015; Frye et al., 2010; McDermott et al., 2012). Presumably, the different effects of testosterone deficiency and subsequent testosterone supplementation are dependent on many factors. The age at hormone deprivation (McDermott et al., 2012; Zuloaga et al., 2011a) as well as the age at androgen replacement (Onaolapo et al., 2016) can be important determinants of the anxiety-like behavior. While the neurobehavioral effects of short-term gonadectomy are frequently reported in the published literature, the consequences of life-long hypoandrogenism have not been described in detail yet.

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In brain, testosterone can be metabolized either by 5α-reductase to dihydrotestosterone, or by aromatase to estradiol. Dihydrotestosterone can be further converted to neuroactive steroids, 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol). It was shown that the anxiolytic-like effect of testosterone in males can be mediated through its 5α-reduced (Aikey et al., 2002; Frye et al., 2010; Osborne et al., 2009), as well as aromatized metabolites (Carrier et al., 2015; Filova et al., 2015). This is the reason why castration does not lead only to a decrease in androgen signaling but may affect estrogen signaling as well. Similarly, supplementation with testosterone can activate both, androgen and estrogen receptors depending on the metabolism of the exogenous testosterone in the target tissue (Chen et al., 2014; Edinger and Frye, 2006; Hodosy et al., 2012; Osborne et al., 2009; Pak et al., 2005; Zuloaga et al., 2008; Zuloaga et al., 2011b).

Aging is associated with a progressive reduction in the activity of the hypothalamo-pituitary-gonadal axis and a subsequent decline in circulating testosterone. In humans, the age-associated hypogonadism is considered to be responsible for some of the cognitive and affective symptoms of aging (Shores et al., 2004; Wahjoepramono et al., 2016; Zhang et al., 2012). Similarly to human, animal experiments confirm that aged hypogonadal males display higher depression (Frye and Walf, 2009) and anxiety-like behavior (Frye et al., 2008a). In addition, these experimental studies showed that testosterone as well as its 5α-reduced and aromatized metabolites can reduce both depression-like (Frye and Walf, 2009) and anxiety-like behavior in aged hypogonadal male mice (Frye et al., 2008a). However, the causality between androgen deficiency and age-associated changes in brain functions needs further clarification.

The primary aim of the present study is to observe potential sex differences in anxiety-like behavior in aged rats and to examine the effects of life-long hypogonadism on anxiety-like behavior in male rats. Furthermore, we aimed to prove whether short-term application of exogenous sex hormones can reverse the effect of long-term hypogonadism on anxiety-like behavior in aged male rats, gonadectomized before puberty.

2. Material and methods

2.1. Animals

Lewis rats (20 females and 48 males, 21-days old) were purchased from Anlab (Prague, Czech Republic). The animals were group-housed (4–5 per cage) and kept in a controlled environment (temperature 25 ± 2 °C and humidity 55 ± 10%) with ad libitum access to food and water and with a 12:12 light-dark cycle. All experimental procedures were carried out according to the Slovak legislation and approved by the institutional Ethics committee.

2.2. Gonadectomy

Male rats were randomly divided into two groups. On postnatal day (PND) 29–31 the animals underwent either gonadectomy (GDX) or sham surgery under general anesthesia (ketamine 100 mg/kg + xylazine 10 mg/kg, applied intraperitoneally). During gonadectomy, both testes were ligated and excised through a small incision cut on the scrotum. In sham surgery, the gonads were gently lifted from and then replaced into the scrotum. At the end of the procedures, the skin was sutured with absorbable silk (size 4–0).

2.3. Behavioral testing

At the age of 12 months the behavior of the animals was tested in the open field, light-dark box and elevated plus maze test. In addition, animals were placed in the PhenoTyper cages to monitor their behavior for 1 h (Noldus Information Technology, Wageningen, Netherlands). All tests were carried out in a dimly lit room. The behavior was recorded using a dedicated camcorder and the files were analyzed using the image and video processing system EthoVision XT 10.0 (Noldus Information Technology, Wageningen, Netherlands).

2.3.1. Open field test

The open field test was conducted in a square shaped (100 × 100 cm) apparatus virtually divided into a central (40 × 40 cm) and a border zone. Animals were placed individually into the central zone of the arena and monitored for 5 min. Time spent in the central zone was evaluated as a parameter of anti-anxiety behavior. Total distance moved and velocity was measured to assess locomotion.

2.3.2. Light-dark box test

The testing box (40 × 55 cm) consisted of two chambers, light part illuminated with 25 lx and a dark part covered by a lid, connected with a passage. The light chamber served as the starting zone and animals were observed for 5 min. As an index of anti-anxiety behavior, the relative time – percentage of the total time spent in light part of the box was assessed.

2.3.3. Elevated plus maze test

The apparatus consisted of two opposite open and two opposite closed arms extending from central platform elevated to a height of 60 cm above the floor. Each animal was placed on the central plate and allowed to explore the maze for 5 min. To assess anti-anxiety-like behavior, relative number of entries into the open arms – open-arm entries/total entries, and time spent on the open arms − time on open arms/(time on open + closed arms) were calculated.

2.3.4. Behavioral Phenotyping

The PhenoTyper cages (Noldus Information Technology, Wageningen, Netherlands) made of clear plexiglas were used to observe the behavioral phenotype of animals during 1 h. The floor of the arena (45 × 45 cm) was virtually divided into a central (20 × 20 cm) and a border zone. In addition, one part of the arena marked as a spot light zone was illuminated by bright white light. In one corner of the arena a small chamber – shelter was placed. To assess locomotor activity total distance moved and velocity were evaluated. As indicators of anti-anxiety-like and exploratory behavior, percentage of time spent in the central zone, shelter or spot light zone relative to total time were calculated. To analyze the dynamics of the behavior time blocks of 10 min were analyzed separately.

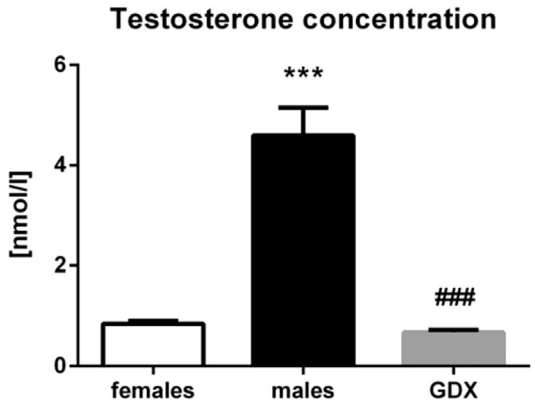

Fig. 1. Testosterone concentration in plasma. Males have significantly higher concentration of testosterone in plasma than females. Gonadectomized males have significantly lower concentration of testosterone in plasma than control males. Data are presented as mean + SEM. GDX – gonadectomized males; * males vs. females; # GDX males vs. control males; *** and ### $p < 0.001$.