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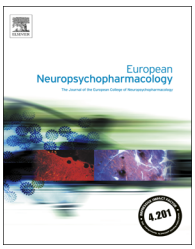

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Beyond the HPA-axis: The role of the gonadal steroid hormone receptors in modulating stress-related responses in an animal model of PTSD

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Abstract
The hypothalamic-pituitary-adrenal (HPA) axis, which plays a major role in the response to stress, and the hypothalamic-pituitary-gonadal (HPG) axis are closely linked with the ability to inhibit the other. Testosterone, a product of the HPG, has many beneficial effects beyond its functions as a sex hormone including anti-anxiety properties. In this study we examined the effect of stress exposure on gonadal hormones, and their efficacy in modulating anxiety-like response in an animal model of PTSD. Male rats were exposed to predator scent stress, followed by analysis of brain expression of androgen receptor (AR) receptor and estrogen receptor α (ERα). The behavioral effects of immediate treatment with testosterone, testosterone receptor antagonist (flutamide) or vehicle were evaluated using the elevated plus-maze, acoustic startle response and trauma-cue response. Levels of circulating corticosterone and testosterone were also measured after treatment. The behavioral effects of delayed testosterone treatment were explored in the same manner. We report that animals whose behavior was extremely disrupted (EBR) selectively displayed significant down-regulation of AR and ERα in the hippocampus. Immediate treatment with flutamide or delayed treatment with testosterone significantly increased prevalence rates of minimal behavioral response (MBR) and decreased prevalence of EBR with favorable behavioral results. Testosterone levels were higher in control un-exposed

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animals, while corticosterone was higher in control exposed animals. This study suggests that gonadal steroid hormones are involved in the neurobiological response to predator scent stress and thus warrant further study as a potential therapeutic avenue for the treatment of anxiety-related disorders.

1. Introduction

Gender is widely accepted as a risk factor for developing post-traumatic stress disorder (PTSD) (Breslau, 2002; Tolin and Foa, 2006). Although female gender is associated with greater susceptibility to develop PTSD after exposure to a traumatic event, findings suggest this may be influenced by type of trauma, personality factors and life-history (Zoladz and Diamond, 2013). Research into the mechanism underlying gender differences in PTSD with the use of animal models provided some interesting and counter-intuitive results. These results suggest that females might be more resilient to stress and that men could be more susceptible to developing an anxiety-like response (Cohen and Yehuda, 2011). As an example, our lab previously showed that gender was associated with specific behavioral patterns: female rats expressed higher baseline anxiety behavior and male rats presented higher sensitivity to the stress paradigm (Mazor et al., 2009). Although the nature of gender differences in rate and response to a stressful event is not clear-cut, the existence of gender differences suggests that gonadal steroid hormones might play a role in the body's response to stress.

Stress has long been known to interrupt normal reproductive behavior (Rivier, 1991; Tilbrook, 2000). The hypothalamic-pituitary-adrenal (HPA) axis plays a pivotal role in the response to stress and reestablishment of homeostasis. Differences in HPA reactivity between males and females have been often demonstrated, but these depend on age, menstrual cycle and species being tested (Handa and Weiser, 2014; Kajantie and Phillips, 2006). The HPA axis and the hypothalamo-pituitary-gonadal (HPG) axis are closely linked (Viau, 2002). The end-product of the HPA axis, corticosterone, has been shown to inhibit the HPG axis and this may well contribute to the impairment of sexual and reproductive functioning under stress (Handa et al., 1994a). Testosterone on the other hand, a product of the HPG axis, can inhibit the functioning of the HPA axis. Testosterone suppresses adrenocorticotropic hormone (ACTH) and corticosterone responses (Handa et al., 1994b) and corticotropin releasing hormone (CRH)—stimulated cortisol (Rubinow et al., 2005). Therefore, existing data supports a close interaction between the HPA and HPG systems.

Gonadal steroid hormones are not only involved in reproductive behavior but are also pivotal in structural and functional aspects of the brain. While stress causes atrophy and suppression of neurogenesis (McEwen, 1999), findings suggest sex hormones exert positive effects on these parameters. Gonadal hormones are involved in neuronal plasticity and synaptic remodeling (Parducz et al., 2006) and are modulators of dendritic morphology in the spinal nucleus of the bulbocavernosus (SNB), ventromedial nucleus of the hippocampus (VMN) and Cornu Ammonis 1 (CA1) region of the hippocampus (Cooke and Woolley, 2005). Testosterone is a positive regulator of synapse density in the CA1 region both in males (Leranth et al., 2003) and females (Leranth et al., 2004) rats. Moreover, it was found to prevent synaptic loss following chronic stress in the SNB (Matsumoto, 2005). Testosterone modifies cognitive skills, mood and behavior (Durdiakova et al., 2011). It is able to produce analgesia, enhanced affect and cognitive abilities (Frye and Seliga, 2001) in addition to anti-depressant effects (Frye and Walf, 2009). In humans, testosterone treatment was useful in alleviating anxiety symptoms in hypogonadism (Cooper, 2000) and in alleviating depressive symptoms (Zarrouf et al., 2009). Multiple imaging studies in humans also reported effects of testosterone on the limbic system (Höfer et al., 2013). These findings suggest gonadal steroid hormones in general, and testosterone in particular, are able to induce beneficial effects on brain and behavior.

In a previous study we found significantly lower levels of plasma testosterone following predator scent stress (PSS) in male rats exhibiting greater anxiety-like response compared to rats exhibiting minimal response or unexposed control (Cohen et al., 2012). In the same manner, stressors as paradoxical sleep deprivation, footshock and cold also led to lower testosterone levels (Andersen et al., 2004). Thus, we sought to examine the effects of manipulating the HPG-axis on stress-related behavioral parameters.

This study employed a controlled, prospectively designed animal model for PTSD using PSS. In this model, populations of exposed rodents are classified according to the degree of their individual behavioral response using standardized "cut-off behavioral criteria" (CBC), creating three distinct groups: "extreme behavioral response" (EBR) and "minimal behavioral response" (MBR) at the extremes, and a middle group of "partial responders" (PBR) (Cohen et al., 2003, 2004). The relative prevalence rates of individuals displaying the different degrees of disrupted behavior provide an indication of the potential efficacy of the drug under study. As the hippocampus is highly affected by stress and is involved in regulating the HPA-axis (Kim and Diamond, 2002), we sought to examine the effects of stress on sex steroid hormones in the hippocampus. Therefore, the first aim of the study was to establish whether single exposure to the PSS results in a long-term effect on the levels of androgen receptor (AR) and estrogen receptor α (ERα) in the hippocampal CA1, CA3 and dentate gyrus (DG) subareas, employing a bank of recently-harvested frozen rat-brains from exposed vs. unexposed rats stored according to CBC classification. The second aim was to perform a controlled, prospective trial to examine the effect of testosterone and an AR antagonist, flutamide, administered immediately after stress exposure on behavioral parameters. Behavioral responses were assessed on the elevated-plus maze (EPM) and acoustic startle response (ASR) tests on day 7 and trauma-cue triggered freezing responses on day 8. Prevalence rates for EBR, MBR and PBR individuals in