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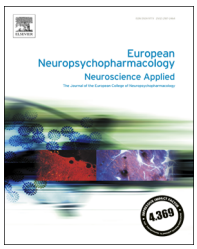

Effects of paternal and peripubertal stress on aggression, anxiety, and metabolic alterations in the lateral septum

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Abstract
Early-life stress and biological predispositions are linked to mood and personality disorders related to aggressive behavior. We previously showed that exposure to peripubertal stress leads to increased anxiety-*like* behaviors and aggression against males and females, as well as increased aggression against females in their male offspring. Here, we investigated whether paternal (pS) and individual (iS) exposure to peripubertal stress may exert additive effects on the long-term programming of anxiety-*like* and aggressive behaviors in rats. Given the key role of the lateral septum (LS) in the regulation of anxiety and aggressive behaviors and the hypothesized alterations in balance between neural excitation and inhibition in aggression-related disorders, markers for these processes were examined in the LS. Peripubertal stress was applied both in naïve male rats and in the offspring of peripubertally stressed males, and anxiety-*like* and aggressive behaviors were assessed at adulthood. Proton magnetic resonance spectroscopy at 6-months, and post-mortem analysis of glutamic acid decarboxylase 67 (GAD67) at 12-months were conducted in LS. We confirmed that aggressive behavior was increased by pS and iS, while only iS increased anxiety-*like* behavior. Individual stress led to reduced GABA, confirmed by reduced GAD67 immunolabelling, and increased glutamate, N-acetyl-aspartate, phosphocholine and creatine; while pS specifically led to reduced phosphocreatine. pS and iS do not interact and exert a differential impact on the analyzed aspects of brain function and anxiety-*like* behaviors. These data support the view that early-life stress can affect the

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behavioral and neurodevelopmental trajectories of individuals and their offspring, which may involve different neurobiological mechanisms.

1. Introduction

Early-life stress and biological predisposition are both frequently underlined as high-risk etiological factors for the development of pathological aggression. For example, early-life adverse experiences have been shown to increase the risk of violence (Duke et al., 2010), and epidemiological studies have shown a high concordance of aggression and criminal offenses among relatives, which suggests that biological predispositions account for an aggression trait (Huesmann et al., 1984). In parallel to findings in humans, we have previously shown that male rats exposed to stress during puberty show an increase in aggressive and anxiety-*like* behaviors (Marquez et al., 2013; Tzanoulinou et al., 2014a, 2014b), with increased aggressive behavior against females also observed in their offspring (Cordero et al., 2012).

Family, twin, and adoption studies suggest that the interaction of biological predisposition and early-life stress increases the risk of developing mental disorders [for review, (Tsuang et al., 2004)]. However, although some studies have found that adopted children of aggressive biological parents have shown a significant increase in aggressive behavior when they were raised in an adverse environment (Cadoret et al., 1995; Caspi et al., 2002; Taylor and Kim-Cohen, 2007), other studies have failed to reproduce these results (Mednick et al., 1984; Willerman et al., 1992). Several factors, including nutrition, exercise, addictions, education, and parental style, are differentially expressed in different human cohorts and may contribute to this discrepancy (Ge et al., 1996; Murray et al., 2012; O'Connor et al., 1998). Thus, using an animal model of peripubertal stress-induced psychopathology that is devoid of some of the limitations intrinsic to human studies, we sought to study whether parental stress would interact with peripubertal stress in the behavioral and neurodevelopmental programing of male rats.

The lateral septum (LS) is one of the critical brain areas that modulates both anxiety and aggression (Albert and Walsh, 1982; Haller et al., 2006; Toth et al., 2010; Veenema and Neumann, 2007). In animals, lesions to the LS increase aggressive behavior (Albert and Walsh, 1982; Clemente and Chase, 1973); moreover, in humans with antisocial personality disorder, those with neurodevelopmental septum abnormalities present higher levels of antisocial personality, psychopathy, and criminal convictions (Raine et al., 2010). The LS is greatly activated during aversive and threatening situations (Duncan et al., 1996) and is involved in the regulation of autonomic (Kubo et al., 2002) and the hypothalamus-pituitary-adrenal (HPA) axis (Herman et al., 2003) responses to stress.

We sought here to investigate the potential neurochemical alterations in the LS in animals exposed to parental and/or individual stress and focused on markers of the balance between neural excitation and inhibition (E/I) and energy metabolism (Bustillo, 2013; Maddock and Buonocore, 2012; Rezin et al., 2009), because both processes have been hypothesized to be involved in dysfunctions in anxiety and aggression (Herman et al., 2004; Miczek et al., 2007). Currently, the existing neurochemical information related to the E/I ratio is scarce. In fact, in humans, this has been frequently provided by proton magnetic resonance spectroscopy (^{1}H MRS) obtained from the whole brain (Pollack et al., 2008); although, when focusing on a single brain region (e.g., the anterior cingulate or insula), the measurements are specifically focused on either glutamate (Modi et al., 2014) or GABA (Rosso et al., 2014) levels. Recently, a study reported a correlation between the CSF glutamate levels and impulsive aggression (Coccaro et al., 2013). In animals, the information from microdialysis studies evaluating neurotransmitter levels (Sustkova-Fiserova et al., 2009) and from protein (Kohl et al., 2013) and gene (Tzanoulinou et al., 2014a, 2014b) expression studies that measured markers of glutamatergic and/or GABAergic transmission (e.g., receptors, transporters) in different brain areas further supports the relevance of investigating potential alterations in the neural E/I imbalance in the context of stress-induced alterations in anxiety and aggression.

2. Experimental procedures

2.1. Animals

The experimental subjects were the male offspring (F1) of Wistar Han rats bred in our animal house (F0), which were the progeny of Wistar Han rats purchased from Charles River Laboratories (Lyon, France). The animals were maintained under controlled conditions (12 h light/dark cycle; lights on at 7:00 a.m.; 22±2 °C), and food and water available *ad libitum*. The males (F0 and F1) from different litters were weaned at postnatal day 21. The male rats from different litters were mixed within each group and were housed at three per standard plastic cage (F0: N=12/group; F1: control father, N=26; F1: stress father, N=22). Male and female Wistar Han rats (8 weeks old) purchased from the same supplier were used as intruders and as partners, respectively. All procedures conformed to the Swiss National Institutional Guidelines on Animal Experimentation and were approved through a license from the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

2.2. Experimental design

The experimental design is depicted in Figure 1. At P28, half of the F0 male rats (N=12) were submitted to the peripubertal stress condition (see details below), and the F0 control animals (N=12) were handled on the days that their experimental counterparts were exposed to stress. Except for the routine husbandry procedures, all F0 male rats were left undisturbed from P43 until