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Behavioral and neuroendocrine consequences of juvenile stress combined with adult immobilization in male rats

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ABSTRACT

Exposure to stress during childhood and adolescence increases vulnerability to developing several psychopathologies in adulthood and alters the activity of the hypothalamic-pituitary-adrenal (HPA) axis, the prototypical stress system. Rodent models of juvenile stress appear to support this hypothesis because juvenile stress can result in reduced activity/exploration and enhanced anxiety, although results are not always consistent. Moreover, an in-depth characterization of changes in the HPA axis is lacking. In the present study, the long-lasting effects of juvenile stress on adult behavior and HPA function were evaluated in male rats. The juvenile stress consisted of a combination of stressors (cat odor, forced swim and footshock) during postnatal days 23–28. Juvenile stress reduced the maximum amplitude of the adrenocorticotropic hormone (ACTH) levels (reduced peak at lights off), without affecting the circadian corticosterone rhythm, but other aspects of the HPA function (negative glucocorticoid feedback, responsiveness to further stressors and brain gene expression of corticotrophin-releasing hormone and corticosteroid receptors) remained unaltered. The behavioral effects of juvenile stress itself at adulthood were modest (decreased activity in the circular corridor) with no evidence of enhanced anxiety. Imposition of an acute severe stressor (immobilization on boards, IMO) did not increase anxiety in control animals, as evaluated one week later in the elevated-plus maze (EPM), but it potentiated the acoustic startle response (ASR). However, acute IMO did enhance anxiety in the EPM, in juvenile stressed rats, thereby suggesting that juvenile stress sensitizes rats to the effects of additional stressors.

Introduction

Adolescence is a transitional stage between childhood and adulthood that includes important changes in personality and cognition (see Casey et al., 2010 for a review). This period represents a critical phase in development during which the nervous system shows unique plasticity, maturation and rearrangement of major neurotransmitter pathways (Romeo, 2003; Spear, 2000). In general, there are dramatic maturational changes in several brain areas important for cognition, emotion, motivation and reactivity to stress, including the prefrontal cortex and other limbic brain structures (see Ernst et al., 2009; Spear, 2000 for a review). In rodents, "adolescence" broadly covers the entire postnatal period ranging from weaning (postnatal day, PND, 21) to adulthood (PND 60). The boundaries of puberty in rodents vary between males and females and between rats and mice (Schneider, 2013), but the period from PND 21 to PND 30–34 is considered prepubertal (Eiland and Romeo, 2013; Laviola et al., 2003). Although several groups during recent years have studied the long-term effects of several interventions between PND 21 and PND 60, the present study focused only on the prepubertal period because there are considerable developmental differences within this period (i.e., Foilb et al., 2011; Klein and Romeo, 2013).

In humans, exposure to stressful experiences during childhood and adolescence has a profound long-lasting impact and increases the risk of developing several psychopathologies later in life (Ehlert, 2013; Heim and Nemeroff, 2001; Teicher et al., 2003). In recent years, several animal models have been proposed to characterize the underlying mechanisms. To our knowledge, the first studies about the long-term effects of prepubertal stress during PND 21 to PND 32 were conducted in outbred rats by Maslova et al. (2002a,b), using a combination of unpredictable stressors. They found an increase in the acoustic startle response (ASR) but no changes in the elevated plus-maze (EPM) behavior.

Moreover, the McCormick laboratory characterized the long-term effects of adolescent social instability initiated during the prepubertal period (usually starting at PND 30) but extending approximately until PND 45. McCormick's model induced anxiety-like behavior in adulthood, altered social behavior and induced a lasting impairment in performance on hippocampal-dependent learning and memory tasks (see McCormick, 2010 for a review). In addition, Richter-Levin's group initiated another extensive series of studies (see Horovitz et al., 2012 for a review) using different combinations of stressors restricted to

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the juvenile period (i.e., an elevated platform, cat odor, forced swim, restraint or footshock) administered approximately from PND 27 to PND 29. In adult male rats, these models typically induced reduced exploration of novel environments and increased anxiety (e.g., Avital et al., 2006; Horovitz et al., 2014; Ilin and Richter-Levin, 2009; Jacobson-Pick and Richter-Levin, 2010, 2012; Jacobson-Pick et al., 2008; Tsoory and Richter-Levin, 2006; Tsoory et al., 2008). Finally, Sandi and colleagues have used another model of prepubertal stress that extends toward the peripubertal period. This procedure consists essentially of the unpredictable exposure to an elevated platform with bright light and/or to synthetic fox odor during 7 days between PND 28 and PND 42. The model induces increased anxiety and depressive-like behavior in adulthood, as well as decreased sociability and greatly enhanced aggression (Cordero et al., 2012, 2013; Márquez et al., 2013). Other similar models of prepubertal stress in rats and mice support the long-lasting increases in anxiety (Brydges et al., 2012; Giovanoli et al., 2013; Weathington et al., 2012; Wilkin et al., 2012; Yee et al., 2011). Thus, it is well recognized that stress during adolescence has enduring effects in adulthood (Green and McCormick, 2013; McCormick and Green, 2013).

In contrast to the attention paid to behavior, particularly anxiety-like behavior, most of the above models have not extensively characterized the long-term effects of prepubertal stress on the hypothalamic-pituitary-adrenal (HPA) axis, the key system in stress research. Most of these studies have only measured corticosterone response to stress at a single time point (Giovanoli et al., 2013; Maslova et al., 2002a; Toledo-Rodríguez and Sandi, 2007; Weathington et al., 2012; Yee et al., 2011), which is clearly insufficient considering the complexity of the HPA axis and the limited value of plasma corticosterone as an index of HPA responsiveness to stress (Armario, 2006). The paucity of data regarding the HPA axis is surprising, given that in humans childhood maltreatment (by itself or in combination with adult stressors) has been associated in adulthood with a dysregulation of basal and stress-induced activity of the HPA axis, although such results have not been always conclusive (see for reviews: Cooke and Weathington, 2014; Danese and McEwen, 2012; Heim and Nemeroff, 2001; Shea et al., 2005; Van Voorhees and Scarpa, 2004).

Some of the long-term consequences of prepubertal stress may be latent, unmasked only after an additional insult in adulthood. This idea is in the framework of the "two-hit" proposal, initially developed by Bayer et al. (1999) and Maynard et al. (2001) in the field of schizophrenia, but that model can be translated to other mental illnesses. Essentially, this conceptualization assumes that some psychopathologies are neurodevelopmental in origin and require some environmental and/or genetic "hits". The "first hit" (genetic background and/or early environmental insult, such as stress, viral infection or exposure to drugs) primes or sensitizes the CNS to the "second hit" (another early or adult environmental insult). Thus, the "first hit" makes the organism more vulnerable to the effects of the "second hit" that precipitates the disease. This idea is similar to the "cumulative stress" hypothesis, which proposes that acute and chronic stressful experiences in adulthood accumulate over early-life adversity, compromising the ability of the organism to manage stress (McEwen, 1998; Taylor, 2010). Alternatively, the "match–mismatch" models predict that after a juvenile stress experience, individuals will show improvements in coping behavior and adaptability to further exposures to stress in adulthood (Claessens et al., 2011; Oitzl et al., 2010; Schmidt, 2011).

Given all of the above, the aims of the present study were three-fold: (i) to characterize, in our conditions, the long-term effects of juvenile stress (restricted to this very limited period of life) on activity and anxiety; (ii) to characterize the long-term consequences of juvenile stress on the peripheral and central functioning of the HPA; and (iii) to test the "two-hit" hypothesis, studying how prior juvenile stress experience can alter the long-term consequences of a single exposure to a severe stressor such as immobilization on boards (IMO). This stressor has been extensively characterized in our laboratory (Belda et al., 2008b) and has been determined to induce changes in activity and anxiety that can last for some days (Belda et al., 2008a), as well as more protracted impairment of spatial memory in the Morris water maze (Andero et al., 2012) and fear extinction (Andero et al., 2011).

Methods

Animals and general procedures

We used 76 male Sprague–Dawley rats, obtained from the breeding center of the Universitat Autònoma de Barcelona (derived from Harlan Interfauna Ibérica, Barcelona, Spain). They were obtained from 13 different mothers; after birth, each litter was culled to 10 pups (sex ratio after culling: 0.65 ± 0.10 males versus females). Pups were weaned at PND 21 and housed in groups of 4 males. After that, they were maintained in opaque polypropylene wire-topped cages with solid bottoms (27.5 × 52.5 × 14.5 cm; Type "1000 cm^2", Panlab S.L.U., Barcelona, Spain) containing sawdust bedding (Ultrasorb, Panlab, S.L.U., Barcelona, Spain). At PND 45, they were housed in pairs, and they remained in these housing conditions until the end of the study. Animals were maintained in standard temperature conditions (21 ± 1 °C) and in a 12-h light/12-h dark schedule (lights on al 8:00 h). Food (SAFE-diet A04, Panlab S.L.U., Barcelona, Spain) and water were available ad libitum. The rats of the same home cage had the same treatment. Except for the juvenile stress (see below), all behavioral manipulations were performed in the morning. The experiments were conducted using two different cohorts, the first corresponding to experiments 1 and 2, which were carried out simultaneously, and the second corresponding to experiment 3. The experimental protocol was approved by the Committee of Ethics of the Universitat Autònoma de Barcelona and the Generalitat de Catalunya, followed the principles of laboratory animal care and was conducted in accordance with the European Communities Council Directives (86/609/EEC).

Animals were handled on 4 days for approximately 2 min a day. During each handling period, each rat was removed from its cage, placed on the tabletop, had its neck and back gently stroked by the experimenter and was gently wrapped with the cloth that would be used for the tail nick. On the last day of handling, animals were exposed to the tail-nick procedure to allow habituation to the procedure (experiments 1 and 2). The tail nick consisted of gently wrapping the animal with a cloth, making a 2-mm incision at the end of one of the tail veins and then massaging the tail while collecting, within 2 min, 300 μl of blood into ice-cold EDTA capillary tubes (Sarsted, Granollers, Spain). This procedure is extensively used because very low resting levels of hormones are obtained under appropriate conditions (Belda et al., 2004; Vahl et al., 2005). The two cage-mates were sampled simultaneously (two experimenters took samples at the same time, and a third gently held the two rats).

Experimental designs

A summary of the experimental designs can be seen in Fig. 1.

Experiment 1

The purpose of this study was to evaluate in adults the long-term effects of juvenile stress on: (i) the circadian rhythm of HPA hormones; (ii) the integrity of the negative glucocorticoid feedback mechanisms, and (iii) the tonic functioning of the HPA axis at the brain level. Twenty rats were used for this experiment (control: n = 10, and juvenile stress: n = 10). After handling, at PND 61, blood samples were taken from rats under basal conditions at 9:00, 15:00, 19:00, 23:00 and again at 9:00 h on the next day. After 7 days of resting, corticosterone was injected (10 mg/kg, sc, dissolved in 0.4 ml of sesame oil) at 13:00 h, and the rats were sampled under basal conditions at 15:00, 19:00 and again at 9:00 h on the next day. After 7 additional days of rest, animals were perfused in basal conditions, and the adrenals and brain were extracted for further analysis. Central functioning of the HPA axis was evaluated by in