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Research report

Effect of chronic stress during adolescence in prefrontal cortex structure and function

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HIGHLIGHTS

- Chronic stress impairs the development of the adolescent brain.
- Chronic stress delays the maturation of extracellular structures called perineuronal nets, which stabilize synaptic contacts on inhibitory neurons, and prefrontal cortex-associated behavior.
- These negative effects of chronic stress on the prefrontal cortex can theoretically occur in humans.

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ABSTRACT

Critical periods of plasticity (CPPs) are defined by developmental intervals wherein neuronal circuits are most susceptible to environmental influences. The CPP of the prefrontal cortex (PFC), which controls executive functions, extends up to early adulthood and, like other cortical areas, reflects the maturation of perineuronal nets (PNNs) surrounding the cell bodies of specialized inhibitory interneurons. The aim of the present work was to evaluate the effect of chronic stress on both structure and function of the adolescent's rat PFC. We subjected P28 rats to stressful situations for 7, 15 and 35 days and evaluated the spatial distribution of histochemically-labeled PNNs in both the Medial Prefrontal Cortex (MPFC) and the Orbitofrontal Cortex (OFC) and PFC-associated behavior as well. Chronic stress affects PFC development, slowing PNN maturation in both the (MPFC) and (OFC) while negatively affecting functions associated with these areas. We speculate upon the risks of prolonged exposure to stressful environments in human adolescents and the possibility of stunted development of executive functions.

1. Introduction

Many neuropsychiatric conditions are diagnosed during adolescence [1,2], when the brain goes through an intense period of synaptic remodeling, which is reflected in a loss of grey matter (GM) volume and concurrent increases in white matter (WM) volume [3]. Chronic stress induces long-lasting changes in the brain and could contribute to an increased vulnerability to mental illness, especially during the so-called critical periods of cortical development [4,5]. Unfortunately, many adolescents are exposed to stressful situations around the world, including war [6] and extreme poverty (UNICEF, 2005). Not surprisingly, epidemiological studies estimate that up to 20% of children and adolescents worldwide suffer from a disabilitating mental illness [7].

Stress is the response of an organism to a threat, either explicit or not, which includes physiological or behavioral

Abbreviations: MPFC, medial prefrontal cortex; PFC, prefrontal cortex; OFC, orbital prefrontal cortex; PNN, perineuronal net; SAAT, spontaneous arm alternation test; Vv, *Vicia villosa* lectin.

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compensatory adaptations to maintain bodily homeostasis [8,9]. The body response to the stressor event proceeds through two distinct but interrelated phases, 1) a rapid fight or flight response due to sympathetic activation of the adrenal medula and the subsequent release of catecholamines in the bloodstream, and 2) a slow response that depends on the activation of the hypothalamic-pituitary-adrenal axis, resulting on the release of glucocorticoids (cortisol in humans and corticosterone in other animals) by the adrenal cortex in the blood circulation [10–15].

Under normal conditions, the stress response is deactivated when the threat is no longer present. This is implemented by an inhibitory feedback mechanism that is controlled by the levels of stress hormones in the circulation. However, when the level of hormones remains elevated for a long period of time, the central nervous system can be cumulatively damaged by the exposure [10,16]. For instance, chronically elevated levels of cortisol can impair the development of the prefrontal cortex (PFC) [14], which has a large density of glucocorticoid receptors [17], causing dendrite retraction and a decline in the number of dendritic spines; both effects leading to an overall decrease in synaptic contacts [18–20].

In humans, the PFC is situated in the anterior portion of the frontal lobe and includes Brodmann areas (BA) 8, 9, 10, 11, 13, 14, 44, 45, 46 and 47/12 [21]. In rodents, the PFC is comprised by the MPFC, which includes the medial precentral, anterior cingulate, prelimbic and infralimbic areas, and the OFC, which is subdivided into medial, ventrolateral, and lateral orbital areas [22,23]. Injuries to the MPFC and the OFC cortex are closely associated with working memory disorders [24,25] and poor judgment and maladaptive decisions due to an inability to anticipate actions' consequences [26], respectively.

Those effects are more evident during the critical period of plasticity of the PFC [14]. Critical periods of plasticity are defined, among other things, by the maturation of GABAergic neurons [27], which make up about 25% of all cortical neurons in primates [28,29]. Although this fraction of GABAergic interneurons in the cortex is relatively constant from neurogenesis to adulthood [30], during postnatal development a subgroup of these cells expressing the Ca^{++}-binding protein parvalbumin gradually accumulate extracellular matrix components around the cell body, such as chondroitin sulfate proteoglycans, forming a lattice-like structure called perineuronal net (PNN). PNNs mature slowly and help stabilize synaptic contacts under environmental constraints [31–35]. Their maturation signals the closing of the critical period of plasticity in several cortical regions [31,32,36,37]. PNNs can be easily revealed histologically with plant lectins with high affinity to N-acetylgalactosamine amino sugars, such as *Wisteria floribunda agglutinin* and *Vicia villosa* [35,38–40].

In the present work, we study the effects of chronic stress on the structure and function of the rat's PFC during the critical period of plasticity. More specifically, we address the effects of chronic stress both on the spatial-temporal distribution of PNN^{+} neurons in the PFC and on tests of executive function during adolescence. To test maladaptive executive function associated with deficits in working memory and bad judgment in decision making we used the spontaneous alternation [41–46] and the open field test [47], respectively.

2. Materials and methods

2.1. Experimental design

All experimental procedures were approved by the Animal Ethics Committee of the Federal University of Pará (BIO069-12). We used 48 male Wistar rats (*Rattus norvegicus*) aged 28 postnatal days (P28). The animals were bred and raised in the University's animal facility and were housed in standard cages with a 12/12 light/dark cycle with free access to food and water. The animals were randomly assigned to either the control (n = 24) or experimental group (n = 24). Each group was subsequently divided into three sub-groups, depending on the length of exposure to either the stressful or the control environment: 7 (n = 8), 15 (n = 8) or 35 (n = 8) days. Behavioral tests were carried out on the last day of exposure to each environment and the animals were immediately sacrificed, after blood was collected to measure corticosterone levels. Researchers tasked with rating behavior were blinded to the groups animals belonged.

2.2. Chronic stress paradigm

Animals of the stressed group were housed in standard cages (0.40 × 0.30 × 0.20 m) with 2 or 3 rats per cage and were subjected to chronic stress once a day according to a protocol similar to the one proposed by Duccotet and coworkers [48]. The stressing events were chosen randomly from the following procedures: forced restraint in a plastic tube for 2 h without access to food or water; forced bath in a cylindrical tank (0.60 m height x 0.30 m diameter) for 30 min in water at 32 °C; pairing with another stressed animal in wet sawdust (18 h); inversion of the light/dark cycle; crowded housing (8 rats living in a cage for 24 h); tail pinch for 10 min; hot air (approx. 38.00 °C) blown from a hair dryer for 10 min [48–53].

2.3. Behavioral tests

Behavior was evaluated with two tests: spontaneous alternation in the elevated plus maze and thigmotaxis. Animals were tested in a single session on the last day of exposure to either the experimental or control environment. Three days before the tests, the experimenter handled the rats for 5 min/day. Thirty minutes before the test the animals were brought to the experimental room. The test apparatuses were cleaned after each session with alcohol 70%. The experimental procedures were videotaped for later analysis.

Thigmotaxis refers to a specific behavior of animals when exploring an open space: they tend to stay close to walls. Thigmotaxis is associated with fear of open spaces and is a valid measure of anxiety and risk evaluation [47]. Thigmotaxis was evaluated in a wooden apparatus (0.60 × 0.60 × 0.35 m) and the dependent variable was the amount of time the animal spent away from a 0.05 m wide strip close to the wall. The animals were placed in the center of the apparatus and their movement was recorded for 5 min [54,55].

Rats spontaneously alternate among arms when exposed to an elevated maze with closed arms. The spontaneous arm alternation test (SAAT) is used to measure spatial working memory in laboratory animals [41–46]. The SAAT was performed in a wooden maze with four closed arms (0.47 × 0.16 × 0.34 m) and an open central space (0.16 × 0.16 m) located 0.5 m away from the floor. The rats were placed individually at the center of the apparatus and observed for 10 min. The alternation behavior was defined as the number of consecutive entries into all four arms without repeated entries and was expressed as percentage of total arm entries [45].

2.4. Corticosterone blood levels

Immediately after the behavioral tests the animals received a mixture of xylazine (Kenzol, Konig, 9 mg/kg) and ketamine chloride (Vetanarcol, Konig, 72 mg/kg) (i.p.) for perfusion and 1 mL of blood was collected by cardiac puncture in order to measure corticosterone levels [56,57]. The biological material was maintained at 37 °C and the blood was centrifuged at 2300 RPM during 7 min. The resultant blood plasma was separated and stored at −30 °C until