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Maternal exercise reverses morphologic changes in amygdala neurons produced by prenatal stress

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ABSTRACT

Objective: Researchers have studied the potential adverse effect of prenatal stress on nervous system in the offspring. In spite of this, the amygdala, an important structure of the limbic system, has not received attention. Otherwise, exercise has been shown to have benefits over neuronal cells, both morphologic and physiologically, but the potential effect on the amygdala has not been studied. Amygdala seems to be important as key therapeutic target for neuropsychiatric disorders. For these reasons, our objective was to evaluate the morphological effect of prenatal stress over neurons belonging to amygdala and the potential beneficial effect of maternal exercise.

Materials and methods: Female mice were submitted to prenatal stress by restriction and the morphologic effects over the male offspring were evaluated. Neurons belonging to lateral and basolateral nuclei from amygdala were measured and analyzed.

Results: A dendritic lengths increase was found in pyramidal and stellate cells in both nuclei when prenatal stress was applied. However, this increase was reversed by maternal exercise until levels similar to control animals.

Conclusion: Maternal stress produces morphologic changes in the neurons belonging to basolateral and lateral nucleus of amygdala, but these changes could be modified by maternal exercise, so this intervention could have a therapeutic effect on behavioral disturbances related to amygdalic disfunction.

1. Introduction

There is increasing evidence about deleterious impact on progeny due to prenatal stress (PS), in both physical and mental levels (Bercovich, Keinan-Boker, & Shasha, 2014; Buss, Davis, Muftuler, Head, & Sandman, 2010; Gutteling et al., 2005; Huizink, De Mediana, Mulder, Visser, & Buitelaar, 2002). Also, PS notably affects to behavioral, social and educational domains (Niederhofer & Reiter, 2004). It has been seen that a rise on maternal glucocorticoid levels, due to stressful situations, induces a fetal glucocorticoid increase (Seckl & Holmes, 2007). An increase in fetal glucocorticoid level could have a deleterious effect on offspring. Mice seem to be useful to study prenatal stress effects on brain (Maccari et al., 2003), and studies that have used this model have shown changes in hippocampus and prefrontal cortex (Bustamante et al., 2010; Fujioka, Fujioka, Ishida, Maekawa, & Nakamura, 2006; Gutiérrez, Pascual, & Bustamante, 2013; Hosseini-sharifabad & Hadinedoushan, 2007; Murmu, 2006; Mychasiuk, Gibb, & Kolb, 2011; Takahashi, 1998; Yang, Han, Cao, Li, & Xu, 2006). These structures process emotional responses (Vertes, 2006). However, the amygdala, another important emotional component (Dalgleish, 2004), has received less attention. Previous findings obtained from studies that evaluated prenatal stress effects on amygdala are contradictory and the underlying mechanisms are still unknown (Tottenham & Sheridam, 2010). Amygdala is formed by neurons which process fear and emotion aspects (Phelps & LeDoux, 2005), and its activity for anxiety and stress responses has been demonstrated (LeDoux, 1994). Otherwise, relation between different stress models and exercise have been well studied in the recent years (Russell, Zigmond, Dimatelis, Daniels, & Mabandla, 2014), and it has been proposed that exercise has profitable effects for prenatally stressed animals (Kim et al., 2013; Mabandla, Kellaway, Daniels, & Russell, 2009). Maternal exercise (ME) during pregnancy has shown positive outcomes for both mother and offspring, in relation to limbic system morphology (Bustamante et al., 2013; Herring et al., 2012; Parnpiansil, Jutapakdeegul, Chentanez, & Kotchabhakdi, 2003; Uysal et al., 2011), but these aspects on amygdala have not been studied.

In this work we propose to study the morphological consequences of PS on both Lateral and Basolateral amygdala nuclei, and the potential ME therapeutic effect. We expect morphological changes on amygdala as higher anxiety levels in prenatally stressed rats have been observed

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(Vallée et al., 1997). In addition we propose that ME could have a modulator effect over these modifications as it has been found a positive effect on other brain structures such as hippocampus (Dayi et al., 2012; Lee et al., 2006), prefrontal (Uysal et al., 2011), and parietal cortex (Bustamante et al., 2013).

2. Materials and methods

2.1. Experimental procedures

2.1.1. Animals and experimental conditions

Ten virgin female mice (CF-1) were mated with adult male mice (2:1 female:male ratio) during a day. Day zero of gestation (G0) was assumed under the following conditions: (i) daily weight gain of the pregnant dams and (ii) presence of vaginal plug. Housing of dams was under standard laboratory conditions: they were housed in transparent Plexiglas cages (30 cm x 19 cm x 13 cm) with sawdust bedding, two dams per cage until parturition day, 12-h inverted light-dark cycle (lights on at 11:00 p.m.), 18 ± 2 °C, and food and water ad libitum. Dams were randomly assigned to the following groups: control (C, n = 3), restraint stressed (RS, n = 3) or restraint stressed + voluntary wheel running (RS + VWR, n = 4). From each dams, 3–4 male offsprings per dams were randomly selected and 320 neurons in total were analyzed. Neurons belonging to Lateral and Basolateral Nuclei, which met the inclusion criteria were selected (2–3 stellate and 2–3 pyramidal neurons per nucleus). All experimental procedures were performed in accordance with protocols approved by the Bioethics Committee of the Pontificia Universidad Católica de Valparaíso and international guidelines. For this reason, our study was conducted using a minimal number of animals.

2.1.2. Prenatal stress procedure

A previously described protocol (Bustamante et al., 2010) was performed for the restraint stress procedure. Briefly, the stress protocol was applied from gestational day 14 (G14) until parturition, three times per day (at 9:00 a.m., 2:00 p.m. and 6:00 p.m.) for 45 min, by placing females mice in plastic cylinders (11 cm long and 4 cm diameter). Control dams were left undisturbed in their cages and were only handled when cleaning was performed three times per week to all cages (C, RS and RS + VWR groups). This model has shown an increased corticosterone levels in plasma of prenatal stressed fetus (Ward & Weisz, 1984), and it has been used in several studies which have applied prenatal stress as a variable (Barros, Duhalde-Vega, Caltana, Brusco, & Antonelli, 2006; Bustamante et al., 2010, 2013; Gutiérrez et al., 2013; Ward and Weisz, 1984)

2.1.3. Voluntary wheel running (VWR) protocol

Female mice from RS + VWR group was subjected to voluntary wheel running protocol from G1 until G17. Protocol was carried out during the dark phase, 4 h per day (2:00–6:00 p.m.), and housing each pregnant female in a cage that contained a running wheel (14 cm diameter and 6 cm width) for voluntary exercise. Food and water were also ad libitum under this protocol. The number of wheel turns per day was recorded with a magnetic sensor connected to a computer. On postnatal day 23 (P23), after weaning, male pups were randomly selected from the three different group dams, conforming the following pup groups: (i) pups born from control dams (C), (ii) pups born from stressed dams (RS) and (iii) pups born from stressed dams subjected to VWR (RS + VWR).

2.1.4. Histological procedures and dendritic analysis

At P52 animals were sacrificed under deep ether anesthesia and their brains were dissected out, fixed and stained with Golgi-Cox-Sholl procedure (Sholl, 1953). After to 45 days of impregnation, the brains were dehydrated in a graded alcohol series (25%–50%–75%–90%–95%–100%, v/v; Merck) and fixed in Paraplast. Coronal sectional (thickness, 120 μm) were cuts using a rotatory microtome, re-hydrated by a graded alcohol series (100%–95%–90%–75%–50%–25%, v/v; Merck), treated with potassium disulfide/oxalic acid (5% dilution; Merck) and finally coverslipped. Previous to morphologic analysis, slides were coded to maximize reliability and avoid experimental bias. Pyramidal and stellate neurons were selected according to Washburn and Moises (Washburn & Moises, 1992), and met the following criteria: they had a well-defined somata shape, showed adequate staining of the soma and dendrites, exhibited uninterrupted dendritic processes, had no extensive dendrites overlapping with neighboring neurons, and were localized in the Lateral and Basolateral nuclei of amygdala, which was carefully delimited by coordinates described in the Rat Stereotaxic Atlas (Paxinos & Watson, 1998). Before starting measurements, neurons were visually inspected for the integrity of soma boundary and dendritic branches, and were discarded if any of the proximal dendritic branches were incomplete. These neurons were located between bregma −2.0 mm and −3.2 mm and selected for analysis on the basis of morphological criteria described in the literature (McDonald, 1982, 1992).

A total of 320 neurons cells were analyzed using an Am Scope light microscope (400×). 5 neurons were selected per both Lateral and Basolateral area in each brain obtained from the three groups. The neurons were imaged using a digital camera (Canon 5D Mark II) and analyzed using Micrometrics SE Premium V-2.8 software, which measured the following: (i) the total dendritic length per neuron (μm), (ii) neuron somata perimeter and (iii) somata area per neuron. All experimental procedures were performed in accordance with protocols that were approved by the Bioethics Committee of the Pontificia Universidad Católica de Valparaíso and international guidelines.

2.2. Statistical analysis

Data were analyzed using a two way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test in STATA 9.1 software. Results were expressed as the mean ± S.E.M. values and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Basolateral Nucleus (BLN)

We found a significant two-way interaction (group x neuron type) in dendritic lengths of the BLN($F(2, 84) = 5{,}623$, $P = 0{,}0051$). Post hoc comparisons showed that pyramidal dendritic lengths were significantly longer in neurons belonging to prenatally stressed animals than control animals ($P = 0{,}00005$). Furthermore, a significant dendritic length reduction was observed in neurons belonging to animals subjected to ME intervention in relation to prenatally stressed animals ($P = 0{,}00005$). Similar data was observed for stellate neurons in which prenatal stress group had significantly longer dendritic lengths than control group ($P = 0{,}005$) with a significant reduction in maternal exercise intervention group ($P = 0{,}0005$) (Fig. 1).

In regard to neuron soma perimeter, it was revealed only a main effect for group ($F(2, 84) = 3{,}552$, $P = 0{,}0330$) without two-way interaction (group x neuron type) ($F(2, 84) = 0{,}3121$, $P = 0{,}7328$). Post hoc comparisons showed no changes. No significant changes were observed in relation to the somata area.

3.2. Lateral Nucleus (LN)

We found that there was not significant interaction (group x neuron type) on dendritic lengths in the LN ($F(2, 84) = 1{,}954$, $P = 0{,}1481$), although there was a significant effect of both group ($F(2, 84) = 34{,}63$, $P < 0{,}0001$) and neuron type ($F(1, 84) = 3{,}969$, $P = 0{,}0496$). Post