Int. J. Devl Neuroscience 31 (2013) 267–273

Contents lists available at SciVerse ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Maternal exercise during pregnancy ameliorates the postnatal neuronal impairments induced by prenatal restraint stress in mice

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ARTICLE INFO

Article history:
Received 12 November 2012
Received in revised form 18 February 2013
Accepted 18 February 2013

Keywords:
Prenatal stress
Maternal voluntary wheel running
Apical dendrites
Parietal cortex
Locomotor behavior

ABSTRACT

Clinical and preclinical studies have demonstrated that prenatal stress (PS) induces neuronal and behavioral disturbances in the offspring. In the present study, we determined whether maternal voluntary wheel running (VWR) during pregnancy could reverse the putative deleterious effects of PS on the neurodevelopment and behavior of the offspring. Pregnant CF-1 mice were randomly assigned to control, restraint stressed or restraint stressed + VWR groups. Dams of the stressed group were subjected to restraint stress between gestational days 14 and delivery, while control pregnant dams remained undisturbed in their home cages. Dams of the restraint stressed + VWR group were subjected to exercise between gestational days 1 and 17. On postnatal day 23 (P23), male pups were assigned to one of the following experimental groups: mice born from control dams, stressed dams or stressed + VWR dams. Locomotor behavior and pyramidal neuronal morphology were evaluated at P23. Animals were then sacrificed, and Golgi-impregnated pyramidal neurons of the parietal cortex were morphometrically analyzed. Here, we present two major findings: first, PS produced significantly diminished dendritic growth of parietal neurons without altered locomotor behavior of the offspring; and second, maternal VWR significantly offset morphological impairments.

Clinical studies have demonstrated that maternal stress during pregnancy is associated with physiological (Tollenaar et al., 2011) and behavioral (de Weerth et al., 2003) disturbances in the offspring. For example, it has been demonstrated that children born from mothers that have suffered from emotional adversity in late pregnancy demonstrate behavioral alterations such as hyperactivity disorder (O'Connor et al., 2002; Huizink et al., 2003). Consistent with these clinical findings, experimental studies have revealed links between prenatal stress (PS) and behavioral disturbances in juvenile offspring, such as increased locomotor activity (Emack and Matthews, 2011) and anxiety-like behaviors (Miyagawa et al., 2011). These abnormalities are likely due to morphological and functional changes in key areas of the brain that control motor and emotional behaviors, such as the prefrontal cortex (Muhammad et al., 2012) and amygdala (Salm et al., 2004; Kraszpulski et al., 2006). In this regard, it has been demonstrated that prenatally stressed animals show altered dendritic outgrowth of pyramidal neurons of the cingulate anterior area of the PFC (Murmu et al., 2006). These findings are not surprising because the PFC is a highly plastic brain area in which neuronal maturation occurs primarily over a critical period in postnatal life (Altman and Bayer, 1990; Kolb, 1976). In addition, the parietal cortex has also demonstrated plastic changes associated with early life stress. Bock et al. (2005) found that the pups of rats subjected the stress of maternal separation during the pre-weaning period exhibit significant changes in the morphology of pyramidal neurons in the somatosensory region of the brain. However, at present, it is still unclear whether pyramidal neurons from this cortical area are vulnerable to the effects of stressful experiences suffered during prenatal life. Thus, our first aim was to determine the effects of PS on the apical dendritic outgrowth of pyramidal neurons in layer II/III of the parietal cortex in offspring evaluated during the post-weaning period (P23). We selected the apical domain of pyramidal neurons for two reasons: first, it has previously been reported that this dendritic domain is more vulnerable to the effects of PS than the basal domain (Murmu et al., 2006; Cerqueira et al., 2007); and second, because the apical dendrites of layer II/III are the targets of the feedback projections coming from the same and other cortical areas (Spratling, 2002). Because the mice exhibit dense topographically organized projections from the somatosensory cortex to the motor cortex (Bayer and Altman, 1991; Porter, 1996), we evaluated their locomotor behaviors using an open field (OF) test, a test that is widely used to

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http://dx.doi.org/10.1016/j.ijdevneu.2013.02.007

assess locomotor and exploratory behaviors in rodents (Walsh and Cummings, 1976).

On the other hand, it has been shown that exercise during pregnancy may prevent or limit adverse maternal and fetal morbidities (Gavard and Artal, 2008; Weissgerber et al., 2006). For instance, controlled exercise in pregnant women results in beneficial effects on their blood pressure parameters (Yeo et al., 2000) and prevents maternal–fetal diseases, such as type 2 diabetes (Dempsey et al., 2004) and preeclampsia (Weissgerber et al., 2004). Thus, several putative mechanisms that may account for the beneficial effects of exercise on maternal/fetal health have been proposed, including the enhancement of placental growth and vascularity, the reduction of oxidative stress and amelioration of endothelial dysfunction (Weissgerber et al., 2004; Sankaralingam et al., 2011).

Furthermore, it has been shown that the children born from mothers who exercised regularly during pregnancy exhibit enhanced oral language scores at the age of five compared with control groups (Clapp, 1996). At present, the mechanisms that account for these cognitive effects are still largely unknown. Interestingly, these findings are, at least partially, similar to the changes observed in rodent studies. Moreover, maternal exercise during pregnancy has been associated with improvements in the spatial learning and short-term memory of juvenile offspring (Parnpiansil et al., 2003; Kim et al., 2007). These changes appear to be related to modifications in the structure and function of specific hippocampal regions, as shown by studies demonstrating that the offspring born from dams subjected to exercise during pregnancy show enhanced hippocampal neurogenesis (Lee et al., 2006). Furthermore, it has been hypothesized that enhancement of the expression of neurotrophins, such as brain-derived neurotrophic factor (BDNF), could, at least partially explain these beneficial effects (Kim et al., 2007). Based on these findings of the effects of maternal exercise, the second aim of this study was to determine whether maternal wheel running exercise during pregnancy ameliorates or reverses the putative deleterious effects of PS on the dendritic morphology of pyramidal neurons in layer II/III of the parietal cortex and locomotor behavior in prenatally stressed mice. Considering the above-mentioned aims, two hypotheses were tested: (i) PS induces a significant decrement in apical dendritic outgrowth of the pyramidal neurons concomitant with an altered locomotor activity in the prenatally stressed mice; and (ii) maternal exercise during pregnancy ameliorates the deleterious effects associated with PS.

1. Experimental procedures

1.1. Animals and experimental conditions

Twenty-four virgin female mice (CF-1) were mated with twenty-four adult male mice and housed for 1 day under standard laboratory conditions; i.e., a 12-h inverted light–dark cycle (lights on at 11:00 p.m.), 18 ± 2 °C, and food and water ad libitum (one male/one female per cage). The day of mating was considered "day zero of pregnancy" (G0), and this assumption was corroborated by the following observations: (i) the daily weight gain of the pregnant dams and (ii) the length of gestation. A cohort of ten female mice fulfilled the above-mentioned criteria and were included in the current study. All of the pregnant females were socially housed in transparent Plexiglas cages (30 cm × 19 cm × 13 cm; 2–3 dams per cage) with sawdust bedding and were randomly assigned to the one of the following three experimental groups: control (C, $n = 3$), restraint stressed (RS, $n = 3$) or RS + VWR ($n = 4$). All pregnant dams remained socially housed until the day before parturition.

1.2. Prenatal stress procedure

The restraint stress procedure was performed according to a previously described protocol (Bustamante et al., 2010). Briefly, from gestational day 14 (G14) until delivery (see Fig. 1B), the pregnant females were subjected to three daily stress sessions at 9.00 a.m., 2.00 p.m. and 6.00 p.m. during which they were placed in plastic cylinders (11 cm long and 4 cm diameter) for 45 min. The control pregnant females were left undisturbed in their home cages (see Fig. 1A) and were handled only when the cages of all groups (C, RS and RS + VWR) were cleaned three times a week.

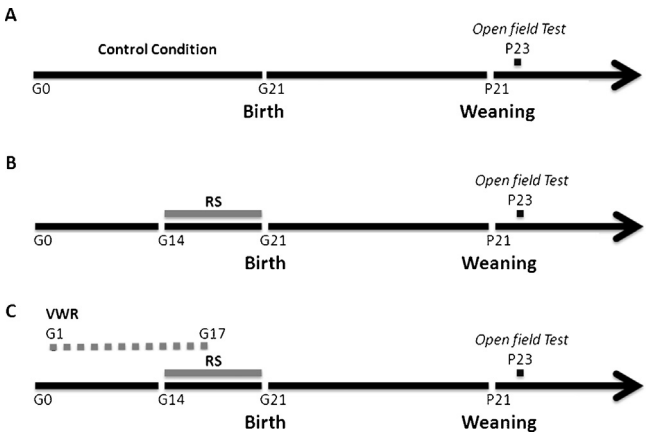

Fig. 1. Timeline showing a summary of the experimental design. G: gestational age; P: postnatal age (days). RS: restraint stress; VWR: voluntary wheel running.

1.3. Voluntary wheel running (VWR) protocol

Between G1 and G17, each female mouse from the RS + VWR group was subjected to the VWR protocol (see Fig. 1C) during the dark phase for 4 h per day (2.00–6.00 p.m.) through housing in a cage that contained a running wheel (diameter, 14 cm; width, 6 cm). Under this condition, the mice had free access to voluntary exercise and food and water ad libitum. The number of daily wheel turns was registered by a magnetic sensor that was placed on the roof of the cage and connected to a computer. After weaning (P23), male pups only were randomly selected from each of the following three groups (to prevent possible litter effects, we used only 1–2 pups/litter/experiment): (i) mice born from a control mother (C), (ii) mice born from a stressed mother (RS) and (iii) mice born from a stressed mother subjected to VWR (RS + VWR).

1.4. Open field (OF) test

On P23, the male mice from each group (C, $n = 5$; RS, $n = 5$ and RS + VWR, $n = 5$) were evaluated using the OF test (Walsh and Cummings, 1976). Briefly, this test was conducted in a square arena (40 cm × 40 cm) that was enclosed by continuous 21 cm-high walls made of black wood. The arena was divided by Anymaze software into 25 equal sized squares containing one central zone (8 cm × 8 cm) and 24 surrounding squares (peripheral zone). The animals were examined during their dark cycle (between 2.00 and 6.00 p.m.), and testing was conducted under dim light (approximately 80 lx). Each mouse was placed in the center of the OF and allowed to freely explore the arena for 90 s. The animals' behaviors were recorded using a camera that was located 40 cm above the apparatus (Logitech Quick cam version 9.5.0), and the data were analyzed using the Anymaze software. The number of lines crossed by each animal (with all four limbs), the total distance traversed and the total time spent in the central zone (a square of 8 cm × 8 cm in area) of the apparatus within 90 s were quantified. The open field apparatus was wiped between each test with a clean alcohol-dipped cloth to eliminate any olfactory clues left by previously tested animals.

1.5. Histological procedures and dendritic analysis

To study the effect of prenatal stress and the potential "therapeutic" effects of VWR on the dendritic outgrowth of pyramidal neurons, mice were sacrificed under deep ether anesthesia, and their brains were immediately dissected out, fixed and stained with the Golgi-Cox-Sholl procedure (Sholl, 1953). After 45 days of slow metallic mercury impregnation, the brains were dehydrated in a graded alcohol series (25%–50%–75%–90%–95%–100%, v/v; Merck) and fixed in Paraplast. Coronal sections (thickness, 120 μm) were cut using a sledge microtome, re-hydrated by in a graded alcohol series (100%–95%–90%–75%–50%–25%, v/v; Merck), treated with potassium disulfide/oxalic acid (5% dilution; Merck) and coverslipped. All slides were coded to avoid experimental bias and to maximize reliability. To qualify for dendritic morphometric evaluation, the pyramidal neurons were required to fulfill the following criteria: (a) have a well-defined somata shape; (b) show adequate staining of the soma and dendrites; (c) exhibit uninterrupted apical dendritic processes; (d) have no extensive dendrites that overlapped with neighboring neurons; and (e) be localized to layer II/III of the parietal cortex (approximately 150–350 μm from the pial surface), which was carefully delimited by coordinates described in the Rat Stereotaxic Atlas (Paxinos and Watson, 1998). A total of 299 pyramidal neurons cells (approximately 20 neurons/animal) were analyzed using an Olympus CX-3 light microscope (400×). The neurons were imaged using a digital camera (5.0 CCD Camera) and analyzed using Micrometrics SE Premium V-2.8 software, which measured the following: (i) the total apical dendritic length per neuron (μm) and (ii) the number of apical branches per neuron. At respect, the branching was determined