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Research Report

Effects of duration and timing of prenatal stress on hippocampal myelination and synaptophysin expression

Jian Xu[a,*,1], Bo Yang[b,1], Chonghuai Yan[a,*], Howard Hu[c], Shizhong Cai[a], Junxia Liu[a], Meiqin Wu[a], Fengxiu Ouyang[a], Xiaoming Shen[a]

[a]Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Institute for Pediatric Research, MOE-Shanghai Key Laboratory of Children's Environmental Health, Shanghai 200092, China
[b]Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China
[c]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada M5T 3M7

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ABSTRACT

The relationship between prenatal stress (PS) exposure and neurodevelopmental deficits remains inconclusive, especially when assessing the role of PS duration and timing and sex-dependent effects. This study explored a sex-specific association between the duration and timing of exposure and the outcomes of PS-induced neurotoxicity in hippocampal microstructure, synaptophysin expression, and neurobehavioral performance in rats. Pregnant rats were randomly assigned to control, PS-ML (exposed to prenatal restraint stress in the mid-to-late period of pregnancy), or PS-L (exposed in the late period of pregnancy) groups, and offspring in each group were divided into two subgroups by sex. Surface-righting reflex test, cliff avoidance test and Morris water maze test showed that neurodevelopmental levels were reduced in PS-treated pups but without significant sex differences. On postnatal day 22, hippocampal microstructure was examined by electron microscopy, and the expression of hippocampal synaptophysin was assessed by western blot. Abnormal ultrastructural appearance of hippocampal neurons and myelin sheaths, more degenerating neurons and higher G-ratios were found in young PS-ML and PS-L rats as well as reduced expression of hippocampal synaptophysin, although PS-ML pups were more greatly affected than PS-L, with males showing slightly greater impairments than females. These findings suggest that hippocampal hypomyelination and decreased synaptophysin expression in neurodevelopment may be a duration and time-dependent effect of prenatal stress exposure, modified slightly by sex.

Abbreviations: PS, prenatal stress; GD, gestational day; PND, postnatal day; PS-ML group, the group receiving prenatal stress either in the mid- to late gestational period of pregnancy; PS-L group, the group receiving prenatal stress in the late pregnancy; MC, male control; FC, female control; MPS-ML, male PS-ML group; FPS-ML, female PS-ML group; MPS-L, male PS-L group; FPS-L, female PS-L group; TEM, transmission electron microscopy; MWM, Morris water maze

*Correspondence to: 1665 Kongjiang Road, Xinhua Hospital, Shanghai 200092, China. Fax: +86 21 6579 1316.
E-mail addresses: jxxinhuachina@gmail.com (J. Xu), yanch@shkeylab-ceh.org (C. Yan).
[1]Contributed equally to this work.

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1. Introduction

Recent epidemiological and animal studies have shown that maternal stress during pregnancy has both short-term and long-term adverse effects on the neurological development of offspring, suggesting that prenatal stress (PS) has profound programming influences on the offspring's brain (Kjaer et al., 2010; Kofman, 2002; Maccari et al., 2003; Mairesse et al., 2012, 2007; Mulder et al., 2002; Sadler et al., 2011; Talge et al., 2007; Van den Hove et al., 2012; Weinstock, 2008; Zuena et al., 2008). In children, PS has been associated with behavioral and cognitive problems as well as attention-deficit hyperactivity disorder and autism (Charil et al., 2010). PS-treated newborn rats showed significant alterations in network properties of hippocampal neurons (Grigoryan and Segal, 2013). However, the underlying mechanisms remain largely unknown.

Recent studies of animal models applying PS stimuli at different stages of pregnancy suggested that the effects of PS on pups' neurological development might be dependent on the timing of stress exposure during gestation (Davis and Sandman, 2010; Kapoor et al., 2009; Koenig et al., 2005; Kofman, 2002; Talge et al., 2007; Yang et al., 2006). However, in real life, if maternal stress starts in the second trimester of pregnancy, it usually does not stop during the third trimester. In addition, the duration of PS exposure usually depends on the timing of PS exposure, and "the duration of PS" and "the timing of PS" are often closely associated. Therefore, in PS animal studies, grouping animals according to the duration of PS exposure, but not according to the timing of PS exposure, may better reflect real-life situations, and can be used to explore the association between both duration and timing of exposure and the outcomes of PS-induced neurotoxicity.

The established paradigm for neurotoxicity research of PS in rats, applying prenatal restraint stress during gestational days (GD) 15–21, is based on the fact that during this period the fetal hippocampus is developing and the hypothalamic-pituitary-adrenal axis starts to secrete its own ACTH and corticosterone (Davis and Sandman, 2010; Lemaire et al., 2000; Maccari et al., 2003; Mairesse et al., 2007; Song et al., 2009; Talge et al., 2007). According to the fetal programming model, however, the development of the central nervous system includes a series of processes that are initiated in sequence and that are dependent upon each other in various ways – such as neuroendocrine and epigenetic mechanisms in response to environmental influences and genetically programmed events – so that interference at one stage may influence later stages of development (Johnston, 1995; Kostović et al., 2002). Therefore, if maternal rats were subjected to PS before GD 15, although the fetal hippocampus has not yet developed nor has the fetal hypothalamic-pituitary-adrenal axis been initiated, such stress may have an effect on a pre-existing neuroendocrine or remodeling variation in an early developmental stage and so might affect subsequent stages of development. Thus it is plausible to expect evidence of exposure duration and time-dependent neurodevelopmental impairment in offspring if PS was extended to an even earlier period of gestation.

There is preliminary evidence that the hormonal response to PS is sexually dimorphic, however, no consistent conclusion has been produced as to which sex may be more susceptible to PS-induced developmental neurotoxicity (García-Cáceres et al., 2010; Schmitz et al., 2002; Weinstock, 2011; Zuena et al., 2008). The hippocampus plays a central role both in learning and memory, and many animal studies suggested that fetal hippocampal development was compromised by PS (Biala et al., 2010; Davis and Sandman, 2010; Kofman, 2002; Maccari et al., 2003; Mairesse et al., 2012; Mueller and Bale, 2007; Schmitz et al., 2002; Song et al., 2009; Weinstock, 2008).

Therefore, the hypotheses of our study are: (1) there is a duration and time-dependent effect of PS exposure on pups' neurological development, (2) damages in hippocampal microstructure and synaptic transmission are possible mechanisms that underlie these effects, and (3) sex differences in the deleterious effects of PS may exist.

2. Results

2.1. *Effects of duration and timing of PS on the growth and development of newborn rats*

No significant differences were observed in gestational length among dams [control: (21.17±0.56) d; PS-ML (exposed to prenatal restraint stress in the mid-to-late period of pregnancy): (20.79±0.78) d; PS-L (exposed in the late period of pregnancy): (20.88±0.61) d, $p>0.05$], and no mortality of newborns was observed in any group. No significant differences existed in birth weight [MC (male control) *vs.* FC (female control) *vs.* MPS-L (male PS-L) *vs.* FPS-L (Female PS-L) *vs.* MPS-ML (male PS-ML) *vs.* FPS-ML (Female PS-ML): (6.10±0.25) g *vs.* (6.19±0.79) g *vs.* (6.30±0.40) g *vs.* (6.10±0.34) g *vs.* (6.38±0.54) g *vs.* (6.44±0.38) g] among the treatment and control groups ($p>0.05$) and between the two sexes ($p>0.05$). Prenatal stress showed no significant effects on litter size or on sex ratio within litters. No significant difference was found in eye-opening days [MC *vs.* FC *vs.* MPS-L *vs.* FPS-L *vs.* MPS-ML *vs.* FPS-ML: (15.50±0.50) d *vs.* (14.75±0.97) d *vs.* (15.39±0.64) d *vs.* (14.88±1.11) d *vs.* (15.39±0.42) d *vs.* (15.35±0.90) d] among the treatment and control groups ($p>0.05$) and between the two sexes ($p>0.05$).

2.2. *Effects of duration and timing of PS on the neurobehavioral levels*

In surface righting reflex test, all pups in the 6 groups showed a progressive improvement in righting response performance from postnatal day (PND) 4 to PND 10. Progressive decrease in righting time was more marked in the control group and least marked in PS-ML group, and statistically significant differences were found among the treatment and control groups ($p=0.015$) and on PNDs ($p=0.000$), but no significant difference was found between the two sexes ($p>0.05$) (Fig. 1A). In cliff avoidance test, the control pups showed a persistent decrease in mean latency time by PND 12. Although pups treated with PS showed similar trends, their mean latency time was relatively longer than that of control pups and positively correlated with the PS exposure-duration. A statistically significant difference in latency time was observed among the treatment and control groups ($p=0.037$) and on PNDs ($p=0.000$), but no significant difference was found between the two sexes ($p>0.05$) (Fig. 1B).