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Original article

Alterations of proliferation and differentiation of hippocampal cells in prenatally stressed rats

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Abstract

Purpose: To clarify the alterations of proliferation and differentiation of hippocampal cells in prenatally stressed rats.

Methods: We investigated the impact of prenatal restraint stress on the hipocampal cell proliferation in the progeny with 5-bromo-2′-deoxyuridine (BrdU), which is a marker of proliferating cells and their progeny. In addition, we observed the differentiation of neural stem cells (NSCs) with double labeling of BrdU/neurofilament (NF), BrdU/glial fibrillary acidic protein (GFAP) in the hipocampus.

Results: Prenatal stress (PS) increased cell proliferation in the dentate gyrus (DG) only in female and neuron differentiation of newly divided cells in the DG and CA4 in both male and female. Moreover, the NF and GFAP-positive cells, but not the BrdU-positive cells, BrdU/NF and BrdU/GFAP-positive cells, were found frequently in the CA3 and CA1 in the offspring of each group.

Conclusions: These results possibly suggest a compensatory adaptive response to neuronal damage or loss in hippocampus induced by PS.

Keywords: Prenatal stress; BrdU; Neurofilament; Glial fibrillary acidic protein; Hippocampus

1. Introduction

Evidences for effects of prenatal stress (PS) on the functional and structural of the hippocampus have been accumulating rapidly in recent years [1]. Research has investigated that mild PS enhanced processes of neurogenesis of hippocampal neurons [2]. Neurogenesis can be divided into three main cellular events: cell proliferation, neuronal differentiation, and cell survival [3], and it is an extremely dynamic process that is regulated in both a negative and positive manner by environmental factors. For example, chronic restraint stress [4] has been shown to enhance survival of new neurons in mice. In contrast, study has shown that PS inhibited neurogenesis of the offspring hippocampus in adult rat [5]. These aforementioned studies examining neurogenesis mainly focused on the different effect of

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stress on the dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis.

Neural stem cell (NSC) is a heterogeneous population of mitotically active, self-renewing and multipotent cells of both the developing and the adult central nervous system [6]. It was demonstrated that NSCs were capable of giving rise to both neurons and glia, which migrate into the granule cell layer and integrate into the hippocampal circuitry with specific afferent and efferent connectivity [7]. Previously, we have found that PS resulted in dendritic atrophy of pyramidal neurons in hippocampal CA3 region, caused a significant hippocampal neurons loss in the juvenile offspring compared with controls [8]. It is interesting to consider possible effects of PS on the neurogenesis after neurons loss or damage in hippocampus.

In this study, we examined cell proliferation in the progeny with BrdU. While, we observed the differentiation of stem cells with NF and GFAP, and double labeling of BrdU/NF, BrdU/GFAP. The present study aim to explore how neurogenesis may happen during the developing hippocampal neurons after exposed to PS.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats were maintained at constant temperature (22 °C) and humidity (60%) on a 12 h light/dark cycle (light on 08:00–20:00 h), freely accessing to food and water throughout the experiment. All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animals Care and Use Committee at Xi'an Jiaotong University. Every effort was made to optimize comfort and minimize the use of animals. Nulliparous female rats weighing 230–250 g were housed with a sexually

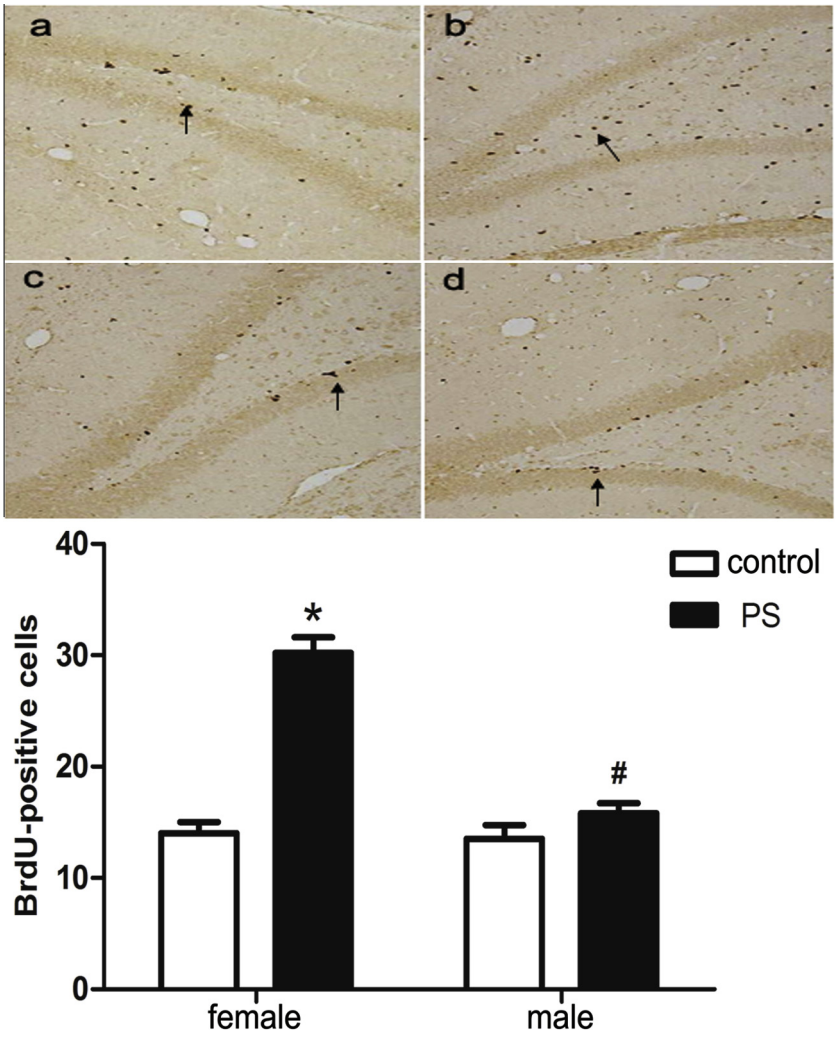

Fig. 1. Effect of PS on cell proliferation in the DG. Brain sections from a control offspring (a, c), and from a prenatally stressed offspring (b, d) were stained for BrdU immunoreactivity (blank arrowheads). Scale bar, 100 μm. Values represent means ± SEM. $n = 10$ per group. $^{\#}p < 0.001$ *vs.* female, $^{*}p < 0.001$ *vs.* CON.