Physiology & Behavior 161 (2016) 166–173

Contents lists available at ScienceDirect

Physiology & Behavior

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

Hippocampal neurogenesis dysfunction linked to depressive-like behaviors in a neuroinflammation induced model of depression

Ming-ming Tang [a,c], Wen-juan Lin [a,b,*], Yu-qin Pan [a,b], Xi-ting Guan [a,c], Ying-cong Li [a]

[a] Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China
[b] Brain-Behavior Research Center, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China
[c] University of Chinese Academy of Sciences, Beijing, China

HIGHLIGHTS

- Repeated central lipopolysaccharide infusions cause neuroinflammation in hippocampus.
- Neuroinflammation induces depressive-like behavior.
- Neuroinflammation impairs adult hippocampal neurogenesis.
- Depressive-like behavior correlates with hippocampal neurogenesis.

ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form 13 April 2016
Accepted 15 April 2016
Available online 19 April 2016

Keywords:
Depressive-like behavior
Neuroinflammation
Neurogenesis
Pro-inflammatory cytokine
Lipopolysaccharide

ABSTRACT

Our previous work found that triple central lipopolysaccharide (LPS) administration could induce depressive-like behaviors and increased central pro-inflammatory cytokines mRNA, hippocampal cytokine mRNA in particular. Since several neuroinflammation-associated conditions have been reported to impair neurogenesis, in this study, we further investigated whether the neuroinflammation induced depression would be associated with hippocampal neurogenesis dysfunction. An animal model of depression induced by triple central lipopolysaccharide (LPS) administration was used. In the hippocampus, the neuroinflammatory state evoked by LPS was marked by an increased production of pro-inflammatory cytokines, including interleukin-1β, interleukin-6, and tumor necrosis factor-α. It was found that rats in the neuroinflammatory state exhibited depressive-like behaviors, including reduced saccharin preference and locomotor activity as well as increased immobility time in the tail suspension test and latency to feed in the novelty suppressed feeding test. Adult hippocampal neurogenesis was concomitantly inhibited, including decreased cell proliferation and newborn cell survival. We also demonstrated that the decreased hippocampal neurogenesis in cell proliferation was significantly correlated with the depressive-like phenotypes of decreased saccharine preference and distance travelled, the core and characteristic symptoms of depression, under neuro inflammation state. These findings provide the first evidence that hippocampal neurogenesis dysfunction is correlated with neuroinflammation-induced depression, which suggests that hippocampal neurogenesis might be one of biological mechanisms underlying depression induced by neruoinflammation.

1. Introduction

It has been proposed that the actions of inflammatory processes, and of pro-inflammatory cytokines in particular, on the brain may account for the pathogenesis of depressive disorders, and this hypothesis has been referred to as the "cytokine or inflammatory hypothesis of depression" [1–5]. Although this hypothesis has been an interesting research focus for more than two decades, how neuronal-immune interactions modulate the depressive phenotype is still not fully understood. Inflammatory processes may affect more than one pathway, which is thought to be important in the pathogenesis of depression, such as monoamine alterations, glutamate neurotransmission, and glucocorticoid receptor resistance [6–8]. Novel theory suggests that adult hippocampal neurogenesis, as a novel pathway, may also be involved in depressive disorders caused by inflammation [9,10].

Neurogenesis is the complex process of generating new neurons from neural stem or progenitor cells that occurs in the brains of many animal species, including humans [11]. Adult neurogenesis primarily occurs in the subventricular zone (SVZ) of the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus [12]. The process of adult neurogenesis involves several steps, including

* Corresponding author at: 16 Lincui Road, Beijing 100101, China.
E-mail address: linwj@psych.ac.cn (W. Lin).

http://dx.doi.org/10.1016/j.physbeh.2016.04.034
0031-9384/

proliferation, specification of the fate of neural progenitors, neuronal migration, neuronal maturation and synaptic integration of the young neurons into the existing neuronal circuitry. It has been reported that adult hippocampal neurogenesis is related to synaptic plasticity, cognitive functioning, and psychiatric diseases [13,14]. Neurogenesis in the dentate gyrus of the hippocampus is negatively regulated by stressful experiences [15] and positively regulated by chronic antidepressant treatment [16]. Recent evidence indicates that neurogenesis is influenced by inflammatory processes [17–19]. Recently, we found that triple central lipopolysaccharide (LPS) administration induced depressive-like behaviors and increased central pro-inflammatory cytokines mRNA, hippocampal cytokine mRNA in particular. We also found that the increased hippocampal TNF-α mRNA expression was the common affected factor in both chronic stress induced and LPS induced models of depression [20]. These results suggest that hippocampal inflammation may have a pivotal role in depression. However, it is unknown how hippocampal neurogenesis is affected by triple central LPS administration and whether the altered hippocampal neurogenesis contributes to the regulation of depression induced by neuroinflammation.

Thus, the purpose of the present study was to determine the relationship between hippocampal neurogenesis and the depressive-like behavior induced by inflammation. In this study, we first examined the neuroinflammatory state in the hippocampus induced by triple central LPS administration by determining the protein expression of IL-1β, IL-6 and TNF-α. We then examined depressive-like behaviors and adult hippocampal neurogenesis under neuroinflammatory state and evaluated the correlation between the degree of depressive-like behaviors and the level of altered neurogenesis.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats that weighed 220–240 g were obtained from Vital River Laboratories (Beijing, China). All of the rats were individually housed in standard stainless steel cages in a temperature- and humidity-controlled room (22 ± 1 °C; 40–60%) with a 12:12 dark/light cycle (lights on at 07:00 a.m.; off at 7:00 p.m.). The rats were given free access to food and water throughout the experiment except for the saccharin preference test time and novelty suppressed feeding test time. The rats were given 5 min of daily handling for 7 days prior to experimental use to minimize stress responses to the experimental manipulation. The experimental procedures were approved by the Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Surgical procedures

After acclimation, the rats were anesthetized with sodium pentobarbital (1%, 35 mg/kg; Merck, Darmstadt, Germany) via an intraperitoneal injection, and guide cannulas were implanted (RWD Life Science Co., Ltd., Shenzhen, China) into the lateral ventricle (AP: −0.9 mm from bregma; LM: 1.1 mm from the sagittal suture; DV: 3.2 mm in depth relative to the skull). The top of the animal's head was shaved, and the head was fixed in a stereotaxic instrument (Stoelting, Wood Dale, IL, USA), with the incisor bar set at 3.3 mm below the interaural line. Body temperature was maintained at 37 °C using a rectal thermometer and a feedback-controlled heating pad (RWD, Shenzhen, China). A 1 mm hole was drilled, and the cannula (outer diameter: 0.64 mm; inner diameter: 0.45 mm) was unilaterally implanted above the lateral ventricle. The guide cannula was fixed to the skull via acrylic resin and two stainless steel screws. At the end of the surgery, a stylet with the same length as the guide cannula was inserted to prevent obstruction. The rats were allowed to recover for ten days in their home cages.

2.3. Drug infusion

All of the injections were performed in freely moving animals using a 5 μl microsyringe attached to a micro infusion pump (RWD, Shenzhen, China). The pro-inflammatory cytokine-inducer LPS (derived from *Escherichia coli* serotype 0111:B4, No: L-2880, Sigma, St Louis, MO, USA) was diluted to 100 ng/μl with sterile saline and was infused intracerebroventricularly (i.c.v.) at a dose of 100 ng/rat (flow rate: 0.5 μl/min). This dose was chosen because it has previously been shown to significantly decrease social exploration and locomotor activity and increase immobility in rats [21,22]. In accordance with our previous studies [20,23], the animals received infusions of LPS or saline once every two days for five days (i.e., on days 1, 3, and 5). For the labelling of S-phase mitotic cells, the thymidine analogue bromodeoxyuridine (BrdU) (Sigma-Aldrich, St Louis, MO, USA) was diluted to 100 μg/μl in sterile saline and was infused i.c.v. at a dose of 200 μg/rat (flow rate: 1 μl/min). This dose could effectively label the newborn cells [24,25]. The animals received two infusions of BrdU at a 6 h interval in one day.

2.4. Experimental procedures

2.4.1. Experiment 1: The effects of central LPS treatment on the protein expression of pro-inflammatory cytokines in the hippocampus

After the rats recovered from surgery, 18 rats were randomly assigned to two groups (9 rats per group) and administered central LPS or saline treatments, 1 h after light on once every two days (on days 1, 3, and 5) to examine the effects of the treatment on the protein expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in the hippocampus (Fig. 1A). The rats were sacrificed 24 h after the last LPS infusion (on day 6). To examine and conform the neuroinflammation state, inflammatory cytokines were determined by two technical methods, i.e. samples from five rats in each group were used for western blotting, and samples from four rats in each group were used for immunohistochemistry.

2.4.2. Experiment 2: The effects of central LPS treatment on depressive-like behaviors and cell proliferation in the hippocampus

The same central LPS treatments were used as in Experiment 1. Twelve rats were randomly assigned to two groups (6 rats per group). The timeline was shown in Fig. 2A. To label the cell proliferation after LPS treatment, all rats were given BrdU infusions on day 6. Behavioral measurements were obtained on day 7. The behavioral tests were conducted between 8:00 and 14:00. The order of testing was balanced by group: one rat from each group in sequence. Brain samples were dissected after the termination of behavioral measurements.

2.4.3. Experiment 3: The effects of central LPS treatment on depressive-like behaviors and newborn cell survival in the hippocampus

In this experiment, the same central LPS treatments were used as in Experiment 1. Twelve rats were randomly divided into two groups (6 rats per group). The timeline was shown in Fig. 3A. To label the newborn cell survival after LPS treatment, all rats were given BrdU infusions on day 0 before the LPS treatment. Behavioral measurements were obtained on day 6. Brain samples were dissected after the termination of behavioral measurements.

2.5. Behavioral measurements

2.5.1. Saccharin preference test (SPT)

Three days of habituation occurred prior to the test. The rats were provided a bottle that contained water and a second bottle with a 0.5% saccharin solution during a 4 h window (8:00–12:00 a.m.) and were deprived of liquid at other times (20 h). On the test day, each rat was given the two bottles (saccharin and water) for 1 h between 8:00 and 9:00 a.m. after a 20 h deprivation of liquid. The intake amount of each