Neuropharmacology 135 (2018) 223–233

Contents lists available at ScienceDirect

Neuropharmacology

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

# Dynamic changes in hippocampal microglia contribute to depressive-like behavior induced by early social isolation

Yu Gong [a, 1], Lijuan Tong [a, 1], Rongrong Yang [b, **, 1], Wenfeng Hu [a], Xingguo Xu [b], Wenjing Wang [a], Peng Wang [a], Xu Lu [a], Minhui Gao [a], Yue Wu [a], Xing Xu [a], Yaru Zhang [a], Zhuo Chen [c], Chao Huang [a, *]

[a] Department of Pharmacology, School of Pharmacy, Nantong University, #19 Qixiu Road, Nantong, Jiangsu Province, 226001, China
[b] Department of Anesthesiology, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, 226001, China
[c] Invasive Technology Department, Nantong First People's Hospital, The Second Affiliated Hospital of Nantong University, #6 North Road Hai'er Xiang, Nantong, Jiangsu Province, 226001, China

ARTICLE INFO

*Article history:*
Received 25 October 2017
Received in revised form
18 March 2018
Accepted 19 March 2018
Available online 21 March 2018

*Keywords:*
Hippocampus
Microglia
Early social isolation
Lipopolysaccharide
Macrophage colony-stimulating factor

ABSTRACT

Depression triggered by early-life stress has begun to attract wide attention due to its severe symptoms and poor treatment outcomes. However, the pathophysiological mechanism for this type of depression remains unclear. Recently, we and others reported that different types of chronic stress induce a significant loss of hippocampal microglia, which is mediated by an initial activation of these microglia. Since early-life stress also promotes microglial activation, we investigated the dynamic changes in hippocampal microglia in mice suffering from depression induced by early social isolation (ESI). Results showed that 8 days of ESI induced depressive-like behaviors in a tail suspension test, forced swim test, sucrose preference test, and open field test, and it also induced a loss and dystrophy of hippocampal microglia. We found that this ESI-induced loss of hippocampal microglia was mediated by both microglial activation and apoptosis. This was demonstrated by the following results: (i) 1 day of ESI induced an obvious activation of hippocampal microglia followed by their apoptosis, and (ii) the blockade of the initial activation of hippocampal microglia by minocycline pretreatment suppressed the ESI-induced apoptosis and loss as well as ESI-induced depressive-like behavior. Lipopolysaccharide (LPS) and macrophage colony-stimulating factor (M-CSF), two activators of microglia, almost completely reversed ESI-induced depressive-like behavior by promoting microglial proliferation in the hippocampus. These results reveal an etiological role of hippocampal microglial loss in ESI-induced depression and demonstrate that the restoration of microglial homeostasis in the hippocampus may serve as a therapeutic strategy for depression induced by early-life stress.



## 1. Introduction

Depression is a common disease that leads to severe social and economic problems. In adult individuals, depression is mainly induced by genetic and/or environmental factors, while in children, different types of early-life stresses, such as traumatic events and poor parental care, are considered risk factors (Amini-Khoei et al., 2017; Liu et al., 2017; Lo Iacono et al., 2015). Depression induced by early-life stress has a different clinical profile, including more severe symptoms, an earlier onset, a more prolonged course of the disease, and poorer treatment outcomes (Miniati et al., 2010; Nanni et al., 2012). Depressed patients who have experienced early-life stress constitute a distinct clinical ecophenotype that can influence the therapeutic efficacy of conventional depression treatments (Andrus et al., 2012; Heim et al., 2008). To date, the exact mechanism underlying depression induced by early-life stress remains largely unknown.

In the past several years, researchers have mainly focused on studying neurons in depression and considered central monoamine dysfunction as a major factor triggering the onset of depression (Dean and Keshavan, 2017; Liu et al., 2017a). Importantly, the most

* Corresponding author.
** Corresponding author.
*E-mail addresses:* charliechao888888@163.com (R. Yang), huachao@ntu.edu.cn (C. Huang).
[1] Yu Gong, Lijuan Tong, and Rongrong Yang contribute equally to this work.

https://doi.org/10.1016/j.neuropharm.2018.03.023
0028-3908/

widely used antidepressants, such as selective serotonin reuptake inhibitors and monoamine oxidase inhibitors, were developed as a result of the monoamine hypothesis of depression (Blier, 2016; Fujita et al., 2017). However, these agents are no longer considered effective antidepressants because numerous clinical practices have reported that only one-third of patients taking these agents exhibit relatively good treatment outcomes, while the other two-thirds only see a therapeutic effect after several weeks of treatment (Fabbri et al., 2013; Schwartz et al., 2016). Thus, it is necessary to find new methods to treat depression. Recently, we (Tong et al., 2017) and others (Kreisel et al., 2014; Yirmiya et al., 2015) reported that the initial activation and subsequent loss of hippocampal microglia mediates the development of depression induced by chronic unpredictable stress (CUS), chronic social defeat stress (CSDS), and chronic restraint stress (CRS). This finding suggests that interrupting these dynamic changes in hippocampal microglia may be a potential therapy for depression.

In previous studies, the neuroinflammatory response was found to mediate depression induced by early-life stress. For example, an increased level of pro-inflammatory cytokine has been observed in the blood of individuals who had traumatic childhoods (Miller and Cole, 2012). Maternal separation can trigger microglial activation in the hippocampus of rat pups (Gracia-Rubio et al., 2016; Roque et al., 2016). Here, we hypothesized that the changes in hippocampal microglia may play a critical role in triggering depression induced by early-life stress. To test this hypothesis, we investigated the dynamic changes in hippocampal microglia in a depression model induced by early social isolation (ESI) during the third postnatal week (postnatal days 14–21; PD 14–21) in mice. ESI consists of less maternal care and social interactions (Lo Iacono et al., 2015). The third postnatal week of brain development is characterized by the maturation of visual, motor, and social abilities (Berardi et al., 2000; Pellis and Pasztor, 1999), and exposing mice to ESI during this period can induce depressive-like behaviors (Lo Iacono et al., 2015). We showed here that ESI induced a significant loss and dystrophy of microglia in the dentate gyrus (DG) of the hippocampus, which was mediated by the initial activation and subsequent apoptosis of hippocampal microglia after short-term ESI. Inhibiting the decline in hippocampal microglia abrogated the depressive-like behavior induced by ESI. These results suggest that restoring microglial homeostasis in the hippocampus may be a novel strategy for the treatment of depression induced by early-life stress.

## 2. Materials and methods

### 2.1. Materials

Lipopolysaccharide (LPS, *Escherichia coli*, serotype 0111: B4) and macrophage colony-stimulating factor (M-CSF) were purchased from Sigma (Saint Louis, MO, USA) and Prospec (Ness-Ziona, Israel), respectively. Minocycline is the product of MedChem Express (Monmouth, NJ, USA).

### 2.2. Animals

Three-week-old DBA/2J@Ico (DBA) male and female mice were purchased from the Model Animal Research Center in Nanjing University (Nanjing, China). Mice were group-housed under standard conditions (12-h light/dark cycle; lights on from 07:00 to 19:00; 23 ± 1 °C ambient temperature; 55 ± 10% relative humidity) with free access to food and water. For the production of pups, the DBA mice were mated at 12 weeks of age, and only pup numbers varying between four and eight were included. Animal experiments were conducted by following internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999 and approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2110836). The researchers were blinded to the group allocation during the experiment and data analysis.

### 2.3. Social isolation procedure and pharmacological treatments

Mouse pups were randomly assigned to the control or ESI group at PD 14. In the control group, mothers and offspring were left undisturbed without cage cleaning until weaning. In the ESI group, each pup was singly housed in a novel cage with clean bedding for 30 min per day from PD 14–21. All of the pups were weaned at PD 22 and were prepared to undergo behavioral testing or gene expression assays at 8–10 weeks of age. The behavioral tests were separated by 3-week intervals according to previous studies (Lo Iacono et al., 2015; Paylor et al., 2006). Since the results of the open field test (OFT) can be affected by inter-test intervals (Paylor et al., 2006), the behavioral experiments in our study were performed in the following order: OFT, tail suspension test (TST), forced swim test (FST), and sucrose preference test (SPT). A schematic diagram for these behavioral experiments is outlined in Fig. 1A. Both LPS and M-CSF were injected intraperitoneally (i.p.) at a dose of 100 μg/kg. LPS at 100 μg/kg has been confirmed to activate microglia and produce depressive-like behaviors (Yirmiya, 1996; Yirmiya et al., 2001; Tong et al., 2017). The M-CSF dose of 100 μg/kg was selected based on previous studies showing that this dosage effectively induces microglial activation and proliferation in chronically stressed mice (Boissonneault et al., 2009; Tong et al., 2017). The behavioral tests were performed 5 h after a single LPS administration or 5 days after M-CSF administration (once daily for 5 consecutive days). Minocycline was administered 2 days before the start of ESI (PD 12) via the drinking water at a dose of 40 mg/kg/day that has been confirmed to effectively counteract chronic stress-induced microglial activation and behavioral changes (Hinwood et al., 2012, 2013).

### 2.4. TST and FST

The TST and FST were performed according to previous studies (Porsolt et al., 1977; Steru et al., 1985). For TST, the mice in different groups (with/without ESI and/or drug treatment) were individually suspended 50 cm above the floor for 6 min by adhesive tape placed approximately 1 cm from the tip of the tail. An investigator blinded to the study then recorded the duration of immobility during the last 4 min of suspension. Mice were considered immobile only when they hung passively and were completely motionless. Any mouse that climbed its tail was excluded from further analysis. For FST, the mice (with/without ESI and/or drug treatment) were individually placed in a clear glass cylinder (25 cm in height and 10 cm in diameter) filled to 10 cm with water at 25 ± 1 °C for 6 min. An investigator blinded to the study then recorded the duration of immobility during the animal's last 4 min in the water. Immobility time was defined as the time spent by the mouse floating in the water without struggling, making only those movements necessary to keep its head above the water.

### 2.5. SPT

The SPT was performed according to previous studies (Liu et al., 2017b; Weng et al., 2016). The mice (with/without ESI and/or drug treatment) were given the choice to drink from two bottles in