Brain, Behavior, and Immunity 53 (2016) 242–254

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

Forced swimming sabotages the morphological and synaptic maturation of newborn granule neurons and triggers a unique pro-inflammatory milieu in the hippocampus

María Llorens-Martín *, Jerónimo Jurado-Arjona, Marta Bolós, Noemí Pallas-Bazarra, Jesús Ávila *

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), c/ Nicolás Cabrera 1, 28049 Madrid, Spain
Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED, ISCIII), c/ Valderrebollo 5, Madrid, Spain

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 23 December 2015
Accepted 23 December 2015
Available online 24 December 2015

Keywords:
Adult hippocampal neurogenesis
Retrovirus
Acute stress
Forced swimming
Inflammation
Microglia

ABSTRACT

Recent experimental data suggest that mood disorders are related to inflammatory phenomena and have led to the "inflammatory hypothesis of depression". Given that the hippocampus is one of the most affected areas in these disorders, we used a model of acute stress (the Porsolt test) to evaluate the consequences of forced swimming on two crucial events related to the pathophysiology of major depression: the functional maturation of newborn granule neurons; and the hippocampal inflammatory milieu. Using PSD95:GFP-expressing retroviruses, we found that forced swimming selectively alters the dendritic morphology of newborn neurons and impairs their connectivity by reducing the number and volume of their postsynaptic densities. In addition, acute stress triggered a series of morphological changes in microglial cells, together with an increase in microglial CD68 expression, thus suggesting the functional and morphological activation of this cell population. Furthermore, we observed an intriguing change in the hippocampal inflammatory milieu in response to forced swimming. Importantly, the levels of several molecules affected by acute stress (such as Interleukin-6 and eotaxin) have been described to also be altered in patients with depression and other mood disorders.

1. Introduction

New neurons are continuously generated in the brain of vertebrates throughout adulthood. Under physiological conditions, this process occurs in two neurogenic regions, namely the subventricular zone of lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). It is widely accepted that adult hippocampal neurogenesis (AHN) is involved in learning and memory (Garthe et al., 2009). In addition, growing evidence indicates that newborn neurons are crucial elements in the regulation of mood and affective behavior. On the one hand, the discovery of the pro-neurogenic effect of antidepressants (Malberg et al., 2000), and, on the other, the observation that increasing AHN is required for the manifestation of the behavioral effects of antidepressants (Santarelli et al., 2003) elicited the 'adult neurogenesis hypothesis' of depression. However, the mechanisms bidirectionally regulating the influence of AHN on mood and *vice versa* seem to be complex and not exempt of controversy (for an example compare (Santarelli et al., 2003) and (Meshi et al., 2006)).

Major depression (MD) is a highly relevant public health issue worldwide. In the past, the monoaminergic hypothesis was the most widely accepted theory explaining depression. However, increasing evidence suggests that MD is related to distinct peripheral and central pro-inflammatory mechanisms (Abelaira et al., 2014; Ceretta et al., 2012; Dantzer et al., 2008; Hoyo-Becerra et al., 2014; Iseme et al., 2014; Menard et al., 2015; Schiepers et al., 2005). Interestingly, some patients suffering from MD show increased levels of pro-inflammatory cytokines, such as IL-1, IL-6, IFN-γ, and TNF-α (Dantzer et al., 2008; Maes et al., 2015; Schiepers et al., 2005; Tavakoli-Ardakani et al., 2015). These observations and other lines of evidence have led to the establishment of the "inflammatory hypothesis" of depression (Huang and Lin, 2015; Maes et al., 2015). One of the most important mechanisms by which stress has been proposed to induce a pro-inflammatory state in the brain is via the increase in glucocorticoid (GC) expression (Horowitz et al., 2015). GCs are known to be elevated after forced swimming and to mediate the behavioral effects of the Porsolt test (Veldhuis et al., 1985). Although GCs inhibit microglial cell activity *in vitro*, they increase microglial proliferation *in vivo*

* Corresponding authors at: Centro de Biología Molecular "Severo Ochoa", Universidad Autónoma de Madrid, c/ Nicolás Cabrera 1, 28049 Madrid, Spain.
E-mail addresses: m.llorens@csic.es (M. Llorens-Martín), javila@cbm.csic.es (J. Ávila).

http://dx.doi.org/10.1016/j.bbi.2015.12.019
0889-1591/

(Nair and Bonneau, 2006; Madrigal et al., 2003). In addition, both chronic stress and GCs prime microglial cells and increase their pro-inflammatory response to various insults (Nair and Bonneau, 2006; Tynan et al., 2010).

Microglial cells are macrophage resident cells that are rapidly activated in response to damage or infections. These cells perform multiple roles including, but are not limited to, surveillance, pathogen recognition, phagocytosis, and synapse pruning and remodeling (Kettenmann et al., 2013; Paolicelli et al., 2014). In addition, the interaction between microglial and neural precursor cells is crucial during AHN (Sierra et al., 2010) and development (Paolicelli et al., 2014).

In general terms, stress is a known potent stimulator of microglial activation and pro-inflammatory cytokine secretion (Cheng et al., 2015; Lee et al., 2015) and a negative regulator of AHN (Gould et al., 1992). Although the consequences of forced swimming on the inflammatory hippocampal milieu have been addressed previously (Hellwig et al., 2015; Sugama et al., 2007), to the best of our knowledge, the link between these changes in microglial activation and the functional maturation of newborn hippocampal neurons has not been studied so far in this model. In this regard, here we analyzed the specific features of this microglial activation and its effects on the maturation of newborn hippocampal granule neurons in a model of acute stress caused by forced swimming. We found that forced swimming drastically impaired the morphological and functional maturation of newborn neurons. Furthermore, it altered microglial activation and triggered changes in the hippocampal inflammatory milieu. Although most of the molecules whose levels appeared to be increased by forced swimming had a pro-inflammatory nature, strong chemoattractant, phagocytic, and neuroprotective components were also observed. This change in the pattern of inflammatory mediators, in turn, may orchestrate the impaired maturation of newborn neurons, a cell population that is damaged in mood and neurodegenerative disorders. These data might be relevant given that inflammation produces long-term alterations in newborn neuron and hippocampal functionality (Llorens-Martin et al., 2014). In addition, the levels of these molecules are also altered in animal models of chronic stress (Frank et al., 2014), and, most relevantly, in patients with MD (Domenici et al., 2010; Maes et al., 2015; Merendino et al., 2004; Tavakoli-Ardakani et al., 2015), thereby suggesting that the alterations in the inflammatory milieu induced by stress are related to the etiological factors behind MD development.

2. Material and methods

2.1. Animals and experimental design

Six-week-old female C57BL/6JRcc mice were obtained from Harlan Laboratories. Animals were subjected to a 2-week habituation period before the experiments began. They were housed in a specific pathogen-free colony facility in accordance with European Community Guidelines (directive 86/609/EEC) and handled following European and local animal care protocols. The detailed experimental design and the description of the experimental groups are shown in Supplementary Fig. S1. Briefly, one group of mice ($n = 3$ per experimental condition) was used to determine the plasma corticosterone levels immediately after the Porsolt test. These levels were compared with those of untreated mice in order to ensure that this manipulation truly represents acute stress for the animals (Supplementary Fig. S1 A). In addition, two independent groups of 14 mice ($n = 7$ per experimental condition) were used for biochemical, histological and molecular determinations (Supplementary Fig. S1 B). These two independent groups of animals were used to perform a Cytokine Protein Array twice. In this type of determination, all the animals belonging to the same experimental condition must be grouped and the resulting mixture is incubated on a single membrane. Hence, for the purpose of statistical comparisons, these arrays were performed on two independent groups of 7 mice. Finally, a group of 4 mice per experimental condition was used for retroviral injections (Supplementary Fig. S1 C). All animals were 4 months old when sacrificed.

2.2. Retroviral stock preparation

We used a retroviral stock (PSD95-GFP) encoding for GFP fused to PSD95 (Kelsch et al., 2008). The PSD95-GFP retrovirus allowed postsynaptic cluster visualization (green channel). Moreover, anti-GFP immunohistochemistry (red channel) allowed visualization of the whole dendritic tree (Kelsch et al., 2008). The plasmids used to produce the PSD95-GFP retrovirus were kindly provided by Prof. Carlos Lois (University of Massachusetts). Retroviral stocks were concentrated to working titers of 1×10^7–2×10^8 pfu/ml by ultracentrifugation (Zhao et al., 2006). Since the retroviruses used are engineered to be replication-incompetent, only dividing cells at the time of surgery can be infected (Kelsch et al., 2008; Zhao et al., 2006).

2.3. Stereotaxic surgery

Mice were anesthetized with Isoflourane and placed in a stereotaxic frame. Coordinates (mm) relative to bregma in the anteroposterior, mediolateral, and dorsoventral axes were as follows: dentate gyrus (DG) [−2.0, 1.4, 2.2]. 2 μl/DG of virus solution was infused at a rate of 0.2 μl/min via a glass micropipette. Animals were 8 weeks old at the time of retroviral injections.

2.4. Forced swimming (the Porsolt test)

Two months after retroviral injections, the Porsolt test was used to induce high-intensity acute stress in animals. In the case of those animals not subjected to the retroviral injections, the Porsolt test was also performed at 4 months of age. Animals were placed in a 12-cm-diameter and 29-cm-tall cylinder filled with water at 23 °C for 6 min on two consecutive days. Behavior was recorded and then assigned to categories, following Detke et al. (Detke et al., 1995): climbing, swimming, and immobility. As previously described (Llorens-Martin et al., 2007), we considered climbing as the vertical position of the mouse with repetitive movement of the limbs, the forelegs striking the glass walls, both hind legs swinging in the water at the same time. We considered swimming to be the more horizontal position of the animal, the hind legs treading water and a clear displacement of the body. To avoid considering the passive displacement of the body through inertia of previous movements as swimming, we took into consideration the movement of legs, as proposed by Gersner et al., (Gersner et al., 2005). The different behaviors were quantified during the two consecutive days of the Porsolt test and are shown in Fig. 1A. The behavioral quantification was performed on all the animals subjected to the Porsolt test ($n = 17$).

2.5. Sacrifice

Twenty-four hours after the last session of the Porsolt test, mice were fully anesthetized by means of an intraperitoneal injection of pentobarbital and transcardially perfused with saline followed by 4% paraformaldehyde in phosphate buffer. Brains were removed and post-fixed overnight in the same fixative. Animals used for biochemical and histological determinations were perfused with saline. The left hemisphere was immediately dissected and frozen,