Neurobiology of Learning and Memory 109 (2014) 62–73

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Voluntary exercise followed by chronic stress strikingly increases mature adult-born hippocampal neurons and prevents stress-induced deficits in 'what–when–where' memory

Estela Castilla-Ortega [a,*], Cristina Rosell-Valle [b], Carmen Pedraza [b], Fernando Rodríguez de Fonseca [a], Guillermo Estivill-Torrús [c], Luis J. Santín [b,*]

[a] Unidad de Gestión Clínica de Salud Mental, Hospital Regional Universitario Carlos Haya, Instituto de Investigación Biomédica de Málaga (IBIMA), Spain
[b] Departamento de Psicobiología y Metodología de las Ciencias del Comportamiento, Universidad de Málaga, and Instituto de Investigación Biomédica de Málaga (IBIMA), E-29071 Málaga, Spain
[c] Unidad de Microscopía and Unidad de Gestión Clínica de Neurociencias, Hospital Regional Universitario Carlos Haya, Instituto de Investigación Biomédica de Málaga (IBIMA), E-29009 Málaga, Spain

ARTICLE INFO

Article history:
Received 10 June 2013
Revised 22 November 2013
Accepted 3 December 2013
Available online 11 December 2013

Keywords:
Object recognition
Episodic-like memory
Adult hippocampal neurogenesis
Intermittent restraint stress
c-Fos
Functional networks

ABSTRACT

We investigated whether voluntary exercise prevents the deleterious effects of chronic stress on episodic-like memory and adult hippocampal neurogenesis. After bromodeoxyuridine (BrdU) administration, mice were assigned to receive standard housing, chronic intermittent restraint stress, voluntary exercise or a combination of both (stress starting on the seventh day of exercise). Twenty-four days later, mice were tested in a 'what–when–where' object recognition memory task. Adult hippocampal neurogenesis (proliferation, differentiation, survival and apoptosis) and c-Fos expression in the hippocampus and extra-hippocampal areas (medial prefrontal cortex, amygdala, paraventricular hypothalamic nucleus, accumbens and perirhinal cortex) were assessed after behavior. Chronic intermittent restraint stress impaired neurogenesis and the 'when' memory, while exercise promoted neurogenesis and improved the 'where' memory. The 'when' and 'where' memories correlated with c-Fos expression in CA1 and the dentate gyrus, respectively. Furthermore, analysis suggested that each treatment induced a distinct pattern of functional connectivity among the areas analyzed for c-Fos. In the animals in which stress and exercise were combined, stress notably reduced the amount of voluntary exercise performed. Nevertheless, exercise still improved memory and counteracted the stress induced-deficits in neurogenesis and behavior. Interestingly, compared with the other three treatments, the stressed exercising animals showed a larger increase in cell survival, the maturation of new neurons and apoptosis in the dentate gyrus, with a considerable increase in the number of 24-day-old BrdU + cells that differentiated into mature neurons. The interaction between exercise and stress in enhancing the number of adult-born hippocampal neurons supports a role of exercise-induced neurogenesis in stressful conditions.

1. Introduction

New cells are constantly generated in the adult hippocampus. The cells mature into neurons that are functionally integrated into the circuits of the dentate gyrus and have a potential role in behavior (Castilla-Ortega, Pedraza, Estivill-Torrus, & Santin, 2011; Deng, Aimone, & Gage, 2010; Jessberger & Kempermann, 2003). These adult-born hippocampal neurons show an enhanced susceptibility to modulation by experience (Castilla-Ortega, Pedraza et al., 2011; Deng et al., 2010), and are highly sensitive to the effects of chronic stress and voluntary exercise. Therefore, while chronic stress downregulates the proliferation, differentiation and survival of new neurons and impairs many forms of hippocampal-dependent memory, voluntary exercise has the opposite effect, potentiating both hippocampal neurogenesis and cognition (Conrad, 2010; Klaus & Amrein, 2012; Schoenfeld & Gould, 2012). Furthermore, voluntary exercise has been shown to be a critical factor in the improvement of neurogenesis and memory that occurs following the broadly used environmental enrichment protocols that combine novel stimulation with free access to a running wheel (Mustroph et al., 2012). Despite the reported ability of voluntary exercise to prevent or enable recovery from stress-induced deficits

* Corresponding authors. Address: Laboratorio de Medicina Regenerativa, Hospital Regional Universitario Carlos Haya de Málaga, Pabellón de Gobierno, sótano, Avenida Carlos Haya 82, 29010 Málaga, Spain. Fax: +34 952 614 102 (E. Castilla-Ortega). Address: Departamento de Psicobiología y Metodología de las CC, Facultad de Psicología, Universidad de Málaga, Campus de Teatinos S/N, 29071 Málaga, Spain. Fax: +34 952 134 142 (L.J. Santín).
E-mail addresses: estela.castilla@fundacionimabis.org (E. Castilla-Ortega), luis@uma.es (L.J. Santín).

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2013.12.001

in hippocampal plasticity and behavior (Head, Singh, & Bugg, 2012; Zheng et al., 2006), few studies that combine these two treatments have focused on adult hippocampal neurogenesis (Kiuchi, Lee, & Mikami, 2012; Nakajima, Ohsawa, Ohta, Ohno, & Mikami, 2010; Yau, Lau, Tong et al., 2011; Yau et al., 2012). Those studies revealed that exercise counteracts the suppressive effects of chronic stress or corticosterone administration on hippocampal cell proliferation (Kiuchi et al., 2012; Nakajima et al., 2010; Yau, Lau, Tong et al., 2011; Yau et al., 2012), but other aspects of neurogenesis such as cell survival have been less frequently assessed and may not benefit from this protective effect (Nakajima et al., 2010). On the other hand, the assessment of adult hippocampal neurogenesis under conditions of stress and voluntary exercise is of great interest because the adult-born neurons may be part of the mechanism by which exercise counteracts the deleterious effects of stress (Snyder, Soumier, Brewer, Pickel, & Cameron, 2011; Yau, Lau, & So, 2011; Yau, Lau, Tong et al., 2011).

Here, we studied the impact of chronic intermittent restraint stress and voluntary exercise, both separately and in combination, on adult hippocampal neurogenesis. In addition, hippocampal dependent memory was evaluated in the three-trial 'what–when–where' object recognition task (Www-Task) developed by Dere, Huston, and De Souza Silva (2005). This task requires mice to remember both the temporal order in which a familiar object was presented ('when' memory) and the place it was presented ('where' memory) because the integrated memory for an event, a time and a place constitutes the basis of episodic-like memory (Dere et al., 2005). The 'when' and 'where' memories are more complex than recognition memories for familiar objects and locations and involve greater hippocampal demand (Albasser, Amin, Lin, Iordanova, & Aggleton, 2012; Castilla-Ortega et al., 2012). A role for the medial prefrontal cortex (mPFC) has also been established, as the mPFC interacts with the hippocampus to solve the 'where' memory (DeVito & Eichenbaum, 2010). On the other hand, functional imaging has revealed that, compared to resting levels, performing the Www-Task increases functional activity in the hippocampus, mPFC, basolateral amygdala (BLA), paraventricular hypothalamic nucleus (PVN), accumbens (Acb) and perirhinal cortex (PRh) (Castilla-Ortega et al., 2012 and unpublished observations). In addition to their potential role in the Www-Task, these extrahippocampal areas are relevant because of their involvement in stress and reward processing (Herman et al., 2003; Tzschentke & Schmidt, 2000). Ninety minutes after the completion of the Www-Task, animals were sacrificed and perfused. The hippocampal and extrahippocampal (mFPC, BLA, PVN, Acb and PRh) c-Fos expression was then assessed as a measure of the functional activity induced by behavior (Barry & Commins, 2011). Moreover, following each experimental treatment, c-Fos expression in the newly born cells was examined to explore their potential involvement in the behavioral task.

2. Experimental procedures

2.1. Animals

Experiments were performed on 3-month-old male mice with a hybrid C57BL/6J×129X1/SvJ background. Six mice were used for each experimental condition, excluding the control group, which included 10 animals for behavioral analysis. Six of these controls animals were then randomly selected for subsequent histology. In all cases, mice were maintained on a 12-h light/dark cycle (lights on at 08:00 a.m), with water and food provided ad libitum. All procedures were performed in accordance with European animal research laws (European Communities Council Directives 86/609/EEC, 98/81/CEE and 2003/65/CE; Commission Recommendation 2007/526/EC) and the Spanish National Guidelines for Animal Experimentation and the Use of Genetically Modified Organisms (Real Decreto 1205/2005 and 178/2004; Ley 32/2007 and 9/2003).

Please note that, as it is reported through the manuscript, the C57BL/6J×129X1/SvJ mice seem to differ from frequently used mouse strains in both their adult hippocampal neurogenesis and cognitive ability (please see the last paragraph of the Discussion for a summary), so some caution is needed to directly extrapolate our findings to mice with a different genetic background.

2.2. BrdU administration

BrdU administration was performed on the first day of the experiment. Mice received four doses of 75 mg/kg BrdU (Sigma, St. Louis, USA) dissolved in saline, which were administered intraperitoneally at 2-h intervals.

2.3. Environmental treatments

The day after BrdU administration, mice were assigned to one of four experimental treatments: standard housing (Control), chronic intermittent and uncontrollable restraint stress (Stress), voluntary exercise (EX) or chronic intermittent restraint stress combined with voluntary exercise (Stress + EX) (Fig. 1A).

The Control condition consisted of individually housing mice in standard cages (11 × 30 cm and 13 cm high) provided with nesting material. The Stress mice also received standard housing conditions, but, beginning 7 days after the administration of BrdU, they were restrained for 3.5 h per day (10:00–13:30 a.m.), excluding the weekends, in 50 ml clear polystyrene conical centrifuge tubes modified with air holes for ventilation. For EX mice, the exercise treatment began 1 day after the BrdU injections, and mice were individually housed in exercise cages consisting of two-floor cages (20 × 26 cm and 27 cm high) provided with nesting material, a ladder and a running wheel equipped with a magnetic counter (Dayang Pet Products, Foshan City, China). For the Stress + EX group, both the stress and exercise treatments were applied, so mice were housed in the exercise cages and, after 6 days of running-wheel adaptation, were exposed to chronic intermittent restraint (Fig. 1A). This experimental desing emphasizes the use of exercise as a 'preventive' approach (Wright & Conrad, 2008), because exercise is allowed to exert its effects for 6 days prior to the onset of chronic stress. Because 6 days seem enough for exercise to induce plastic changes within the hippocampus (Adlard, Perreau, & Cotman, 2005; Fabel et al., 2003), exercise may establish a protective environment that could counteract the deleterious consequences of the subsequently applied stressor.

As a measure of voluntary exercise, wheel running was monitored daily in both the EX and Stress + EX groups. The distance run (number of rotations multiplied by the wheel perimeter) was averaged every three days for analysis across the exercise protocol. In addition, the total distance run was calculated as the mean distance run per day.

2.4. Behavioral analysis in the 'what–when–where' object recognition task

On day 24, all mice were tested using the behavioral protocol depicted in Fig. 1B. Mice received 5 min of habituation to an open-field arena (40 × 40 cm). After 60 min, four copies of an object were positioned in the apparatus in a triangular shape and mice allowed to explore for 10 min (Sample 1). Ninety minutes later, four copies of a new object were placed in the corners and the mice allowed to explore for 10 min (Sample 2). The Test Trial began 90 min later and lasted another 10 min. Two objects from the second sample (recent objects) were placed in their previous