Behavioural Brain Research 317 (2017) 286–291

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Neural oscillations as a bridge between glutamatergic system and emotional behaviors in simulated microgravity-induced mice

Xueliang Shang [1], Bo Xu [1], Qun Li [1], Baohui Zhai, Xiaxia Xu, Tao Zhang *

College of Life Sciences, Key Laboratory of Bioactive Materials Ministry of Education and State Key Laboratory of Medicinal Chemical Biology, Nankai University, 300071 Tianjin, PR China

HIGHLIGHTS

- Mice were exposed to hindlimb unloading for 14 days.
- Behavior test, neural oscillatory analysis, Western and HPLC were performed.
- Microgravity mice show anxiety-like behaviors, NR2A/2B and glutamate levels decrease.
- There were significant differences of oscillatory patterns between two groups.
- Neural oscillations as a bridge between glutamatergic system and emotional behaviors.

ARTICLE INFO

Article history:
Received 10 August 2016
Received in revised form
23 September 2016
Accepted 27 September 2016
Available online 28 September 2016

Keywords:
Simulated microgravity
Neural oscillations
Emotional behaviors
NR2A/2B
Glutamate

ABSTRACT

This study aims to investigate if neural oscillations can play a role as a bridge between the alteration of glutamatergic system and emotional behaviors in simulated microgravity (SM) mice. Adult male C57BL/6J mice were randomly divided into two groups: SM and control groups. The animal model was established by hindlimb unloading (HU). The mice were exposed to HU continued for 14 days. Weight and sucrose consumption were measured. The degree of anxious and depressive was evaluated by Open field test and Elevated plus maze test. Local field potentials were recorded in the hippocampal perforant path (PP) and dentate gyrus (DG) regions. The NMDAR2A/2B (NR2A/2B) subunits expression and glutamate level were measured by Western and high performance liquid chromatography (HPLC), respectively. After 14 days, SM mice exhibited depressive-like and anxiety-like behaviors, while the expression of NR2A/2B subunits and the glutamate level were significantly decreased in the SM group. Moreover, the power distribution of theta (3–8 Hz) was decreased by HU, which further significantly attenuated the identical-frequency strength of phase synchronization and the neural information flow at theta rhythm on the PP-DG pathway. The theta-gamma phase synchronization strength was also significantly reduced by HU. The data imply that the neural oscillations measurements is a sign of the emotional behaviors impairment and the glutamatergic system change induced by HU.

1. Introduction

Neural oscillations are generated by interaction and influence of neurons with each other through excitatory and inhibitory synaptic connections. As known, the classification for oscillations is usually identified with different frequencies, delta 1–3 Hz, theta 3–8 Hz, alpha 8–13 Hz, beta 13–30 Hz and gamma 30–100 Hz at the same time in the same brain regions [1]. Among the five rhythms, theta and gamma rhythms are deemed to be most related to cognitive functions [2,3]. Furthermore, it is well known that there is a very close relationship between *N*-methyl-D-aspartic acid receptors (NMDARs) and neural activity rhythms [4]. It was reported that blockade of NMDAR by ketamine, as a non-selective NMDAR antagonist, could alter the cross-frequency coupling (CFC) between theta and gamma rhythms in the hippocampus [5]. In addition, the blockade of NMDAR by NR2A-preferring antagonist (PEAQX and NVP-AAM077) and NR2B-selective (ifenprodil and Ro25-6985) effectively diminished the low-frequency modulation of gamma oscillations [6]. However, the role of neural oscillations, in which cognitive deficits are induced by glutamatergic system in hindlimb unloading (HU)-induced rodents, is yet to be illuminated.

* Corresponding author.
E-mail address: zhangtao@nankai.edu.cn (T. Zhang).
[1] Xueliang Shang, Bo Xu and Qun Li have contributed equally to this work.

http://dx.doi.org/10.1016/j.bbr.2016.09.063
0166-4328/

Numerous studies have reported that there is a significant influence of microgravity on osteo-muscular, immune and cardiovascular systems [7,8]. Moreover, neurotransmitters related with cognitive functioning are also affected by microgravity [9]. It suggests that microgravity disturbs cognitive processes, learning capacity and neurotransmitters, possibly as a consequence of the gross physiological, hormonal and cardiovascular changes. For example, the cardiovascular changes, induced by microgravity, may inhibit the ascending reticular formation activation and cortical frontal functioning and this in turn may impair several neural networks, and perceptual, motor and cognitive processes [10]. Indeed, microgravity ground simulation studies provided some evidences to the hypothesis of a microgravity-induced cognitive impairment. In addition, a previous study showed that two genes (Grin1 and Itga3), involved in learning and memory, were significantly altered in the brains of 2-week HU mice using cDNA microarrays [11]. Another investigation with human subjects showed that cortical activities were also affected apart from the known physiological changes induced by simulated microgravity [12]. This general physiological pattern may unmask the risk of astronauts to develop several specific cognitive deficits in space.

In the study, a hypothesis has been raised that neural synchronization as the critical "middle ground" connecting glutamatergic system with cognitive behaviors may play a role of bridge between molecular biology and behavior. Accordingly, a HU mouse-model was established and the alterations of cognitive behaviors were examined. The functional role of neural oscillations was investigated by measuring not only theta and gamma identical-frequency synchronization but also theta-gamma cross-frequency couplings. In order to identify a potential mechanism, the protein expression of NR2A/2B subunits and the level of glutamate were determined.

2. Materials and methods

Twelve male C57BL/6 mice (25–30 g body weight) were purchased from the Laboratory Animal Center of Academy of Military Medical Science of People's Liberation Army, and reared in standard rodent cages in the animal house of Medical School, Nankai University, under the condition of a constant temperature of 25 °C (±2 °C) and a 12 h light/dark cycle (lights on at 7 a.m.). Food and water were freely available during all phases of the experiment, with the exception of model establishing and sucrose consumption phases. After 4 days' habituation to the environment, the animals were randomly divided into two groups, which are control group (CON, n = 6) and simulated microgravity group (SM, n = 6). Each animal was housed separately in a cage [13]. Every effort was made to minimize the number of animals used and their suffering. All experiments were carried out according to the protocol approved by the Ethical Commission at Nankai University and in accordance with the practices outlined in the NIH Guide for the Care and Use of Laboratory Animals. The HU procedure was conducted for 14 days, according to the modification method of Emily R. and John M. Lawler [13,14]. Sham mice were treated similarly except for elevation of the hind limbs. Body weight measurement and sucrose preference test (SPT) were performed. All behavioral experiments were carried out on the day after the last SPT. Each test were performed in the dark room of the light cycle. Afterward, the signals of local field potentials at both the hippocampal perforant path (PP) and dentate gyrus (DG) areas were recorded for off-line analysis. The following approaches provide the neural oscillations assessment at theta frequency band (3–8 Hz) and gamma frequency band (30–90 Hz), which was further divided into low gamma (LG, 30–50 Hz) and high gamma (HG, 50–90 Hz) respectively. The approaches were included in the analysis, such as the power spectrum measurement, phase locking value, evolution map approach, and cross-frequency coupling. In addition, Western blotting and High performance liquid chromatography (HPLC) were performed to measure the NR2A/2B subunits expression and glutamate level.

All the details were provided in the Supplementary materials.

3. Results

3.1. Body weight measuring and sucrose preference test (SPT)

There was a significant difference of the body weight gain ($t_{(10)} = 5.239$, $p < 0.001$, Table 1) between the CON group and the SM group after the HU procedure.

Table 1 further showed the effect of HU on the sucrose consumption percentage. It was found that there was no statistical difference of groups before HU procedure ($t_{(10)} = 0.189$, $p = 0.854$). However, A student *t*-test showed that the sucrose consumption was significantly lower in the SM group than that in the CON group after HU procedure ($t_{(10)} = 2.235$, $p < 0.05$).

3.2. HU impacted the locomotor activity and increased the degree of anxiety in SM mice

There was a significant effect on locomotor activity and anxiety induced by HU procedure in OFT and EPM (Fig. 1). A student *t*-test showed that there were significant differences of the total distance ($t_{(10)} = 2.769$, $p < 0.05$, Fig. 1a), the central area entries ($t_{(10)} = 2.535$, $p < 0.05$, Fig. 1b), and the open arms entries ($t_{(10)} = 2.480$, $p < 0.05$, Fig. 1c) between the CON group and the SM group.

3.3. The protein expression of NR2A/2B subunits and the level of glutamate were significantly reduced by HU

Two types of proteins that were related to synaptic plasticity, bands at about 163 kDa and 180 kDa were detected by NR2A and NR2 B antibodies respectively (Fig. 1d). Student *t*-test showed that the protein levels of NR2A and NR2B were statistically decreased in the SM group compared to that in the CON group (NR2A: $t_{(6)} = 6.964$, $p < 0.001$, Fig. 1e; NR2B: $t_{(4)} = 6.214$, $p < 0.01$, Fig. 1f). In addition, the level of glutamate was also measured in the hippocampus. The data showed that the level of glutamate was significantly down-regulated in the SM group than CON group ($t_{(4)} = 5.216$, $p < 0.01$, Fig. 1g).

3.4. Neurodynamical analysis

3.4.1. Power spectral analysis in both PP and DG regions

In order to figure out how the brain rhythms in LFPs was impacted by HU, the power spectra were measured before and after HU treatment. The alteration of power spectra distribution could be detected after HU (Fig. 2), which induced a visible decrease at theta frequency bands in the PP (Fig. 2a and c) and DG (Fig. 2b and d) regions, and an observable increase at HG frequency bands in the PP (Fig. 2a and c) and DG (Fig. 2b and d) regions. It was found that the percentage power in the theta frequency range was significantly decreased in the SM group compared to that in the CON group in both the PP region ($t_{(9)} = 2.882$, $p < 0.05$, Fig. 2e) and the DG region ($t_{(9)} = 2.531$, $p < 0.05$, Fig. 2f). However, there were no statistical differences of percentage power distribution in the both low and high gamma frequency ranges between the CON group and the SM group in either the PP area or the DG area (Fig. 2e and f)

3.4.2. The radial distance values (r) of circular distribution was reduced by HU

In order to quantitatively analyze the cross frequency theta-gamma phase coupling, the radial distance values (r) of circular distribution from the difference between m*theta phase and