Environmental enrichment condition does not alter glutamine synthetase activity in the hippocampus and cerebral cortex of Swiss albino mice

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Abstract

Environmental enrichment (EE) promotes morphological changes in astrocytes and gliogenesis, as well as an increment in the synapses number of hippocampus. In addition, exposure to EE is known to increase the astrocytic number and volume in cortical regions. The glutamine synthetase (GS) is an astrocyte enzyme that converts glutamate into glutamine, which play an important role in the glutamate/glutamine cycle. Here, we hypothesized that the GS activity would be increased in the cortex and hippocampus of mice living in EE. For this, mice were assigned randomly in two housing conditions: standard condition (SC) or enriched environment (EE) and the GS activity was evaluated after 9 weeks. Our results showed that EE did not promote any significant change in GS activity in both regions tested. Future studies must focus on the temporal analyses of GS activity after EE conditions as well in other species and/or strains and during distinct age of life in order to understand the factors affecting the GS activity after exposition to EE conditions.

Introduction

Environmental enrichment (EE) is a form of manipulation in which captive animals are exposed to complex conditions through alterations in the physical and social environment [1]. EE manipulation occurs by the introduction of objects (e.g. running wheels, shelters, etc.) with different textures, colors, shapes, sizes, and also by changing their places in the home cage, which lead to enhanced of sensory, cognitive and physical stimulation [2]. It has been demonstrated that EE improve the cognitive capacity of rats [3], mice [4], horses [5] and it also promotes a reduction in the risk of dementia in human [6].

Exposition to EE is known to promote an improvement in the neuroplasticity of several brain areas [1], as well as stimulation of hippocampal response such as morphological changes in both neurons [7,8] and astrocytes [4,9]. In addition, EE is known to improve the neurogenesis [10], gliogenesis [11] and synaptogenesis [12]. Likewise, increase in volume and number of astrocytes have also been reported after EE in cortical regions [13,14]. In the visual cortex, the contact between astrocytic processes and synapses increases after EE conditions [15].

Additionally, it has been demonstrated that mice exposed to short periods under EE conditions promote improvement in the glutamatergic pathways in the cortex. The expression level of the postsynaptic density-95 protein (PSD95), the anchoring of the N-methyl-D-aspartate glutamate receptor (NMDA) in postsynaptic membrane, and calmodulin, the downstream of NMDA receptor, are up-regulated after EE [16]. Furthermore, mice subject to 40 days of EE present an increase in the levels of GluR2 and GluR4 (AMPA subunits) in the hippocampus [17]. In this way, a short period of EE promote improvement in the PSD95, Ras and reelin gene expression in hippocampus of NMDAR1-knockout (KO) and suggest that these signal pathways are possibly involving in the beneficial effects of EE on memory improvement in this KO mice [18]. Accordingly, young Wistar rats exposed to EE for three months showed higher hippocampal glutamate and GABA concentrations [19].

Glutamate uptake in astrocytes is currently known to be performed by sodium-dependent glutamatergic transporters, GLT-1 and GLAST. Once inside astrocytes, glutamate may be converted to glutamine in the presence of ammonia by the activity of glutamine synthetase (GS). Glutamine is then transferred to neurons where it can be converted back to glutamate by phosphate-activated glutaminase. It is well documented that GS activity plays a crucial role to maintain glutamate release during increased activity of glutamatergic synapses [20]. Thus, astrocytes play an important role in glutamatergic synapses, including uptake and turnover of glutamate. Considering it, in the present study we tested the hypothesis that GS activity improved hippocampus and cortex of animals living under EE conditions.
Material and methods

Animals and approval

Male albino Swiss mice (21 days old) were obtained from Central Animal Facility of Universidade Federal de Santa Catarina. Mice were kept in experimental cages under two different conditions with water and food ad libitum, controlled light/dark photoperiod cycle (12:12 h; lights on at 7:00 a.m.) and room temperature adjusted to 21 ± 1°C. All experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and Brazilian Society for Neuroscience and Behavior (SBNNeC). Recommendations for Animal Care and approved by the Ethical Committee of the Universidade Federal de Santa Catarina (UFSC). All efforts were made in order to minimize the number of animals used and their suffering.

Experimental procedure

Groups composed by 20 albino Swiss mice were randomly exposed in two housing conditions: standard condition (SC; control group) or enriched environment (EE; treatment group). Groups under SC were housing in apparatus of Plexiglas box (38 cm x 32 cm x 16 cm) containing just sawdust. EE groups were kept in Plexiglas box (38 cm x 32 cm x 16 cm) connected to a three-story metal cage (28 cm x 21 cm x 50 cm) containing sawdust, two running wheels and a variety of objects including wood and plastic objects, nesting material and hiding places, such as tunnels in order to represent eco-ethological expansions for mice including the sense of security and to provide a place where they could avoid open spaces and luminosity, a natural behavior of wild mice. Additional cognitive stimulation regarding the formation of spatial mapping was provided by changing the objects or shifting their positions twice a week in the EE treatment. In both, SC and EE, treatments animals were kept for 63 days (9 weeks).

Glutamine synthetase activity

Activity of GS enzyme was measured as adapted from Shapiro and Stadtman [21,22] and Stadtman et al. [23] and modified by Vandresen-Filho et al. [24]. Two groups containing five mice each were killed by decapitation. Hippocampi and cerebral cortex were rapidly removed and homogenized in imidazole-HCl buffer (80 mM, pH 7.0). The assay mixture contained 80 mM imidazole-HCl buffer, 30 mM glutamine, 3 mM MnCl2, 30 mM hydroxylamine-HCl, 20 mM sodium arsenate, 0.4 mM, ADP and 50 μl of the tissue homogenate. The reaction stopped after 30 min at 37°C by adding 100 μl of a mixture containing 4/1/0.5/6.5 (v/v/v%) of 10% (w/v) ferric chloride, 24% (w/v) trichloroacetic acid, 6 M HCl and water. The reaction product, γ-glutamylhydroxamate, was measured at 540 nm and converted to the amount of product formed by comparison with a standard curve of γ-glutamylhydroxamate ranging from 100 to 1000 μM. The activity of GS enzyme was expressed in unit(s) per milligram of protein in the sample per minute.

Statistical analyses

Comparison of GS activity on hippocampus and cortex among groups (SC vs. EE) were performed using an unpaired two-tailed Student’s t test, with p<0.05 as indicative of statistical significance. All data were expressed as means ± standard error.

Results

The GS activity measured in the hippocampal homogenates between SC and EE groups do not present significant differences (t8=1.281, p=0.2361; Figure 1A). In the same way, the GS activity measured in the cortical homogenates between SC and EE groups do not present significant differences (t8=1.842, p=0.1028; Figure 1B).

Discussion

Considering the pivotal role of glutamatergic transmission in neural plasticity and the improvement in dendritic complexity [7], synaptogenesis [12], complexity of astrocyte morphology [9] and gliogenesis [11], we would expect that GS activity might be improved in hippocampus of mice exposed to EE conditions when compared with mice living in control conditions. Furthermore, taking into account the increment in the volume and number of astrocytes in cortical regions after EE [13,14] and the contact between astrocytic processes and synapses increment after exposition to EE conditions [15], an improvement of GS activity in cortex regions of mice exposed to EE conditions when compared with mice living in control conditions was also expected. However, in the present study, our results showed that albino Swiss mice maintained under EE conditions for 9 weeks not presented alteration in the GS activity in both hippocampus and cortex regions. Such results do not allow us to accept the hypothesis proposed in the present study.

Previous studies have been showed that EE housing promotes alterations in the morphology of neurons [7], synaptic elements [12]...
and also astrocytes [9]. Additionally, synaptic elements (e.g. PSD95, synaptophycin, calmodulin) and neurotrophic factors in the regions were improved in animals exposed to EE conditions [16]. Despite such findings, several divergences results can be found in the literature about the effect of animals living under EE conditions; BDNF levels in the hippocampus, for example, have been shown to be increased after EE exposure, but also no changes have been reported [3]. These different effects of EE conditions can be generated by intrinsic responses of animal species or strain used in the experiments as well their age and also by the time to perform the analysis during the protocol [25].

An improvement of gliogenesis in the dentate gyrus in old Swiss albino mice was reported [11]. Additionally, morphological changes in astrocytes of the Stratum Radiatum of CA1 in the hippocampus was observed in C57 mice [9]. However, Swiss albino mice present different responses to EE conditions when compared with other strains or species: no changes in the neurogenesis [26] while there is an increase in the neurogenesis in dentate gyrus in BALB/cByJ mice after EE housing [27]. The hypo-responsiveness of Swiss albino mice to EE can be one possible explanation for no changes in the GS activity observed in the present study. Further analysis of GS activity in other strains or species is still necessary in order to elucidate such inconsistent results.

Besides above explanations, GS activity also present divergences responses. Wistar rats exposed to forced exercise for 4 weeks showed an increase activity of GS in the hippocampus [28], however, no changes were also observed with the same specie/strain and with the same protocol of forced exercise but for a longer period (12 weeks) [29]. Likewise, Mora-Gallegos et al. [19] showed that young Wistar rats exposed to EE present improve in the glutamate and GABA concentration in hippocampus, however, adult rats of the same strain exposure to the same EE protocol, does not change the concentration of glutamate and GABA. It this view is known that the neuronal-astrocyte unit is affected by age in the hippocampus [30]. Here, no changes were observed in the GS activity in the hippocampus of albino Swiss mice under EE conditions after 9 weeks, which can be generated by a transitory effect in the GS activity.

Another suitable explanation behind our results may involve the differential response to corticosterone between GS and glial fibrillary acidic protein (GFAP) [31]. Mc Quaid et al. [32] observed an increase in the aggressive behaviour and higher plasma corticosterone in rats exposed to forced exercise for 4 weeks showed an increase activity of GS in the hippocampus [28], however, no changes were observed with the same specie/strain and with the same protocol of forced exercise but for a longer period (12 weeks) [29]. Likewise, Mora-Gallegos et al. [19] showed that young Wistar rats exposed to EE present improve in the glutamate and GABA concentration in hippocampus, however, adult rats of the same strain exposure to the same EE protocol, does not change the concentration of glutamate and GABA. It this view is known that the neuronal-astrocyte unit is affected by age in the hippocampus [30]. Here, no changes were observed in the GS activity in the hippocampus of albino Swiss mice under EE conditions after 9 weeks, which can be generated by a transitory effect in the GS activity.

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Both regions study here (cortex and hippocampus) are rich in receptor and EE condition have been reported to increase the expression levels of PSD-95 and calmodulin in the cortex [16], spine density [12] and also an improvement in the PSD95, Ras and reelin gene expression in hippocampus. Thus, could be that the improvement of synapses [12] and the activation of NMDA receptor [16], both reported after EE exposure, modulating negatively the GS activity.

In summary, our results showed no alteration of GS activity in the hippocampus and cerebral cortex of young male albino Swiss mice exposed to EE conditions after 9 weeks. Future studies must focus on the temporal analyses of GS activity after EE conditions as well in other species and/or strains and during distinct age of life. Such new analyses may provide a better understanding of the participation of astrocytes in the neuroplasticity promoted by environmental changes.

**Highlights**

- EE does not alter Glutamine Synthetase activity in hippocampus of Swiss albino mice;
- EE does not alter Glutamine Synthetase activity in cortex of Swiss albino mice;
- Swiss albino mice are hyporesponsive to EE;
- Future studies must focus on the temporal analyses of GS activity after EE conditions.

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