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Influence of environmental enrichment on an object recognition task in CF1 mice

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ABSTRACT

Environmental enrichment (EE) is an experimental model for studying neuroplasticity. EE is used to investigate behavioral modifications associated with gene–environmental interaction. The object recognition task (ORT) evaluates animals' ability to learn about their environment, which depends on their innate instinct. By using young CF1 mice, the present study evaluated the effect of 8 weeks of EE on the ORT. Our results indicate that EE decreased the time the animals spent exploring familiar and unfamiliar objects and total time spent exploring both objects, without affecting the capacity of discrimination of objects. These findings indicate a more propitious behavior for species survival in animals subjected to EE, including rapid exploration and learning about the environment.

1. Introduction

Domesticated animals, such as rats (*Rattus norvegicus*) and cats (*Felis catus*), present brains 8–33% smaller than their wild congeners (when corrected for body size), with the greatest reduction usually seen in the forebrain [1]. These findings have been attributed to genetic changes resulting from artificial selection for traits such as docility. Conversely, animals maintained in enriched environments tend to have larger brain structures, increased neurogenesis, higher learning ability and less stereotyped behaviors than those developed in standard conditions [1].

Environmental enrichment (EE), an experimental model that allows the study of neuroplasticity, increases physical activity, learning experiences, visual inputs and social interactions [2,3]. EE promotes neuroplasticity in the hippocampus and cerebral cortex due to an increase in the levels of neurotrophins [4,5], changes in cell proliferation [6,7], changes in astrocyte shape [8], and increase in dendritic branching and synaptogenesis [9,10]. EE also causes chromatin remodeling and histone acetylation, which regulates DNA activity and therefore the protein synthesis [11,12].

In the natural world, the juvenile stage is preparatory for the next stages of life: dispersal, habitat selection, settlement and residency in a new habitat. Prior to leaving their birth habitat, the juveniles need to be equipped with physiological, morphological, and behavioral tools [13]. The wild and laboratory animals need to learn about their environment in order to enlarge and select the behavioral repertory, which is specie-specific. The literature does not present a consensus about the cognitive and behavioral changes promoted by EE in distinct animals and strains in diverse behavioral tasks [14,15]. Therefore, the ethological perspective is important for selection of behavioral tasks used for evaluating alterations occasioned by EE [16].

The object recognition task (ORT) has been widely used to evaluate the effects of pharmacological and genetic interventions on memory processes [17–19]. This task deals with the natural motivation of the animals to explore novelty (as new/unfamiliar objects), an innate instinct that drives animals to learn about their environment (discrimination ratio). Additionally this task present the adaptation session, this session is important for habituating the animals of the apparatus, especially in mice for attenuating the important factor of eco-ethological behavior of their, i.e. present thigmotaxis [16]. In the natural world, mice need to display rapid exploration and knowledge on the environment. This behavior is important for species preservation, such as mating or eating, and minimizing associated risks, such as exposure to predators or rivals [20]; this ethological perspective is evaluated by the ORT paradigm. The hippocampus seems to present a central role in this task, for processes involved both in memory and in environmental interactions [21–23].

Abbreviations: BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; EE, environmental enrichment; TrkB, tyrosine kinase receptor.

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The hippocampus exerts a vital role in learning and memory processes [24,25]. EE can modify synaptic physiology in hippocampal neurons and influence hippocampal neurogenesis [26]. Spatial and nonspatial memories present deficits in animals following hippocampal lesions [27], but exposure of animals to EE induces spontaneous recovery after these lesions [28].

Enriched housing stimulates the production of specific neurotrophic factors that promote cell proliferation and/or survival of newborn hippocampal neurons [5]. One of the candidates mediating the effects of EE on hippocampal neurogenesis is BDNF (acting via TrkB receptors) [5]; however, there is no consensus whether BDNF and TrkB hippocampal levels are influenced by EE. In fact, several articles show an increase in hippocampal BDNF levels in animals exposed to EE [4,5,29] whereas other studies show no changes in the BDNF and TrkB levels in the hippocampus [30–33]. It should be noted however that these studies differed in the protocol employed, including the time of exposure to EE.

Our study evaluated changes in behavioral parameters and in BDNF and TrkB immunocontent in CF1 mice submitted to 8 weeks of EE. The first goal of this study was to evaluate the influence of EE on the behavioral response in the ORT, and the second goal was to associate the behavioral findings to hippocampal BDNF and TrkB immunocontent.

2. Material and methods

2.1. Animals

Male albino CF1 mice were obtained from State Foundation for Health Science Research (FEPPS, Porto Alegre, RS, Brasil). All experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and Brazilian Society for Neuroscience and Behavior (SBNeC) Recommendations for Animal Care and approved by the ethical committee from Universidade Federal do Rio Grande do Sul. All efforts were made to minimize the number of animals and their suffering.

2.2. Housing conditions

Animals ($n=60$) were weaned at 21 days and assigned randomly to standard or enriched housing immediately after weaning for 60 days. All animals were kept in a temperature-controlled colony room with food and water available *ad libitum*, and maintained on a 12-h light/dark cycle (light on at 7:00 A.M.). Standard housing consisted of a 27 cm × 16 cm × 12 cm acrylic box with sawdust containing groups of 5 mice. Enriched housing consisted of a 38 cm × 32 cm × 16 cm acrylic box connected to a 28 cm × 21 cm × 50 cm three-story metal cage with sawdust, housing 10 mice at a time. The enrichment housing apparatus contained two running wheels and a variety of objects, including wood and plastic objects, tunnels, hiding places and nesting material, where the mice were kept out of luminosity, the natural behavior of wild mice. The EE model presented the possibility of changing in the objects and/or their positions in the enriched housing, which might provide additional cognitive stimulation regarding the formation of spatial map [34,35].

2.3. Object recognition task

The ORT was performed in an apparatus consisting of a painted wood small chamber: 25 cm × 25 cm × 40 cm. Before the experimental sessions, the animals were habituated to the experimental room for 60 min in dim light conditions. A light bulb was switched on during the experimental sessions. The light intensity was equal in the different parts of the apparatus. The objects were placed equidistant from two corners, 12 cm apart from the wall. Mice were placed individually into the chamber. In the adaptation sessions, the mice explored the apparatus during 10 min, with no object. In training sessions, performed 24 h later, 2 similar objects were utilized, not familiar to the mice. In test sessions, performed 90 min later, the two objects, familiar and novel, were presented. The objects employed were two glass bottles presenting the same texture and size, but with different shapes and colors (white and amber). The objects were not known to have ethological significance for the mice. Discrimination ratio was expressed by the ratio TN/(TN + TF), (TN time spent exploring the novel object; TF time spent exploring familiar object), both in the training and test sessions. Between the sessions the objects were cleaned with 70% ethanol solution. Exploration was defined by directing the nose to the object at a distance less than 2 cm and/or touching the object with the nose or forepaws. The time of exploration was measured by 3 blinded observers, with the use of chronometers. Animals that explored the objects less than 3 s in a session were excluded from the study (according to [19]).

2.4. Immunoblotting

After the behavioral experiments, mice were killed by cervical displacement; the whole hippocampus was dissected out and immediately homogenized in 5% SDS with protease inhibitors cocktail. The protein content was determined by using Bicinchoninic acid assay and bovine serum albumin (BSA) as standard. Hippocampal homogenates (80 µg protein/ sample) were separated in SDS-PAGE (12%) and transferred to nitrocellulose membranes. Membranes were blocked with 5% BSA for 2 h. After blocking, membranes were incubated for 24 h at 4 °C with rabbit anti-TrkB antibody (1:1000), mouse anti-BDNF antibody (1:500) or mouse anti-actin antibody (1:1000) overnight, and followed by incubation with secondary antibodies anti-rabbit (1:3000) and anti-mouse (1:2000) for 2 h at room temperature and developed with ECL kit. The densitometric analyses were performed using public domain NIH Image Program (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). As an additional control of the protein loading, membranes were stained with the Ponceau S stain.

2.5. Statistical evaluation

For the behavioral parameters, the statistical differences were analyzed using parametric analysis (two-way ANOVA) followed by Bonferroni post hoc test. For immunoblotting parameters, the multiple comparisons between groups were analyzed by using the parametric analysis of the *t* unpaired test. Statistically significant differences were considered when $p<0.05$.

3. Results

During the study, 3 animals of control group and 8 animals of EE group were excluded because they explored the objects less than 3 s in a session (see Material and methods).

The time spent in the exploration of both objects decreased in the test session compared to training session in the control group ($n=27$) [89.5 ± 24.9 s and 54.5 ± 21.5; $F(1,94)=44.84$ $p<0.001$] and in the EE group ($n=22$) [30.5 ± 17.3 s and 13.5 ± 8.0, $F(1,94)=44.84$ $p<0.001$]; these findings indicate habituation to the ORT in both groups. Control group spent more time exploring both objects compared to EE group in training and test sessions [$F(1,94)=165.4$ $p<0.001$], which could indicate that EE decreased the levels of curiosity and interest for the objects (both objects were not ethologically relevant and were unfamiliar to animals). In fact the EE group presented more rapid exploration and equal capacity of learning about the environment, which is a more propitious behaviors for species survival. Interaction between housing conditions (EE or control) and session type (training or test) presented $F(1,94)=5.321$, $p=0.0232$ (Fig. 1).

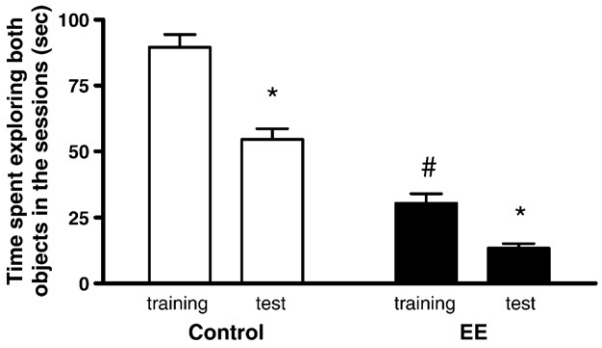

Fig. 1. Total time (in seconds) recorded for the objects exploration in the training and test sessions, for control and EE groups of mice (n 27–22 animals in each group). Results are presented as means ± S.E.M. of the seconds spent in both objects during 10 min. The test session was performed 90 min after the training session. $^{*}p \leq 0.01$ indicates significant difference for the time spent in both objects between training and test sessions. $\#p \leq 0.001$ indicates significant difference for the time spent in both objects between control and EE groups. Two-way ANOVA followed by Bonferroni post hoc test.

The discrimination ratio was similar in control and EE groups and increased in test sessions compared to training sessions [control: 0.51 ± 0.04 vs. 0.63 ± 0.07; $F(1,94) = 50.45$ $p < 0.001$; EE: 0.50 ± 0.09 vs. 0.60 ± 0.1; $F(1,94) = 50.45$ $p < 0{,}001$] (Fig. 2), indicating that the animals learned about the environment.

The time spent exploring the familiar object was shorter than the time spent exploring the unfamiliar object in both groups ($F(1,94) = 18.33$, $p < 0.001$), indicating a capacity of object discrimination in control [20.48 ± 9.46 and 34.04 ± 13.63 $F(1,94) = 18.33$ $p < 0.001$], and EE [5.53 ± 3.78 and 7.55 ± 4.36 $(1,94) = 18.33$ $p < 0.001$] groups. However, the time spent in the exploration of either familiar or unfamiliar objects by the control group was higher than by the EE group, both in the unfamiliar and familiar objects [$F(1,94) = 129.7$ $p < 0.001$] (Fig. 3). This latter result could indicate that EE in fact decreased the levels of curiosity and interest for the objects and/or that the EE group habituates faster than control group; at any event, a lower time required for exploration of objects is a more propitious behavior to survival of mice. Interaction between housing conditions (EE or control) and object exploration time (familiar or unfamiliar) presented a statistical significance ($F(1,94) = 10.05$, $p = 0.002$; see Fig. 3).

The BDNF and TrkB immunocontent in the whole hippocampus presented no statistical difference between groups [BDNF: $F(5, 4) = 1.033$, $p = 0.73$]; [TrkB: $F(4, 5) = 1.912$ $p = 0.94$].

4. Discussion

In this study, we showed that EE strongly decreased the time that the mice spent exploring the objects, both in training and test sessions,

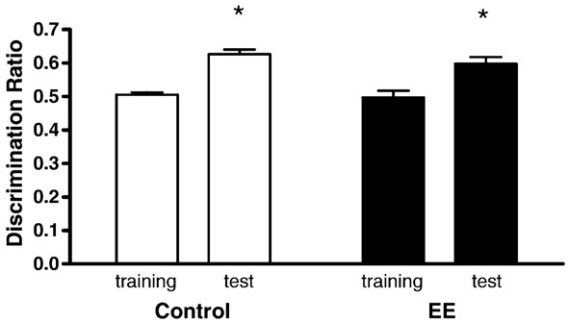

Fig. 2. Discrimination ratio for the objects in the training and test sessions for control and EE groups of mice: ($n = 27$–22 animals in each group). Results are presented as means ± S.E.M. of the discrimination ratio. The test session was performed 90 min after the training session. $^{*}p \leq 0.001$ indicates difference from the discrimination ratio between training and test session. Two-way ANOVA followed by Bonferroni post hoc test.

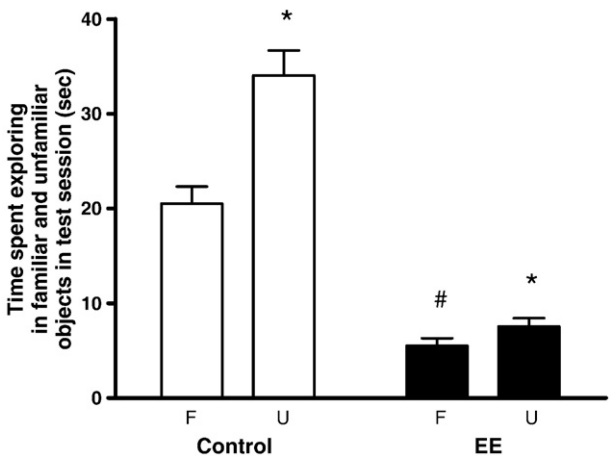

Fig. 3. Time spent in familiar and unfamiliar objects (in seconds) in test sessions, for control and EE groups of mice (n 27–22 animals in each group). Results are presented as means ± S.E.M. of the time spent in objects during 10 min. The test session was performed 90 min after the training session. $^{*}p \leq 0.01$ indicates significant difference for the time spent between familiar and unfamiliar objects in test sessions (two-way ANOVA followed by Bonferroni post hoc test). Labels: F familiar object, U unfamiliar object.

without affecting the discrimination ratio of the objects. This could indicate that EE caused a lessening of the interest to objects utilized in ORT (objects were not ethologically relevant and were unfamiliar to animals), without affecting the ORT paradigm. Our results show that both groups presented a decrease in the time spent exploring the familiar object in test session and these findings indicate that the animals learned about the environment. EE group spent less time in both objects in both sessions, which indicates a more rapid exploration, minimizing associated costs, in spite of the equal performance of both groups in the capacity of discrimination. These animals presented no changes in the hippocampal immunocontent of BDNF and TrkB proteins.

Although the literature presents few studies about behavioral effects of EE in CF1 albino mice, we previously demonstrated that EE decreases the exploration in the second day of exposure to an open field arena [15]; this behavioral change suggests a more propitious behavior in mice exposed to EE, such as decrease in time exposed to predators, and/or improved memory of the exposure to the open field apparatus in the first day [15].

The ORT evaluates natural behavior of rodents, such as approaching and exploring novel objects rather than familiar objects. Thus this task deals with the natural motivation of the animals to explore novelty, an innate instinct that animals use to recognize their environment [18]. Here, the EE group expended less time exploring the objects, which could indicate reduction of motivation, curiosity and/or interest for objects, probably because these animals previously experienced more stimulating environmental conditions (learning, social and physical), which make the novelties not so appealing. Renner demonstrated an increase in the time spent exploring the objects, but their protocols employed different material and methods than the ones used in our study, as, for instance, hemioctogonal arena, familiar and unfamiliar objects in both training and test sessions, and the species of animals studied (rat strain (Berkeley S1)) [36]. However, in the study by Bruel-Jugerman et al., employing Sprague–Dawley rats, the authors have demonstrated a slight reduction in the time spent in the objects by the EE group in training sessions [37]. Together with our present results, we believe that the discrepancy among these findings is possibly related to different factors, including different animals or strain employed, as well as different EE protocols and particularities of the ORT used.

However, here the discrimination ratio was not affected by EE, which could be considered that the memory was actually improved, since EE mice could correctly discriminate using less time exploring the objects. In fact the ethology of the mice indicates the necessity of

rapid exploration and knowledge about the environment for species maintenance, such as mating or food, and minimizing associated costs, such as exposure to predators or rivals [20]; thus, these findings could indicate an increase in behavioral ability of CF-1 albino mice exposed to EE.

In the literature there is a consensus that several behavioral differences among the strains and other genetic and epigenetic variables are more pronounced after EE [14], possibly associated to gene–environment interactions. Concerning EE, there is no consensus about its behavioral effects in different species (rats and mice) and strains (Berkeley, Sprague–Dawley and CF1) on distinct behavioral paradigms, such as open field [15] and water maze [14] and others. Thus, the behavioral response of laboratory animals submitted to EE is more similar to the ethological responses presented by wild animals, and the EE may attenuate artificial selection for traits; this fact is one of the likely responsible for the conflicting findings among different species, these conflicting findings, corroborate the Charles Darwin postulate, each species has developed the individual repertoire of behaviors which has been formed by its differences of the evolutionary history [38].

A recent study suggests that EE effects might be mediated, at least in part, by chromatin remodeling and histone acetylation [11], indicating an involvement of DNA transcription and subsequent increase in protein formation. The literature reports different genetic models for evaluating environment condition [27], including BDNF heterozygous [39], demonstrating gene–environment interactions. However, in our study we did not find any changes in the immunocontent of the hippocampal proteins BDNF and TrkB. This result does not exclude that other neurotrophins could be involved on synaptic plasticity and behavioral modulation by EE exposure in the specific mouse strain (male albino CF1 mice) used here.

Previous studies showed distinct results concerning the neurotrophin levels in the hippocampus of animals maintained in the enrichment housing, with some studies reporting increase in BDNF-TrkB [4,5,29] whereas others, including ours, did not find any changes [30–33]. The divergence among these results might be due to differences in the enrichment protocols and distinct animals or strain employed; for example, Bindou et al., which also did not find changes in BDNF levels, used EE for 10 days (6 h day) in Wistar rats after lesion of ventral subiculum [27], whereas Ickes et al., reported changes, used Sprague–Dawley rats maintained for 12 months in EE condition. The discrepancy in these findings demonstrates the importance of more studies for the understanding of the variation of response in neurotrophins and their receptors, as BDNF and TrkB, in distinct animal species or strains exposed to different protocols of EE.

In summary, the present study demonstrated equal discrimination ratio by control and EE groups. On the other hand, the EE group presented a decrease in the time spent exploring both objects evaluated together, and in the time expended exploring familiar and unfamiliar objects when evaluated separately. These results could indicate that the EE mice present diminished levels of motivation, curiosity and/or interest in exploring the objects utilized in ORT (objects were not ethologically relevant and were unfamiliar to animals). In an ethologic perspective, the EE group presented a more propitious behavior for species survival, including rapid exploration and knowledge about the environment. In view of these findings, further studies in different animal models or strains, including wild animals and/or variations of EE protocols, are important for understanding potential gene–environment interactions leading to different behavioral profiles.

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