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Enriched environment effects on behavior, memory and BDNF in low and high exploratory mice

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ABSTRACT

Environmental enrichment (EE) has been largely used to investigate behavioral modifications and neuroplasticity in the adult brain both in normal and pathological conditions. The interaction between individual behavioral traits with EE responsiveness has not been investigated within the same strain. By using two extremes of CF1 mice that differ by their exploratory behavior in the Open Field (OF) task (Kazlauckas V, 2005), denominated as Low (LE) and High (HE) Exploratory Mice, the present study evaluated if EE during adulthood could modify the putative differences between LE and HE mice on exploratory behavior, memory performance and hippocampal BDNF levels. To this end, we investigated the effect of adult LE and HE mice after 2 months of enriched or standard housing conditions on the open field, on novel object recognition, on the inhibitory avoidance task and on hippocampal BDNF immunocontent. LE showed low exploratory behavior, less retention in the inhibitory avoidance and lower hippocampal BDNF levels. EE enhanced exploratory behavior, memory performance and hippocampal BDNF levels both in LE and HE mice. Importantly, the general profile of LE mice submitted to EE was similar to HE mice housed in standard conditions. These results show that internalized behavior of LE mice can be significantly modified by exposure to an enriched environment even during adulthood. These observations may contribute to investigate biological mechanisms and therapeutical interventions for individuals with internalized psychiatric disorders.

1. Introduction

Enriched housing is an environmental condition that provides enhanced possibilities of complex inanimate and social stimulation as compared to standard laboratory conditions [1]. This protocol has been largely used to investigate behavioral modifications and neuroplasticity by increasing physical activity, learning experiences, visuals inputs, and social interactions [2–5]. It has been observed that rodents submitted to an enriched environment even during adulthood present biochemical, morphological and functional changes in the adult brain both in normal and pathological conditions [5,6].

The exposure to environmental enrichment (EE) induces plastic changes particularly at the level of the hippocampus and cerebral cortex [5,7]. In rodents, hippocampal expression of BDNF and NGF neurotrophins increases after taking part in spatial learning tasks or performing physical exercise [8,9]. In addition, these neurotrophins are correlated to an improved performance in learning and memory tasks [8,10–13].

Previous studies using the anxious BALB/c strain as a possible model of neophobia and the nonanxious C57BL/6 strain revealed that BALB/c was more affected by EE than C57BL/6 [14,15], suggesting that EE could have a strain specific effect on rodents behavior. EE also improved Spontaneously Hypertensive Rats (SHR) performance in open field habituation, water maze spatial reference, social and object recognition tasks, whereas non-cognitive traits, such as nociception and hypertension, were not affected by EE [16,17].

A study with Roman high- and low-avoidance rats (RHA/Verh and RLA/Verh), which represent low emotional/anxious and high novelty seeker vs. high emotional/anxious and low novelty seeker profiles, respectively, showed that early-life EE increased head-dipping behavior in both rat lines, without affecting locomotor activity. They reported that these genetically divergent novelty seeking patterns can be enduringly modified to the point of considerably reducing the between-line differences by early life rearing in an enriched environment [18].

Within the same strain, the interaction between individual behavioral traits and EE responsiveness during adulthood has not been investigated. Our group has characterized two extremes of mice that differ by their exploratory behavior in the Open Field (OF) task [19], denominated as Low (LE) and High (HE) Exploratory Mice. HE mice present less anxiety-like behavior, more aggression against intruders,

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higher avoidance of conditioned punishment (electric footshock), and better performance in a maze with positive reinforcement (food) compared to LE mice [19]. Thus, LE and HE mice may represent a model for internalized and externalized behaviors and disorders in humans. Internalized disorders, such as generalized anxiety, major depression and phobias show a common trait called neuroticism, with high fear, sensitivity and distress, whereas externalized disorders, such as antisocial personality disorder and drug abuse are characterized by impulsivity and aggression [20].

The purpose of the present study was to investigate if the influence of EE during adulthood could modify the putative differences between LE and HE mice on exploratory behavior, memory performance and hippocampal BDNF levels, or at least significantly affect LE behavior towards a less internalized profile. To this end, we evaluated adult LE and HE mice after 2 months of standard (ST) or enriched (EE) housing conditions with the open field for exploratory behavior, the novel object recognition and the inhibitory avoidance tasks for memory. After behavioral analysis, the hippocampal immunocontent of BDNF was determined.

2. Materials and methods

2.1. Animals

Male albino CF1 mice (2 months), weighing approximately 35–40 g, were obtained from State Foundation for Health Science Research (FEPPS, Porto Alegre, RS, Brasil). They were housed in groups of six to eight in standard conditions of temperature and humidity, in a 12 h light/dark cycle (lights on at 7:00 am), with access to food and water *ad libitum*. Sawdust was changed 2 times a week. 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 were followed throughout all the experiments in accordance to the project approved by the ethical committee from Universidade Federal do Rio Grande do Sul. All efforts were made to minimize the number of animals employed in the present study and their suffering.

2.2. LE and HE mice selection

Two batches of eighty mice each were selected into low (LE) and high exploratory (HE) mice, according to their exploratory behavior in the central area of the open field (OF), as described in our previous research [19]. This test was used to separate the two different mice populations depending on the animal's response to a novel object in a new environment. Briefly, the animal was placed in an open-field (50 cm × 50 cm × 50 cm) with an object (a white cylinder of 1.5 cm radius and 5 cm high) in the center of the arena to stimulate exploration. Exploratory behavior was video recorded for 5 min, and the time spent by the animal in and out of an imaginary center square of 30 cm × 30 cm was analyzed using the ANYmaze software (Stoelting, Woods Dale). From 160 mice screened, the bottom and top 25% explorers of the central area of the arena composed the LE and HE exploratory groups, respectively. All mice were kept within their same housing groups and were randomly allocated to enriched environment (LE–EE, n = 18 and HE–EE, n = 23) or standard housing conditions (LE–ST, n = 22 and HE–ST, n = 17).

These four groups were tested after 2 months of environmental enrichment or standard conditions. Mice were appropriately identified and remained in their respective home cages without changing housemates until the end of behavioral testing.

2.3. Housing conditions

Standard housing conditions consisted of a 27 cm × 16 cm × 12 cm acrylic box with sawdust containing groups of 6–8 mice. Enriched housing conditions consisted of 38 cm × 32 cm × 16 cm acrylic box with sawdust containing 8 mice. The apparatus contained one running wheel and a variety of objects, including wood and plastic objects, tunnels, hiding places and nesting materials where the animals could be out of luminosity. The objects were changed 2 times a week.

2.4. Behavioral tasks

The behavioral tasks were conducted in two independent cohorts of LE and HE mice. One group was tested in the open field and novel object recognition task, and the other group was tested in the open field, in the novel object recognition task and in the inhibitory avoidance task.

2.4.1. Open field after EE

The open field task was performed as previously described above in 2.2 LE and HE mice selection.

2.4.2. Novel object recognition task

Novel object recognition task (NOR) was performed in an apparatus consisting of a small black wood chamber (25 cm × 25 cm × 40 cm). Before the experimental sessions, animals were habituated to the experimental room for 60 min in dim light conditions. A light bulb was switched on during the experimental sessions, with uniform light intensity in the different parts of the apparatus. The objects were placed equidistant from two corners, 12 cm apart from the wall. Two observers blind to the housing conditions performed the behavioral evaluation. Mice had been acclimated in the apparatus during ten minutes twenty-four hours before sample session. The sample session consisted of placing a mouse in the apparatus containing two identical objects, and allowed it to explore for 10 min. Each mouse was always placed in the apparatus facing the wall. In the discrimination sessions, performed 1.5 h and 24 h later, one familiar (used in the sample session) and a novel object were presented. The objects employed were 2 kinds of small bottles with different shape and color (white and amber) presenting the same texture and size. The objects do not have ethological significance for mice. Objects were cleaned between sessions with 70% ethanol solution. Exploration was defined as directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose or forepaws. The time of exploration was manually recorded. Animals presenting less than 3 s of exploration time were excluded from the experiment. The following parameters were analyzed: (a) the discrimination ratio, analyzed and expressed by the ratio of total time spent exploring the novel object by the total time spent in both objects and (b) time spent exploring both objects during the sample and discrimination sessions in seconds. The discrimination for sample session was calculated by the ratio between the time spent on one of the objects randomly chosen and the total time of exploration for both objects in the sample session [21].

2.4.3. Inhibitory avoidance

The inhibitory avoidance task was assessed in an acrylic box (50 × 25 × 25 cm) with parallel stainless-steel bars (1 mm diameter) spaced 1 cm apart as the floor. A platform (2 cm high and 4 cm × 6 cm wide) was placed in the center of the box. In the training session, mice were placed on the platform and the latency to step-down onto the floor with the four paws was recorded; immediately after stepping-down mice received a 0.4 mA, 2 s footshock and were placed in their home cage. The test session was performed 1.5 hours after training (short-term memory) or 24 h after training (long-term memory). No footshock was given in the test session, and step-down latencies (180 s ceiling) were taken as a measure of retention.

2.5. Immunoblotting

After behavioral analysis mice were sacrificed by decapitation and the whole hippocampus was dissected out immediately after the end of the experiments. Hippocampi were homogenized in 5% SDS solution containing A protease inhibitor cocktail (Sigma, São Paulo/Brazil) and kept at −70 °C. Protein content was further determined by using bicinchoninic acid assay using bovine serum albumin (BSA) as standard (Pierce, São Paulo/Brazil). Hippocampal extracts were diluted to a final protein concentration 2 μg/μl in SDS-PAGE buffer and 85 μg of the samples and dual-color prestained molecular weight standards (Bio-Rad, Porto Alegre, Brazil) were separated by SDS-PAGE (16% with 4% concentrating gel). After electro-transfer, the membranes were incubated overnight with Tris–buffered saline 0.1% Tween-20 (TBS-T) containing 3% BSA. After blocking, the membranes were incubated for 24 h at 4 °C with mouse anti-BDNF antibody (1:500, Sigma, São Paulo, Brazil) or with mouse anti-β-tubulin antibody (1:1000; Sigma, São Paulo, Brazil). After primary antibodies incubation, membranes were washed and incubated with horseradish peroxidase conjugated secondary antibodies for 2 h at room temperature and developed with ECL (Amersham, São Paulo/Brazil). The autoradiographic films were scanned, and densitometric analyses were performed using Image J software. As an additional control of the protein loading, membranes were stained with Ponceau S. The results were presented by BDNF/ β-tubulin density.

2.6. Statistical analysis

Differences in exploratory profile between LE and HE in the open field task were analyzed using Student's t-test. For open field exploration after EE and BDNF immunocontent determination, differences were analyzed using two-way ANOVA with groups (LE/HE) and housing conditions (ST/EE) as independent variables, followed by Bonferroni to compare each EE to their ST group. In the novel object recognition test, we used Three-way ANOVA with differences between groups, housing conditions and trials and also used the test of within subjects contrast. Separate analyses were performed in order to test for specific differences between groups. In the inhibitory avoidance test, step-down latencies are expressed as medians (interquartile ranges). Differences between training and test session were analyzed by Wilcoxon and difference between groups were analyzed by Kruskal Wallis followed by Dunn's multiple comparison tests. Graphpad Prism 5 and SPSS16.0 softwares were used, and significant differences were considered when $P<0.05$. Except for inhibitory avoidance, results are expressed as mean + S.E.M.

3. Results

3.1. LE and HE phenotypes selection

LE and HE mice were selected in the open field task according to their exploratory behavior (n = 40 in each group). LE mice spent 14.8 ± 1.4 % of the time in the central area of the arena compared to 41.6 ± 1.1 % for HE mice ($P<0.001$, Fig. 1A). Locomotor activity did not differ between LE and HE groups (Fig. 1B).

3.2. Effects of environmental enrichment in the open field task

EE conditions significantly increased exploration of the central area in both LE and HE groups [$F(1, 72) = 19.44$; $P<0.001$]. After two months under standard or enriched housing conditions, the exploratory behavior of HE groups remained higher than their respective LE groups

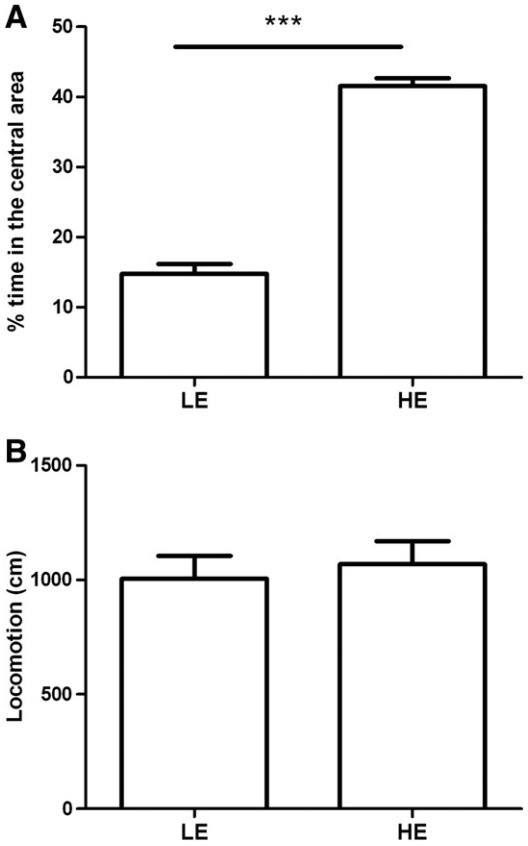

Fig. 1. Selection of low (LE) and high (HE) exploratory mice behavioral pattern. Animals were subjected to the open field task with a central object, and time spent in the central area (A) and locomotor activity (B) were recorded during five minutes. LE (n = 40) and HE (n = 40) mice were evaluated for time spent in the central area (A) and locomotion (cm) (B). Results are presented as mean + S.E.M. Statistical analysis was performed using Student's *t* test. *** $P<0.001$.

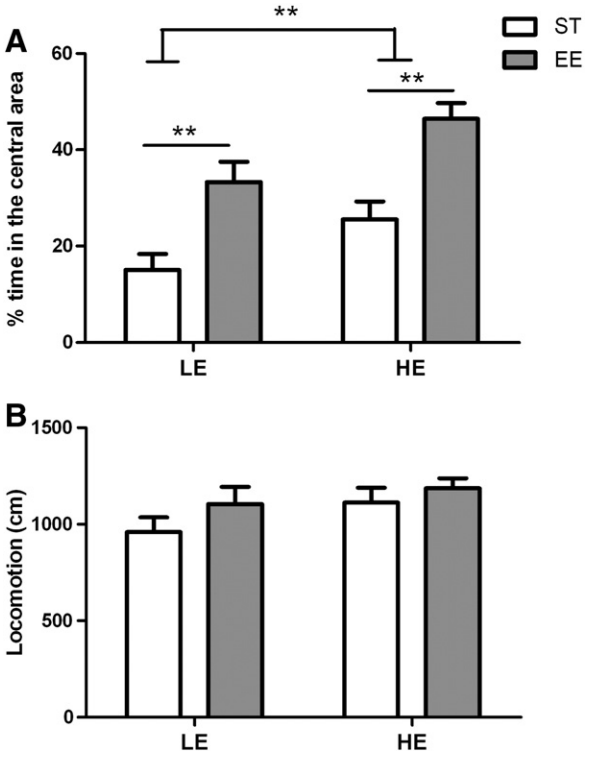

Fig. 2. Exploratory and locomotor activities in the open field task after environmental enrichment. LE and HE standard (ST) and enriched (EE) mice were subjected to the open field task with a central object, and time spent in the central area (A) and locomotor activity (B) were recorded during five minutes. White bars represent the LE and HE ST groups and gray bars represent LE and HE EE groups. Results are presented as mean + S.E.M. Statistical analysis was performed by Two-way ANOVA followed by Bonferroni post hoc test. ** $P<0.001$. LE–ST (n = 20); LE–EE (n = 19); HE–ST (n = 19); HE–EE (n = 19).

[F (1, 72) = 32.35; $P<0.001$], but LE–EE was not different from HE–ST ($P>0.1$) (Fig. 2A). Locomotor activity did not change for any group after environmental enrichment exposure (Fig. 2B).

3.3. Novel object recognition task

The performance of both groups was improved by enriched environment as shown in Fig. 3A. All groups had an increase in the discrimination ratio across trials [F (2, 94) = 1 06.47, $P<0.05$] and there was a significant interaction [F (2, 94) = 11.29, $P<0.05$]. The test of within subjects contrast revealed a quadratic relation between trials vs. housing conditions (F (1, 47) = 13.9, $P<0.05$), showing that enriched groups reached the most prominent performance in this task in the second trial, whereas standards groups reached the best performance only in the third trial.

LE groups showed lower exploration of both objects when compared to HE [F (1, 47) = 26.65, $P<0.05$]. Also, EE in both LE and HE groups decreased object exploration compared to standard [F (1, 47) = 16.66, $P<0.05$]. We observed a trend for interaction ($P=0.051$) indicating that this effect of EE is more prominent in HE groups (Fig. 3B).

3.4. Inhibitory avoidance task

In the inhibitory avoidance task, latency to step-down in the training session was similar for all groups (data not shown). All four groups significantly increased latency to step-down at 1.5 h and 24 h after training session (Fig. 4, $^{*}P<0.05$ and $^{**}P<0.001$). Under standard housing conditions, HE showed higher retention than LE mice at 24 h

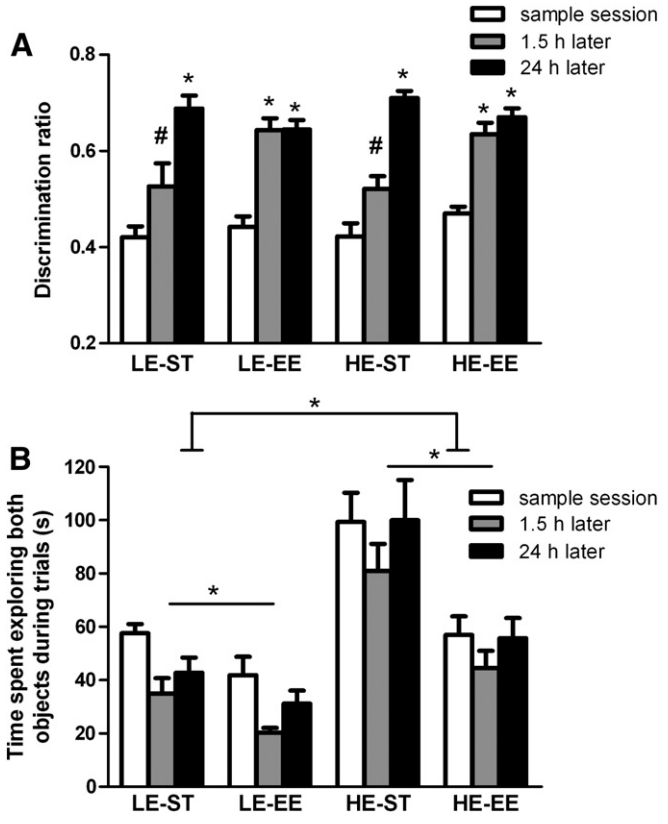

Fig. 3. Novel object recognition task. LE and HE standard (ST) and enriched (EE) mice were evaluated for: (A) discrimination ratio for the sample and discrimination sessions and (B) total time spent exploring both objects during the sample and discrimination sessions. Results are presented as mean + S.E.M. Statistical analysis was performed by three-way ANOVA with differences between groups, housing conditions and trials. * $P<0.05$ indicates difference between sample and discrimination sessions within groups. # $P<0.05$ indicates difference between sample and discrimination sessions within groups and differences between discrimination sessions within groups [LE (ST and EE)]; [HE (ST and EE)]. LE–ST (n = 12); LE–EE (n = 13); HE–ST (n = 11); HE–EE (n = 15).

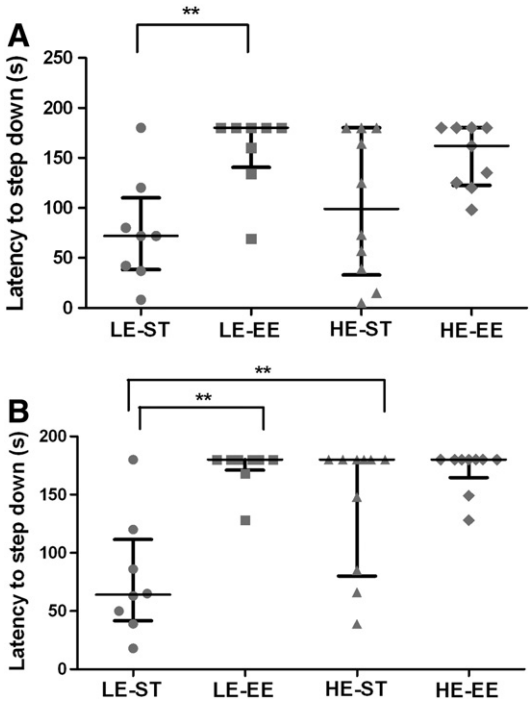

Fig. 4. Effect of environmental enrichment in the inhibitory avoidance task in mice tested 1.5 h (A) and 24 h (B) after the training session. Results are presented as dots and dash represents median (interquartile range) values. Differences between groups were analyzed by Kruskal Wallis followed by Dunn's multiple comparison tests. LE–ST (n = 8); LE–EE (n = 8); HE–ST (n = 10); HE–EE (n = 9). ** $P<0.001$ between groups. (ST): standard and (EE): enriched.

($P<0.001$) but not at the 1.5 h after training. LE–EE had significantly higher step-down latency compared to LE–ST both 1.5 h and 24 h after training ($P<0.001$, Fig. 4A and B). The performance of HE under standard and enriched conditions was not different as revealed by similar latencies to step-down in the test session. Thus, these results indicate that environmental enrichment promotes an improvement in performance of inhibitory avoidance task especially in LE mice.

3.5. Hippocampal BDNF immunocontent

BDNF levels increased in LE and HE groups after environmental enrichment [F (1, 18) = 5.56; $P<0.05$], and also both HE groups

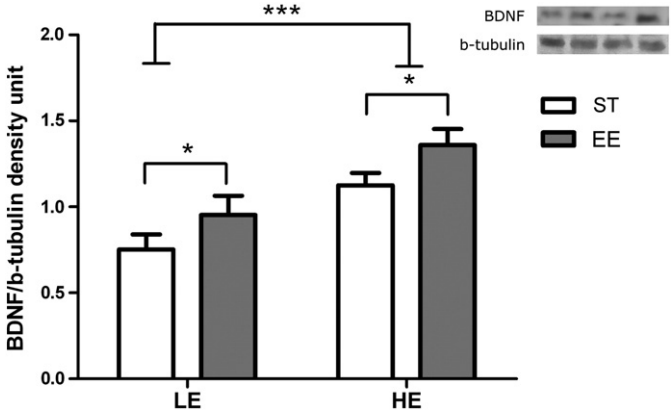

Fig. 5. Hippocampal BDNF immunocontent for LE and HE standard (ST) and enriched (EE) mice. White bars represent the LE and HE ST groups and gray bars represent LE and HE EE groups. Results are presented as mean + S.E.M. Statistical analysis was performed by Two-way ANOVA followed by Bonferroni post hoc test. *** $P<0.001$ between LE and HE groups and * $P<0.05$ between ST and EE groups. LE–ST (n = 6); LE–EE (n = 5); HE–ST (n = 5); HE–EE (n = 6).

presented higher levels of BDNF when compared to LE groups [F (1, 18) = 17.80; $P<0.001$] (Fig. 5). Hippocampal BDNF levels were not significantly different between LE–EE and HE–ST groups ($P>0.10$).

4. Discussion

This study showed that environmental enrichment enhanced exploratory behavior, memory performance and hippocampal BDNF levels both in LE and HE mice. Trait differences in exploratory behavior and hippocampal BDNF levels remained within the same housing conditions. Importantly, the general profile of LE mice submitted to environmental enrichment was similar to HE mice housed in standard conditions. These results show that the more internalized behavior, inferior cognitive performance and neurotrophic strength of LE mice can be significantly modified by exposure to an enriched environment even during adulthood. However, overall these results do not support that individual trait differences between HA and LE mice can be substantially modified by environmental interventions.

Postweaning rats exposed to enriched environment explored more the central area of the open field when compared to rats housed in isolation or standard conditions [22]. Furthermore, rats exposed to an environmental enrichment presented reduced latency to explore the novel open field and faster exploration of novel objects in the NOR task [23]. These studies corroborate our findings of increased OF central area exploration in adult mice exposed to EE, which occurred in both LE and HE groups. However, in the NOR task, EE lead to reduced time exploring objects. This was particularly evident in HE mice, which explored both objects extensively in standard conditions, in agreement with these mice natural behavioral patterns [19]. Thus, depending on the parameter analyzed and time of exposure to novelty, EE may have different effects on exploratory activity. In general, EE seems to change mice exploratory behavior towards an immediate start of exploration, but for shorter periods of time. Thus, this faster habituation induced by an enriched environment may lead to higher exploration in a short protocol (5′ min in the OF) and lower exploration in a longer protocol (10′ in the NOR task).

Enrichment protocol at postweaning and during adulthood also decreases locomotion in rodents probably by increasing habituation [3,17,24,25]. However, Fernandez-Teruel et al. (2002) reported that EE did not produce changes in the locomotor activity in their Roman rats [18] which corroborates our study, that locomotor activity in the OF task did not change after environmental enrichment, but our protocol was shorter (5′ compared to 10′ in other studies) and the OF had an object in the central area, which may have changed the locomotor pattern.

Improved learning and memory by environmental enrichment is one of the most consistent findings in the literature [5,10,13,26,27]. Therefore, to further understand the effect of enriched conditions in LE and HE mice, we analyzed their behavioral performance in the novel object recognition task, which accesses memory based on the natural motivation of animals to explore novelty in a familiar context. This task has been widely used to evaluate the effects of pharmacological, genetic or environmental interventions on memory processes [28]. The hippocampus seems to play a central role in this task, both in memory processes and in environmental interactions [29,30]. A previous study, using CF1 male mice, showed that mice in EE correctly discriminate objects using less time exploring objects [2]. Our results showed that LE–ST and HE–ST mice presented similar memory performance in the NOR task. Furthermore, LE and HE submitted to EE clearly discriminated the novel object in the 1.5 h test whereas the standard groups showed this performance only in the 24 h test after training. These results suggest that EE can especially improve recognition memory.

Another task that deals with the animal's natural exploratory behavior is the inhibitory avoidance task which consists in the animal's ability to avoid a conditioned punishment repressing their tendency to explore beyond the safe areas [31]. The trait difference was clearly evident when long-term memory was assessed (24 h after training), since the retention for LE–ST was lower than HE–ST, confirming our previous observation [19]. Thus, this trait difference was reflected for long- but not for short-term memory. Short and long-term memories present different molecular mechanisms, which includes protein synthesis for long-term memory. Recently, studies in rodents using the inhibitory avoidance task showed that BDNF induces memory persistence by transforming a nonlasting long-term memory trace into a persistent memory trace, revealing that BDNF is essential for long-term memory persistence [32]. Thus, the lower density of BDNF presented for LE mice compared to HE housed in the same conditions could help to explain the lower performance for long-term memory in the inhibitory avoidance task.

Interestingly, the environmental enrichment significantly improved the performance in the inhibitory avoidance task only for LE mice. Probably, the performance for HE mice was not modified due to a ceiling effect. Considering that the consolidation of memory is a process that lasts a few hours through which memories are transformed from a labile into a more stable state, probably interventions could be more effective for consolidation than acquisition. Previous studies have demonstrated significant alterations in the BDNF protein levels in several brain regions as a result of an enriched environment, providing a possible biochemical basis for its behavioral and morphological alterations [11,33]. Given that LE presented lower density of BDNF than HE mice housed in the same conditions, the environmental enrichment promoted an increase on BNDF immunocontent in both groups. Once more the increase on BDNF immunocontent caused by EE may be involved in the environmental enrichment benefits on memory presented mainly for LE–EE mice when compared with their LE counterparts. Thus, memory and BDNF levels depend on both trait and environmental conditions.

In conclusion, trait behavior, memory and neurobiological markers can be substantially modified by environmental interventions in adult mice. More specifically, internalized traits, as in LE mice and in patients with internalized psychiatric disorders, may be attenuated by exposure to an enriched environment even during adulthood.

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