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Increase of glucocorticoid receptor expression after environmental enrichment: Relations to spatial memory, exploration and anxiety-related behaviors

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HIGHLIGHTS

- EE reduced anxiety-like behaviors on the elevated-zero maze.
- EE improved the performance in the 4-RAWM.
- EE condition increased GRs' expression in the dorsal hippocampus.
- High GRs' expression could produce changes in cognition, anxiety levels and behavior.

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ABSTRACT

Environmental enrichment (EE) produces a remarkable degree of structural and functional plasticity in the hippocampus and possible mediators of these changes, such as glucocorticoid receptors (GRs), are of considerable interest. GRs are richly expressed in the hippocampus and they are involved in the adaptation to stressors and facilitate active coping in anxious situations. In this study, we assessed the effect of an EE protocol (24 h/day during 69 days) in adult Wistar rats on the activity in the elevated-zero maze (EZM), performance in the holeboard task (HB) and we also examined the changes in the glucocorticoid receptors (GRs) expression in the dorsal hippocampus (CA1, CA3 and DG). Our EE protocol reduced anxious behaviors in the EZM, so the animals spent more time and made more entries into the open sections. In the HB task, the enriched group showed more explorative behavior, a reduction of anxiety-related behaviors and a better cognitive performance compared to non-enriched animals. With regard to the GR expression, the EE condition produced an increase in the number of immunopositive cells for GRs in CA1, CA3 and DG. These results suggest that the better performance of enriched animals could be mediated in part by the increase of GRs in the dorsal hippocampus, which may alter the hippocampal neuronal function and accordingly, the anxiety levels, the spatial memory performance and the exploration levels in these animals.

1. Introduction

The hippocampus has attracted the attention of many researchers, owing to its different role in several behavioral processes [1–3]. It has a long-established role in certain forms of memory, above all in spatial memory [4–6], but new functions have been attributed to the hippocampus in recent years. For example, it has been involved both in motivated-explorative behavior [7–9] and in the control of anxiety responses, through negative feedback on the hypothalamic–pituitary–adrenal axis (HPA) [10,11].

The negative feedback on the HPA axis is mediated by mineralocorticoid and glucocorticoid receptors (MRs and GRs) [12]. Interestingly, the activation of GRs has significant effects on the structure and function of the brain [13]. This type of receptor has been associated with learning and memory processes [14]. Thus, recent studies have revealed that GRs may affect learning and memory processes by interacting with glutamatergic mechanisms [15]. Specifically, GRs seem to be involved in the elimination of non-relevant behaviors and in the acquisition and

Abbreviations: EE, environmental enrichment; PCA, principal component analysis; HB, holeboard task; MRs, mineralocorticoid receptors; GRs, glucocorticoid receptors; GCs, glucocorticoids; EZM, elevated-zero maze; EPM, elevated-plus maze; OF, open field; CA1–CA3, hippocampal cornu ammonis; DG, dentate gyrus; HPA, hypothalamic–pituitary–adrenal axis; LTP, long-term potentiation; CE, coefficient of error; CV, coefficient of variation; CORT, corticosterone; CRF, corticotrophin-releasing factor; DAB, diaminobenzidine.

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consolidation of recently acquired information [16–18]. According to this, the administration of a GR antagonist or a GR antisense oligonucleotide directly into the hippocampus before learning impaired retention behavior in the Porsolt swimming task [19] and GR-knockout mice show impaired memory consolidation [20]. On the other hand, a decrease of forebrain GRs in male rodents induces an increase of depression-like and anxiety-like behaviors, along with a reduction of explorative behaviors [21].

In addition to its different roles, the hippocampus has shown a remarkable degree of structural and functional plasticity after environmental enrichment (EE) condition [22–24]. The molecular substrate of the effects of EE on hippocampal plasticity is multifactorial. Thereby, EE has shown to enhance the long-term potentiation (LTP), neurogenesis, dendritic spine growth, neurotrophin expression, dendritic branching, presynaptic vesicle number and density of dendritic spines in the hippocampus [25–29]. With regard to GRs, some studies have found that EE enhances the GR expression in the hippocampus and its positive impact on cognitive, motivational and anxiety-related behaviors [30–32]. Hence, the more complex and diverse explorative behavior, the reduction of fearfulness and anxiety and the improved acquisition and retention of several learning tasks in enriched animals, could be mediated in part by GR expression [33–36]. EE has shown to increase the exploration in novel situations, such as in the open-field test [33], the performance in spatial [35,36] and non-spatial memory tasks, such as object recognition [34] and a reduction of defecation in the open field as well as more entries into the open arms of the elevated plus-maze [33]. Therefore EE, by inducing GRs, may alter the hippocampal neuronal function, anxiety levels, cognition and behavior [32]. This increase could be related to a better regulation of the HPA axis in an anxious situation and constitute a stress resilience mechanism for the animals [37–39]. In contrast, other studies have also found a reduction of GRs after EE condition [40,41], so the data are controversial maybe due to the different EE protocols employed.

Given the above evidence about the impact of the EE condition on the structural and functional plasticity in the hippocampus, we decided to assess the effect of continuous EE (24 h/day) on the performance in the elevated-zero maze (EZM) and in the holeboard task (HB) in young male rats. We have employed these behavioral tests because on the one hand, the EZM is a validated test to assess anxiety and it has several advantages in comparison with the elevated-plus maze (EPM), such as the absence of the central area [42] and on the other hand, the HB task allows us to study simultaneously the effect of the EE on memory performance, anxiety levels and explorative behavior [43]. We also wanted to discover if our EE protocol produced changes in the GR protein expression in the dorsal hippocampus and if so, study the possible relationship with the behavioral measures registered. In this study, we further show the utility of the principal component analysis (PCA) in analyzing behavioral measures.

2. Material and methods

2.1. Animals

Forty male Albino Wistar rats aged between 30 and 45 days old from the vivarium of the University of Oviedo were used. All the animals had ad libitum access to the same rodent pellets and tap water and they were maintained at constant room temperature (20–21 °C), with a relative humidity of 65–70% and artificial light–dark cycle of 12 h (08:00–20:00 h light/20:00–08:00 h dark). All the procedures complied with the Directive 86/609/EEC (The Council Directive of the European Community) concerning the protection of animals used for experimental and other scientific purposes. The National legislation, in agreement with this Directive, is defined in Royal Decree no. 1201/2005.

First, all the animals were handled daily for 5 days for a period of 10 min to avoid stress reactions to subsequent manipulation. Next, all the rats performed a neurological screening to test the sensorimotor orientation, the co-ordinated limb and the neurological function. Neurologic assessment was performed as described earlier in Santin et al. (2009) [44]. The neurological deficit in each test was evaluated in a three-point scale: 0 = absent, 1 = mild, 2 = severe. All the animals were in a good state of health, scoring 0 in all tests performed. After this, the rats were randomly assigned to four groups: control group (CO: 176.5–197.9 g; $n = 10$), environmental enrichment group (EE: 172.1–182.56 g; $n = 10$) environmental enrichment + holeboard group (EE + HB: 170.1–198.5 g; $n = 10$) and holeboard group (HB: 183.1–200.3 g; $n = 10$). The animals were kept in their respective experimental conditions (EE housing condition or standard housing condition) until behavioral experiments were carried out and also throughout the whole behavioral testing, 69 days in total. The CO group consisted of 2 groups of 5 rats kept in 2 different standard cages without learning and EE experiences.

2.2. Rearing conditions

The EE and EE + HB groups were housed in two large cages of 100 cm × 95 cm × 54 cm (each experimental group in a different cage, 10 rats per cage) 24 h/day for 69 days. The stimulating objects were similar in both cages. The cages contained sawdust and various objects such as toys, a running wheel, ropes, plastic tubes of different diameters, a yellow platform, a wooden house, odorous and sound objects, and nesting materials. Throughout the enrichment period, the yellow platform was kept in the cages, while the toys and constructions were changed once a week. The cages were cleaned twice a week and the feeding boxes were moved to different points of the cages to encourage explorative behaviors in the animals.

The CO and HB groups were housed in groups of five in standard cages during 69 days (20 cm × 35 cm × 55 cm) containing sawdust, but without stimulating objects or constructions. The feeding box was kept in the same position throughout the rats' life. After the 69 days in the EE or standard housing condition, we determined again the weights of all the animals (CO: 245.07–257.9 g; EE: 258.8–270.3 g; HB: 269.9–278.6 g; EE + HB: 255.4–268.3 g).

2.3. Elevated zero-maze (EZM)

The day prior to the HB task, the EE + HB and HB groups were assessed in the EZM in one session of 5 min. This maze was made of black acrylic in a circular track 10 cm wide, 81 cm in diameter, and elevated 82 cm from the floor (Noldus Information Technology). It was divided into four sections of equal lengths, two open sections and two closed sections with black acrylic walls 35 cm in height. We followed the same behavioral protocol described in Sampedro-Piquero et al. (2013) [35]. Behavioral measures taken included: (a) closed head dips (the number of times the rat looked over the edge of the maze while a portion of the body was in the closed sections); (b) open head dips (the number of times the rat looked over the edge of the maze while its body was completely in the open sections); (c) duration in open sections; (d) latency (the time before the first entry into the open section); (e) the number of entries into the open sections; (f) climbing (rat maintains an erect posture leaning against the wall); (g) fecal boli; (h) duration in contracted body elongation (duration in this body posture); (i) duration in normal body elongation (duration in this body posture); (j) duration in stretched body elongation (duration in this body posture); (k) total velocity (cm/s); and (l) distance traveled (cm). The movements of the rats were recorded with a camera connected to a computer running the EthoVision 3.1. software (EthoVision 3.1; Noldus Information Technology, Leesburg, VA).

2.4. Holeboard task (HB)

The EE + HB and HB groups were trained on a board (70 × 70 cm) with 16 regularly arranged holes (3 cm diameter, 1 cm deep) with