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Research report

Effects of environmental enrichment on anxiety responses, spatial memory and cytochrome c oxidase activity in adult rats

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ABSTRACT

We have studied the effect of an environmental enrichment (EE) protocol in adult Wistar rats on the activity in the elevated zero-maze (EZM), performance in the radial-arm water maze (RAWM) and we have also examined the changes in the neuronal metabolic activity of several brain regions related to anxiety response and spatial memory through cytochrome c oxidase histochemistry (COx). Our EE protocol had anxiolytic effect in the EZM; the animals spent more time and made more entries into the open quadrants, they had lower latency to enter into the open quadrant and lower levels of defecation. Also, the EE group showed fewer working memory and reference memory errors, as well as lesser distance travelled in the first day of the spatial training. In relation to the neuronal metabolic activity, EE reduced the COx activity in brain regions related to anxiety response, such as the infralimbic cortex, the paraventricular thalamic and hypothalamic nucleus, the basolateral amygdala, and the ventral hippocampus. Interestingly, there were no significant differences between groups in the dorsal hippocampus, more related to spatial cognition. These results suggest a beneficial effect of EE on spatial memory as a result of reducing anxiety levels and the COx activity in brain regions involved in anxiety response. We also found a differential pattern of activation inside the hippocampus, suggesting that the dorsal hippocampus has a preferential involvement in spatial learning and memory, whereas the ventral hippocampus has a role in anxiety response.

1. Introduction

Anxiety response can be characterized as behavioural and neuroendocrine activation associated with exposure to threatening stimulus. Over a century of behavioural research has revealed a powerful influence of anxiety on learning and memory (James, 1890; Yerkes and Dodson, 1908; Hebb, 1955; McGaugh, 2000). The literature in this area lacks consistency, with studies reporting that high levels of anxiety can enhance, impair or have no effect on learning and memory (Conrad, 2005; Diamond, 2005; Lupien et al., 2005). The majority of studies examining anxiety effects on memory in rats use spatial learning tasks (Diamond and Rose, 1994; Luine et al., 1994). The relationship between anxiety and memory performance follows an inverted U-shaped curve, with better performance when anxiety levels are in an optimal level (Salehi et al., 2012). For example, Herrero et al. (2006) have found that rats with high and low levels of anxiety show different performance in the acquisition and in the retrieval of spatial information. Interestingly, high levels of anxiety influence differently the spatial reference and working memory domains in the radial-arm water maze (RAWM), so the spatial reference memory domain decays sooner than the spatial working memory domain (Hutchinson et al., 2012).

In this context, several studies have reported an anxiolytic effect and improvement of memory function after aerobic exercise and environmental enrichment (EE) protocols (Petrosini et al., 2009; Hutchinson et al., 2012; Sciolino and Holmes, 2012; Kennard and

Abbreviations: EE, environmental enrichment; EZM, elevated zero-maze; 4-RAWM, four-arm radial water maze; COx, cytochrome c oxidase histochemistry; HPA, hypothalamic–pituitary–adrenal axis; GC, glucocorticoids; LTP, long-term potentiation; EPM, elevated plus-maze; CO, control group; SPL, spatial learning group; EE+SPL, environmental enrichment + spatial learning group; EE, environmental enrichment group; TbE, time by entries; MO, medial orbital cortex; Cg, cingulate cortex; PL, prelimbic cortex; IL, infralimbic cortex; Acb, accumbens nucleus; BNST, bed nucleus stria terminalis; CeA, central amygdala; BlA, basolateral amygdala; PVNt, paraventricular thalamic nucleus; PVNh, paraventricular hypothalamic nucleus; dCA1 dCA3, dorsal hippocampal cornu ammonis; dDG, dorsal dentate gyrus; vCA1 vCA3, ventral hippocampal cornu ammonis; vDG, ventral dentate gyrus; RM ANOVA, ANOVA of repeated measures; MANOVA, multivariate analysis of variance; OD, optical density.

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Woodruff-Pak, 2012; Pang and Hannan, 2013). Our research group has found for example, that a protocol of aerobic exercise applied over a period of 2 months in aged rats reduced the cytochrome c oxidase activity (COx) in brain regions traditionally involved in anxiety response such as the amygdala or the bed nucleus of the stria terminalis and this training slightly enhanced the memory performance in the RAWM (Sampedro-Piquero et al., 2013a,b). In the case of EE, this complex housing condition has shown to have important benefits on spatial cognition, maybe due to the effect on the brain plasticity in frontal and parietal cortex, hippocampus, striatum and cerebellum (Ekstrand et al., 2008; Leggio et al., 2005; Vazquez-Sanroman et al., 2013) and also, it reduces the anxiety in a novel situation giving the animals more control over their environment (Van de Weerd et al., 2002). This last positive effect can be explained if we consider the EE as a eustressor that makes the hypothalamic–pituitary–adrenal axis (HPA) more adaptive to the future stressors diminishing the emotional reactivity (Larsson et al., 2002). For example, in unconditioned tests such as the elevated plus-maze (EPM) or the elevated zero-maze (EZM), the animals show a reduction of anxiety with low levels of defecation, more entries into the open sections and lesser latency to enter into the open section (Friske and Gammie, 2005; Heredia et al., 2012). During the cognitive testing, Harris et al. (2009) conclude that the cognitive benefits of EE occur because rats are less anxious during the cognitive task.

It is unknown if the EE condition influences neuronal metabolic activation recruited during a spatial memory task and if this activation changes as a result of lower levels of anxiety. A common method for measuring the neuronal oxidative metabolism, after prolonged stimulation or training in behavioural tasks, is the Cytochrome c oxidase (COx) histochemistry (Begega et al., 2012; Riha et al., 2008; Rubio et al., 2012; Mendez-Lopez et al., 2009a). COx is a mitochondrial enzyme involved in the oxidative phosphorylation process in which ATP is generated for sustaining neuronal functions (Wong-Riley, 1989). This method is suitable to detect regional brain activity changes relative to control conditions (Gonzalez-Lima and Cada, 1994). COx histochemistry has been extensively used in studies of learning and memory in animal species (Begega et al., 2010; Bruchey and Gonzalez-Lima, 2008; Puga et al., 2007; Sakata et al., 2005) and some studies have related high COx activity in brain regions such as the amygdala or the medial mammillary body with high levels of anxiety during the cognitive testing (Sampedro-Piquero et al., 2013a,b; Leger et al., 2012; Conejo et al., 2004).

We assessed the effect of EE on anxiety-related behaviours in the EZM and its impact on working memory and reference memory in the RAWM. We chose to expose the animals only 3 h per day to the EE condition as other studies had already found a positive effect on anxiety response and cognition with restricted daily exposure (Widman et al., 1992; Widman and Rosellini, 1990). We also analyzed the possible changes in the neuronal metabolic activity of several brain regions related to anxiety response and spatial cognition.

2. Materials and methods

2.1. Subjects

Forty male 3-month-old Wistar rats from the vivarium of the University of Oviedo were used. The subjects were housed in groups of five animals. All the animals had ad libitum access to food and tap water and 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). Since rats (Wistar strain) were used as experimental animals, the experimental procedures applied in this research were previously reviewed and accepted by a bioethics committee of the University of Oviedo. In addition, the procedures and manipulation of the animals used in this study were carried out according to 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.

Fig. 1. A typical enriched setting enhancing motor, sensory, cognitive and social stimulation in rats is illustrated in the Fig. 1.

The rats were randomly assigned to four groups: control group (CO: 300.5–346.7 g; $n = 10$), environmental enrichment group (EE: 284.5–342.6 g; $n = 10$), environmental enrichment + spatial learning group (EE + SPL: 298.7–327.2 g; $n = 10$) and spatial learning group (SPL: 302.6–350.3 g; $n = 10$). The CO group was used as a reference of basal COx activity and it consisted of two groups of five rats kept in standard cages without any learning or environmental enrichment experience. When evaluating the impact of EE, it is initially important to consider the use of appropriate control groups. In general, it is recommended that laboratory animals are housed in social groups, because rats reared in isolation display a behavioural pattern called "*social isolation syndrome*" which is associated with hyperactivity in novel environments and poor adaptability, as well as higher impulsivity compared to rats housed in groups (Simpson and Kelly, 2011).

2.2. Environmental enrichment

The EE and EE + SPL groups were housed in large cages of 100 cm × 95 cm × 54 cm (each experimental group in a different cage, ten rats per cage) for a period of 3 h every day (10:00 am/13:00 pm). We ensured that we always put the same group of ten rats together in the EE cages and the stimulating objects were similar in both cages. The rest of the day, the animals submitted to EE were housed in groups of five in standard cages without stimulating objects, as were the control group. In this case, the distribution of the ten rats of each group in the two standard cages was random to ensure that all rats had lived together and so, avoid possible fighting between them. The EE cages contained various objects like toys, running wheels, ropes, plastic tubes of different diameters, platforms, wooden houses, odorous and sound objects and nesting materials. To ensure novelty, the configuration of the cages was changed once a week and the cages were cleaned twice a week. EE rats were placed and maintained in this condition two months before the start of the behavioural testing. The animals were exposed to the EE condition for only 3 h per day (Fig. 1).

2.3. Elevated zero-maze (EZM)

The day before the spatial task, SPL and EE + SPL groups were assessed in the EZM. This maze was constructed 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 quadrants of equal lengths, two open quadrants and two closed quadrants with black acrylic walls 35 cm in height. The session consisted of five minutes, under the same lighting conditions, and the animal was placed in the center of a closed quadrant. When an animal had finished the test, the maze was cleaned with 70% ethanol in order to eliminate odor and start with the next rat. Behavioural 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