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Environmental enrichment for adult rats: Effects on trait and state anxiety

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HIGHLIGHTS

- The effects on anxiety of late-life environmental enrichment were investigated in adult rats.
- Both trait (free-exploratory paradigm) and state (elevated plus-maze test) anxiety were tested.
- Environmental enrichment decreased trait anxiety of highly anxious rats.
- Environmental enrichment did not affect state anxiety.

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ABSTRACT

Experimental evidence indicates that enriched environment (EE) induces neurobiological and behavioural alterations. EE in early life improves learning and memory and reduces trait and state anxiety. However, the effect of EE established in adulthood has rarely been investigated. Thus, the aim of this study was to evaluate the possibility of modifying the levels of trait and/or state anxiety of adult rats exposed to EE. Seventy adult Wistar male rats were first tested in the free-exploratory paradigm (FEP) and were categorized according to their levels of trait anxiety (high, medium and low). Subsequently, half of the animals from each category returned to their home cages (standard condition: SC) and the other half was transferred to an enriched environment (enriched condition: EC). After three weeks, all animals were again tested in FEP. Seven to 10 days later, fifty of the seventy animals were tested on the elevated plus-maze test (EPM). In FEP, EE reduced locomotor activity in the second exposition independently of the anxiety category and, it decreased the levels of trait anxiety of highly anxious rats. No effect of EE was observed on EPM. In conclusion, EE established in adulthood was able to reduce high trait anxiety, a major risk factor for anxiety disorders.

1. Introduction

Previous studies have demonstrated the influence of the environment on brain regulation and behaviour [1]. In these studies, the animals have been kept in an enriched environment (EE), *i.e.*, housing condition containing different objects (assorted colourful toys, tunnels, running wheels, nesting material, ladders, *etc.*) which are frequently changed during the experiment [2,3]. These conditions provide enhanced sensory, cognitive and motor stimulation in comparison to standard housing conditions [2].

It is well documented that EE induces a number of neuroanatomical, neurochemical and behavioural alterations [4,5]. Behaviourally, the EE exerts positive effects on learning and memory [6] and decreases levels of anxiety [7,8]. However, most of the anxiety studies used the elevated plus-maze test. This model confronts the animals with an anxiety provoking situation, modelling the so-called state anxiety, which is the anxiety a subject experiences at a particular moment in time, when facing threat. However, there is another concept, trait anxiety, considered to be an enduring feature of an individual, relatively stable over time [9], and particularly important in anxiety patients, as they tend to present greater anxious trait in comparison to healthy subjects [10].

In 1999, Chapillon et al. [11] assessed the influence of environmental enrichment on trait anxiety levels in mice. In this study,

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BALB/c mice, known as highly emotional, were reared under either enriched or standard conditions and, when adults, were tested on the free-exploratory paradigm, a model of trait anxiety [12,13]. It was observed that animals reared in EE presented a lower anxiety profile in adulthood, in comparison to animals reared in the standard environment, demonstrating the capability of environmental enrichment to modify a personality trait. However, as in most studies in this field, Chapillon's work established the environmental enrichment early in life, in order to observe its effects in adulthood. Exposure to EE at different ages has rarely been investigated.

Bearing all this in mind and taking brain plasticity into consideration [14], it is reasonable to question if animals reared in standard cages since birth and moved to an enriched environment in adulthood could still benefit from its effects. Thus the aim of the present study was to evaluate the possibility of modifying the levels of trait and/or state anxiety of adult rats exposed to EE.

2. Animals, materials and methods

2.1. Animals

Seventy adult (10 weeks) Wistar male rats, obtained from our own colony, were used in the experiment. The animals were kept five per cage (41 cm × 34 cm × 18 cm), in a temperature (22–24 °C) and light (12 h/12 h light/dark cycle, lights on at 06:00 a.m.) controlled room, with water and food *ad libitum*.

Experimental procedures were approved by the local ethical committee (Universidade Federal de Sergipe) and complied with both national (Brazilian National Council on the Control of Animal Experimentation – Law 11.794, of October 8, 2008) and international guidelines (European Community's Council Directive of November 24, 1986–86/609/EEC) for the care of animals.

2.2. Enriched environment

The enriched environment consisted of an arena (100 cm in diameter and 70 cm high), maintained in the same room where the animals were kept since birth, which contained different objects (tunnels, sticks and blocks of several sizes, made of wood or plastic and a running wheel) and nesting material, that were changed three times a week. At each time, the animals were presented with one stick, four wooden blocks and three plastic blocks, which varied in colour and size; they were also presented with fresh nesting material and a tunnel, made of PVC tubes, interconnected in a different way each time. The running wheel was available to the animals at all times. The floor of the arena, which contained five animals at a time, was covered with fresh sawdust and allowed free access to food and water.

2.3. Free-exploratory paradigm

The free-exploratory paradigm (FEP) was set up as described by Antunes et al. [15]. The apparatus consisted of a wooden box, divided into two compartments, with each compartment further subdivided into three exploratory units (20 cm × 20 cm), interconnected by small openings. The two compartments were separated by a removable partition. The box was placed on a stand in the rat home room. Approximately 24 h before testing, the partition was installed and an animal was put into one-half of the apparatus and left there until the test time, in order to become familiarized with it. This familiar half had fresh zeolites (Zoocel Biotério® – Celta Brasil, Cotia, Brazil) covering the floor and the animal had free access to food and water. On the test day, the partition, between the familiar and the novel compartments, was removed and the animal was observed for 15 min, under infra-red light. The following parameters were measured: total distance travelled (TDT), and the time spent in each compartment, from which the percentage of time spent in the novel side (%TNS) was calculated – a parameter considered a reliable measure of trait anxiety in rats [13].

Table 1
TDT results obtained from FEP.

Anxiety category	Type of environment	TDT (m)	
		FEP1	FEP2
High (%TNS < 69.53)	SC (n = 9)	17.04 ± 1.76	17.33 ± 2.33
	EC (n = 8)	17.65 ± 1.90[a]	14.60 ± 1.59[b]
Medium (69.53 < %TNS < 83.14)	SC (n = 14)	20.56 ± 1.18	19.67 ± 1.17
	EC (n = 16)	21.60 ± 1.24[a]	16.50 ± 0.74[b]
Low (%TNS > 83.14)	SC (n = 9)	20.51 ± 1.55	19.12 ± 1.20
	EC (n = 8)	23.58 ± 2.72[a]	17.86 ± 2.32[b]

Data are presented as mean ± SEM.
FEP: free-exploratory paradigm; TDT: total distance travelled; %TNS: percentage of time spent in the novel side; SC: standard condition; EC: enriched condition. Data with the same letter were analyzed as a group, since anxiety category did not interact with the other factors (trial and environment). a *vs* b: $p < 0.01$.

2.4. Elevated plus-maze

The elevated plus-maze (EPM) was set up as described by Pellow et al. [16], except for the lighting, which was red [11]. The apparatus consisted of a wooden maze with two closed arms (50 cm × 10 cm × 40 cm) and two open arms (50 cm × 10 cm) connected by an open central area (10 cm × 10 cm). The arms were arranged such that those of the same type were opposite each other. The maze was positioned 50 cm above the floor. Animals were individually put into the centre of the maze and allowed to explore the apparatus for 5 min. The following parameters were measured: total distance travelled (TDT), percentage of time spent in the open arms (%TOA) and percentage of entries into the open arms (%EOA).

2.5. Procedure

Ten-week-old rats, reared in standard conditions (as described in Section 2.1), were first tested in FEP (FEP1). The obtained results (Table 1) were used to classify the animals according to the %TNS, as presenting high (<1st quartile), medium (≥1st quartile and ≤3rd quartile) or low (>3rd quartile) levels of trait anxiety. Three to six days after FEP1, half of the animals from each category remained in their home cages (standard condition: SC) while the other half was transferred to the enriched environment (enriched condition: EC). After three weeks, all animals were again tested in FEP (FEP2) and returned to their respective environments. Seven to 10 days later, fifty of the seventy animals were tested on EPM.

The two behavioural tests were performed in the dark phase of the light/dark cycle, between 7:00 and 8:00 p.m., and the observed parameters were recorded using a computerized system for animal tracking (Anymaze, Stoelting Co., Wood Dale, IL, USA). Both apparatuses were cleaned using a 10% ethanol solution after each test to eliminate possible odour cues left by previous rats.

2.6. Statistical analysis

All data obtained from FEP were analyzed by three-way ANOVA for repeated measures [factor 1: environment (EC or SC); factor 2: anxiety category (low, medium or high); factor 3: trial (FEP1 or FEP2)]. In the case of significant interaction among all three factors, the analyses were followed by fixing one of the factors and conducting two-way ANOVA for repeated measures on the other