NEUROSCIENCE
RESEARCH ARTICLE
A. Begega et al. / Neuroscience 516 (2023) 75–90

Reorganization of Brain Networks as a Substrate of Resilience: An Analysis of Cytochrome c Oxidase Activity in Rats

Azucena Begega, [a]* **Ignacio Cuesta Lopez,** [a] **Marcelino Cuesta Izquierdo,** [b] **Claudia I. Jove,** [a] **Román D Moreno-Fernández** [c] **and Matias López** [b]

[a] *Laboratory of Neuroscience, Institute of Neuroscience of Principality of Asturias, INEUROPA, Faculty of Psychology, Plaza Feijoo s/n, 33003 Oviedo, Spain*

[b] *Department of Psychology, Institute of Neuroscience of Principality of Asturias, INEUROPA, Faculty of Psychology, Plaza Feijoo s/n, 33003 Oviedo, Spain*

[c] *Faculty of Education and Psychology, University Francisco de Vitoria, Pozuelo de Alarcón, 28223 Madrid, Spain*

Abstract—The unpredictable chronic mild stress (UCMS) model has been used to induce depressive-like symptoms in animal models, showing adequate predictive validity. Our work aims to evaluate the effects of environmental enrichment (EE) on resilience in this experimental model of depression. We also aim to assess changes in brain connectivity using cytochrome c oxidase histochemistry in cerebral regions related to cognitive-affective processes associated with depressive disorder: dorsal hippocampus, prefrontal cortex, amygdala, accumbens, and habenula nuclei. Five groups of rats were used: UCMS, EE, EE + UCMS (enrichment + stress), BG (basal level of brain activity), and CONT (behavioral tests only). We assessed the hedonic responses elicited by sucrose solution using a consumption test; the anxiety level was evaluated using the elevated zero maze test, and the unconditioned fear responses were assessed by the cat odor test. The behavioral results showed that the UCMS protocol induces elevated anhedonia and anxiety. But these responses are attenuated previous exposure to EE. Regarding brain activity, the UCMS group showed greater activity in the habenula compared to the EE + UCMS group. EE induced a functional reorganization of brain activity. The EE + UCMS and UCMS groups showed different patterns of connections between brain regions. Our results showed that EE favors greater resilience and could reduce vulnerability to disorders such as depression and anxiety, modifying metabolic brain activity.

Key words: brain networks, resilience, environmental enrichment, cytochrome c oxidase, rat.

INTRODUCTION

Chronic stress can cause or worsen many serious health problems, including depression, anxiety, and neurogenerative diseases, such as Alzheimer's dementia, which affects the older population. Currently, the World Health Organization estimates that 4.4% of the global population suffers from depression and 3.6% from anxiety disorder (WHO, 2017; Schulz, 2020). Despite the development of pharmacological treatments for depression with antidepressants, this disorder shows low remission and high relapse rates. The efficacy of pharmacological treatments is very low, as only 30% of depressive patients will reach remission (Francis and Lobo, 2016). Furthermore, depression is typically accompanied by emotional symptoms, such as loss of interest, sadness, and the inability to feel pleasure, known as anhedonia. This symptom is considered key in depressive disorders and represents a prognostic indicator of treatment-resistant major depressive disorder (MDD) (Scheggi et al., 2018; Coccurello, 2019; Stanton et al., 2019), hopelessness and anxiety (Allen et al., 2018), as well as cognitive symptoms, such as attention dysfunction, and alterations of the autonomic nervous system, causing weight loss and sleep disturbances (Grahek et al., 2018). A key factor for the development of depression is chronic stress (McEwen and Akil, 2020). The unpredictable chronic mild stress model (UCMS) has been used to induce depressive-like symptoms in animal models, showing adequate predictive validity. This model requires exposure to a wide variety of unpredictable uncontrollable stressful stimuli that induce anhedonia

*Corresponding author.
E-mail address: begega@uniovi.es (A. Begega).

Abbreviations: ACC, Anterior Cingulate Cortex; AMG, Amygdalae complex; CCO, cytochrome c oxidase; dCA1, dCA3, dorsal Cornu Amonnis; dGD, dorsal Gyrus dentate; EZM, Elevated Zero Maze; Hab, habenula nucleus; HPA, Hypothalamus - Pituitary -Adrenal glands; IL, infralimbic region; LhB, lateral habenula; mPFC, medial Prefrontal Cortex;; NAc, accumbens nucleus; PrL, prelimbic cortex; RTMg, Rostrotegmental nucleus; Sg ACC, subgenual anterior cingulate region; UCMS, unpredictable chronic mild stress; VTA, ventral tegmental area.

https://doi.org/10.1016/j.neuroscience.2023.01.012
0306-4522/

(Antoniuk et al., 2019; Fornaguera and Brenes, 2019). Previous research has used the UCMS protocol at early ages of postnatal development to study whether early stress exposure can increase psychiatric disorder vulnerability later in life. However, the effect of chronic stress exposure during adulthood has been less addressed. It would be interesting to study the regulatory mechanisms of chronic stress in adulthood. Few studies have examined this issue in female animals (e.g., Baker et al., 2006) or compared both sexes (e.g., Burke et al., 2016; Konkle et al. 2003), with inconsistent results. In general, it has been observed that female rats show less anhedonia than male rats when consuming sucrose; in contrast, sucrose preference is unaffected by stressors. In addition, in the case of female rats, it has been observed that the phase of the estrous cycle is a relevant factor in brain dynamics. It has been observed that the presence of estrogens favors neuroplasticity, protects against oxidative stress, maintains mitochondrial activity levels, and increases the release of endorphins. However, the estrous cycle in female rats is short (4–5 days), and the phases that compose it only last a few hours. This short duration adds a greater difficulty (increased variability of the results, need for a larger sample, etc.) when accurately determining the effects of estrogen on brain activity. In general, it is not only of interest to know the consequences of stress but also the mechanisms associated with allostasis and recovery during adult life. These mechanisms could control the deleterious effects of stress and reduce vulnerability to depression and anxiety. Chronic stress exposure does not induce a permanent impact in all cases. Some subjects who suffer chronic stress episodes do not show negative consequences and instead are resilient. Resilience is defined as the subject's ability to recover after a situation of chronic stress (Lambert et al., 2020). Resilient individuals do not show vulnerability to depression, addictions, and neurodegenerative diseases after a prolonged situation of chronic stress. It would be interesting to study the neurobiological mechanisms of resilient individuals and determine how we could promote or favor resilience.

Emotional and cognitive symptoms of depression involve a vast brain network. The hyperactivity of brain areas involved in the response of the hypothalamic–pituitaryadrenal (HPA) axis in situations of chronic stress produces a sustained increase in cortisol levels (Menke, 2019). The amygdala plays a central role, with an activating influence on the HPA axis. Contrary to the amygdala, the hippocampus and the medial prefrontal cortex (mPFc) exert an inhibitory effect on the HPA axis, acting as homeostatic control mechanisms. Both regions have been repeatedly implicated in the pathophysiology and progression of depression. In fact, continuous secretion of cortisol reduces hippocampal volume in individuals with MDD and loss of synaptic contacts' density in the CA3 hippocampal region (McEwen, 2016; Ortiz and Conrad, 2018; Belleau et al., 2019). The hippocampal DG is also affected by chronic stress. Sustained stress causes a reduction in DG volume affecting granular neurons and neurogenesis (Umschweif et al., 2021). Interventions such as exercise, environmental enrichment (EE), or fluoxetine treatment facilitate neurogenesis and induce greater resilience. The mPFc is also affected by stress. This brain region plays a role in executive functions, including, among others, attention, cognitive flexibility, decision-making, and working memory, and it is also involved in the processing of aversive and appetitive stimuli. Impairments in these functions may persist among many MDD patients after recovery from depression (Bortolato et al., 2016). Functional imaging studies show mPFc hyperactivity in pharmacological treatment resistant MDD patients, with the subgenual anterior cingulate cortex (Sg ACC) being the most hyperactivated region. This increased activity of the Sg ACC is also shown in MDD patients after chronic stress exposure (Roca et al., 2016; Girotti et al., 2017; Bittar and Labonté, 2021). Studies in rodents also confirm similar effects on the infralimbic region (IL), the rodent anatomical homolog of the Sg ACC. This hyperactivity supports the hypothesis of the IL playing a role in the motivational neural circuit through its connections with the nucleus accumbens (NAc). The NAc is part of the brain circuit of reinforcement and regulates emotional stimulus–response associations (Dutcher and Creswell, 2018). This region integrates afferents from the basolateral amygdala (BLA), mPFc and hippocampus. In addition, the NAc maintains GABAergic connections with the ventral tegmental area (VTA). It has been proposed that Gabaergic neurons in the NAc are related to resilience and low risk of depression (Zhu et al., 2017). The habenula (Hab) is also part of the brain network involved in depression and plays an adaptive regulatory role in stress regulation due to its connectivity with the HPA axis. In fact, the CRH1 receptors of the lateral Hab are activated by restraint conditions. The Hab drives motivational symptoms in MDD. Severe anhedonic symptoms are associated with the Hab's hyperactivity observed in MDD. For all these reasons, the Hab plays a relevant role in the brain network that regulates reinforcement, motivation, and hedonic state, due to its connections with the NAc and VTA (Aizawa and Zhu, 2019; Gold and Kadriu, 2019).

Recent studies have identified a complex network of brain regions related to emotional and cognitive control, which participates in depressive disorder (Allen et al., 2018). The interest is focused on how to prevent depression and its symptoms. Different interventions were proposed to reduce the effects of chronic stress and thus promote resilience (Hearing et al., 2016). Environmental enrichment (EE) is one of these interventions (Lucassen et al., 2016; Smith, 2019). EE is an experimental paradigm used to explore how a complex, stimulating environment can impact overall health. In laboratory animal experiments, EE housing conditions typically include larger-than-standard cages, abundant bedding, running wheels, mazes, toys, and shelters, which are rearranged regularly to further increase novelty and exploratory behavior. EE reduces anxiety and improves allocentric spatial memory in aged rats (Sampedro-Piquero et al., 2013; Sampedro-Piquero and Begega, 2017). EE can act as a stress inoculator, lessening the negative effects of chronic stress on depressive symptoms by increasing resilience(Ashokan et al., 2016; Mahati et al., 2016). Con-

sidering that EE has a beneficial impact on the brain, those subjects subjected to an EE protocol could show brain functional restructuring, and the new configurations of brain networks would show differences between vulnerable subjects versus resilient subjects (Gonzalez-Lima et al., 2014). The precise neurobiological mechanisms of how EE improves performance in this behavioral paradigm are still unknown. The EE protocols increase neurogenesis, dendritic arborization and spinogenesis, expression and release of neurotransmitters, mainly serotonergic neurotransmission, and trophic factors such as BDNF (Jha et al., 2011). Studies in mice show that, after exposure to EE, repetitive motor behavior is reduced (Hattori et al., 2007). This effect is related to an increase in neuronal activity, with an increase in dendritic spine density in subthalamic nucleus and globus pallidus (Bechar et al, 2016). In the case of depressive disorder, hippocampal neurogenesis is compromised. Stress is one of the most important factors in reducing the number of granular neurons in the DG. Thus, the effects of prenatal stress in rats cause a decrease in the dendritic area, the density of spines, and the granular cells in the Hippocampus. These effects are reduced by the EE, showing an increase in markers of synaptic plasticity including the neural cell adhesion molecule (N-CAM) synaptophysin (SYP) in the cortex and hippocampus (McCreary and Metz, 2016). These benefits are observed even after the application of EE for 6 h during 14 days. In this case, an improvement in spatial learning, a decrease in anxiety, anhedonia, and despair behavior is observed in depressive-like subjects. These changes are accompanied by an alteration in the hippocampal 5-HT 1A receptors (Mahati K. et al. 2016). Authors as Schloesser et al. (2010) mention that this greater neurogenic response associated with EE facilitates greater adaptability because the newborn cells in the DG may be important for the animal to perceive a similar negative environment (SC), and to then adopt new coping strategies to combat it. This suggests that EE increases stress resiliency and promotes adaptive coping (Schloesser et al., 2010). Chronic stress influences brain metabolism, affecting mitochondrial functioning in regions related to stress control. The activity of cytochrome c oxidase (CCO), the terminal enzyme in the mitochondrial electron transport chain, reflects long-term trends in energy demands, linking information about neurometabolic profiles and behavioral phenotypes (Harro et al., 2011; Allen et al., 2018). EE promotes positive effects, as it helps to maintain redox homeostasis, which may reduce susceptibility to stress and its oxidative impact (Pang and Hannan, 2013; Queen et al., 2020).

The main of this study is to examine the functional organization of the brain regions affected by stress using a UCMS protocol, the protective effect of EE on these networks and their behavioral consequences. We aimed to analyze the functional effect of chronic stress induced by UCMS on CCO activity in brain regions related to the depressive disorder and its symptoms. We expect to establish the behavioral pattern of resilient versus vulnerable subjects and the brain networks associated with these behaviors, analyzing whether those resilient subjects show a reorganization of brain connections compared to the group of vulnerable subjects.

EXPERIMENTAL PROCEDURES

Subjects

Sixty-five adult male rats supplied by the University of Sevilla vivarium (Spain) were used. They were 7 weeks old at the start of the experiment and with a mean weight of 200-250gr. Upon arrival, the rats were accommodated in the animal facilities of the University of Oviedo with a pattern of 12 hours of light and dark (8:00–20:00 light/20:00–8:00 dark), relative humidity of 65–70%, and a temperature of 20 ± 1 °C. All the procedures were carried out during the day and followed the Directive 2010/63/EU of the European Parliament and the Spanish regulation for the protection of animals used in experimentation. The different procedures were implemented once the weight of the animals had stabilized after arrival at the laboratory to 250–300 grams and the subjects were 12 weeks old.

The animals were randomly assigned to: Basal group (BG, n:10), housed under normal conditions without any intervention and used to establish basal levels of CCO activity; Control group (CONT, n:15), housed under normal conditions and submitted to behavioral tests (sucrose consume, anxiety, and cat odor tests); Unpredictable Chronic Mild Stress group (UCMS, n:15), submitted to a UCMS protocol and the behavioral tasks discussed above; Environmental Enrichment group (EE, n:10), housed under novel stimuli conditions that ensure exposure to novelty, interaction and physical activity; Environmental Enrichment in Unpredictable Chronic Mild Stress group (EE + UCMS, n:15), exposed to EE before experiencing chronic stress by UCMS protocol and behavioral tests. The total number of subjects necessary to reach a test power of 1- β: 95% was calculated. We used the Gpower sofware and to analyze the centrality indices with the JASP program has been calculated.

Within each condition, subjects were randomly divided into three cages (55 cm × 20 cm × 35 cm) of five animals each. They had unlimited access to both food and water.

Unpredictable Chronic Mild Stress (UCMS)

Chronic exposure to moderate and unpredictable stressors (UCMS) is a chronic-stress paradigm based on the successive and repeated application of different stressors that, when presented individually, would not necessarily involve risk to subjects but, when combined, can induce long-lasting behavioral and brain alterations. This protocol has proven to be a powerful model of animal depression, with high levels of predictive validity (subjects subjected to this condition show a response to antidepressants comparable to that observed in cases of depression in humans), face validity (it is related to anhedonia, weight reduction, and alterations in the hippocampus, prefrontal areas, and the normal functioning of the HPA axis), and construct validity (as

chronic exposure to stress is an important risk factor for developing depression)(Willner,2017a; 2017b).

During the four consecutive weeks in which the protocol was carried out, rats were exposed to different stressors: (a) abnormal cage inclination (45° for 6 hours), (b) wet sawdust (300 ml of water poured on the sawdust for 6 hours), (c) food deprivation (total deprivation for 24 hours), d) water deprivation (total deprivation for 5 hours + 1 hour with empty bottle), e) crowding (grouping of 7–8 animals in the same cage, 6 hours), f) immobilization (immobilization in a restraint tube for 5–15 minutes), g) flashing light (300 flashes/second for 6 hours). All the stressors were administered to them in their home cages. Each stressor is applied only once a week. In addition, the presentation sequence is modified every week. Only the duration of restraint is modified each week: from 5 minutes in the first week to 20 minutes in the last week. The duration of the rest of the stressors remains constant throughout the chronic stress protocol.

Environmental Enrichment

The Environmental Enrichment (EE) protocol was applied for 4 consecutive weeks. For this purpose, the 15 animals were grouped in a cage (100 cm × 95 cm × 54 cm). The cage contained different types of objects, ropes, wooden platforms, and plastic tubes of different shapes. Once a week, the arrangement of these objects was changed, and some of them were replaced by new ones, to ensure the presence of novel stimuli. Compared to the standard housing condition, EE favors exercise and interaction of the rodents both with the environment and each other. To ensure the animals' welfare, they were weighed weekly; a time used to thoroughly clean the cage.

Anhedonia: licking behaviour analysis

The anhedonia protocol took place in a dimly lit room containing 8 custom-made drinking boxes measuring 42 × 25 × 20 cm, with acrylic walls, steel mesh flooring, and wire mesh lids. Drinking bottles (50 ml) with metal spouts could be inserted at one end of each box. A contact-sensitive lickometer registered the licks made by rats to the nearest 0.01 sec, and MED-PC software (Med Associates, Inc., Fairfax, VT) controlled the equipment and recorded the data. The anhedonia protocol involves analyzing the microstructure of licking behavior during voluntary consumption (Davis and Smith, 1992; Dwyer, 2012). The ingestive behavior of rats consuming fluids consists of sustained runs of licks separated by pauses of varying length (clusters), with each cluster separated by pauses, typically > 0.5 s. The mean number of licks per cluster (lick cluster size) provides a measure of hedonic value of the fluid which is dissociable from consumption levels and directly related to the nature and concentration of the ingested solution. Lick cluster size monotonically increases with the concentration of palatable sweet solutions, whereas it decreases monotonically with the increasing concentration of unpalatable quinine solutions (Davis and Smith, 1992; Spector et al., 1998). In the context of ingestive behavior, there is evidence that incentive devaluation not only results in reduced intake but also in a decrease in the hedonic value or palatability of the devalued reward (Grigson et al., 1993; Strickland et al., 2018).

In this study, the experimental procedure for assessing anhedonia consisted of two phases: Pre and Post. Before the start of the experiment, the rats received two sessions of habituation to the drinking boxes during which they had access to a bottle containing water for 10 min. The Pre- phase consisted of three 10-min sessions (one per day) during which rats had access to a bottle containing a 4% sucrose solution. In the Post-phase (two days), the rats were given the sucrose solution in the drinking boxes for 10 min. Consumption, total number of licks, and lick cluster size were recorded across the experimental sessions. Four hours before each experimental session, the rats were moved to a water-deprivation schedule with 60-min access to water in the home cage per day, given approximately 1 h after the experimental sessions. Between phases Pre and Post, subjects in groups UCMS and EE + UCMS received the treatment described above (mild stress and enrichment + stress, respectively) for four weeks to assess the effect of these manipulations on the hedonic evaluation of the sucrose solution Fig. 1.

Anxiety: Elevated Zero Maze

The apparatus used for assessing anxiety in CONT, UCMS, and EE + UCMS groups was an Elevated Zero Maze (EZM). The EZM is a variant of the elevated plus maze (Czéh et al., 2016). It consists of a circular platform constructed in Perspex, 6.1 cm wide and 40 cm in diameter, elevated 72.4 cm from the ground, and divided into four quadrants of the same size. Two of these parts are protected by vertical black acrylic walls (20.3 cm high), giving the animals a sense of protection. In contrast to the previous ones, the other two sections are devoid of walls, giving greater environmental exposure.

The device was situated in an experimental room specially equipped to carry out this type of test. The session was carried out one week after passing the preference test. Each session was recorded with a video camera connected to a computer equipped with a video tracking program (Ethovision Pro, Noldus Information Technologies, Wageningen, The Netherlands). The following variables were recorded: a) number of entries into the open area, b) time spent in each area, c) latency of entry into the open arm, d) time by entry (this variable is a correction of the time spent by the animal in the open arm considering the level of activity). All of them are considered anxiety indexes. The animals were transferred individually from the animal house to the room where the experiment took place. Then, the experimenter placed the animal in the center of one of the protected arms for 5 minutes. Each subject was exposed only once to the maze to avoid the risk of habituation.

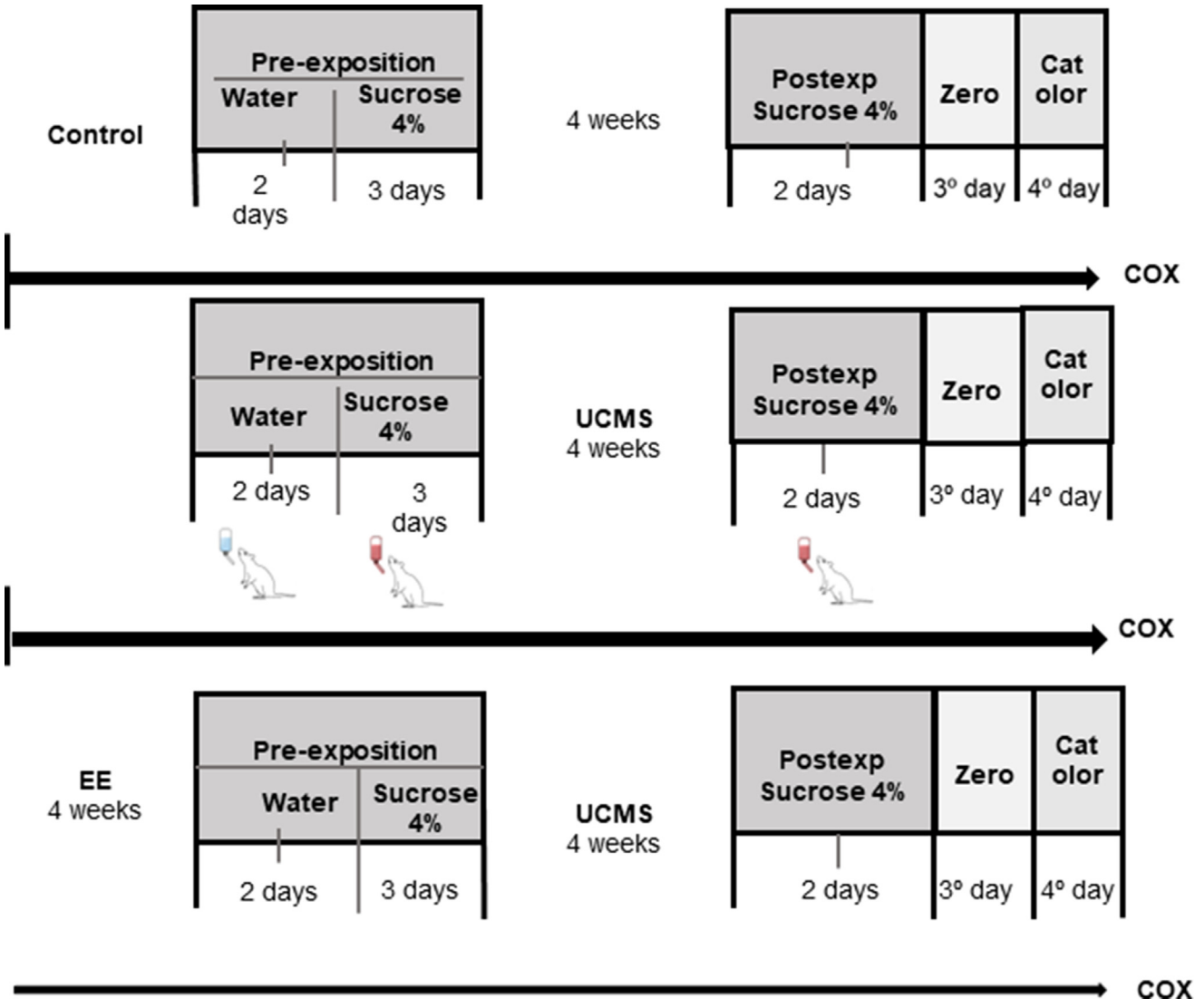


Fig. 1. Global timing of the experimental design. Experimental groups of male Wistar (control, UCMS, and EE + UCMS). Pre-phase of sucrose consumption. Unpredictable chronic stress paradigm (four weeks); post-phase of sucrose consumption, zero maze, and cat odor tasks.

Unconditioned fear: Cat odor test

The exposure cage consisted of a rectangular arena (20 cm × 60 cm × 22 cm) divided into three areas (near odor zone, intermediate odor zone, and remote odor zone (20 cm × 20 cm)). To assess the rats' behavior related to the closeness of a predator, we used three towels that had been in daily contact with domestic cats for two months. The towels were stored in a refrigerator at 4 °C when they were removed from the cat, and 15 min before starting the test, the odor stimulus was at room temperature (Sampedro-Piquero, et al., 2016). Twenty-four hours after the habituation session (5 min), in which the rats were habituated to the cage context in one session with no odor present, the rats were exposed to a cat odor stimulus (a towel) presented inside a cylinder with grid bars, allowing the rat to smell the odor for 5 min. In the experimental session, each rat was placed in the central zone opposite the zone containing the odor stimulus. Each session was recorded with a video camera connected to a computer equipped with a video tracking program (Ethovision Pro, Noldus Information Technologies, Wageningen, The Netherlands). We recorded: a) the total time in each zone, b) the duration of normal posture, and rearing and stretching behavior. A stretch-attend posture occurs during risk assessment and is a component of defensive behaviors.

Cytochrome c oxidase (CCO): analysis of brain activity

Once the behavioral evaluation was completed, the animals were decapitated. The brain tissue was extracted intact and frozen in isopentane at −40 °C (Sigma-Aldrich, Madrid, Spain). Subsequently, the brain tissue was sectioned at 30 micrometers with the aid of a cryostat (Leica CM1900, Germany). The sections were mounted on non-gelatinized slides and fixed at room temperature, and then stored at −40 °C until staining.

CCO enzyme is a membrane protein involved in the electron transport chain responsible for ATP synthesis and, therefore, closely related to energy production at the cellular level. This enzyme is considered a direct indicator of neuronal activity in brain regions (53). For its quantification, we followed the histochemical analysis protocol for the study of CCO described by Gonzalez-Lima and Cada (1994). The sections were fixed for 5 minutes in 0.1 M phosphate buffer, with 10% sucrose (w/v) and 0.5% glutaraldehyde (v/v), pH 7.6. They were then passed through four successive baths of 5 minutes each in 0.1 M phosphate buffer, 10% (w/v) sucrose, and then placed for 10 minutes in 0.05 M Tris buffer, pH 7.6. They were placed in 0.1 M phosphate buffer at 37 °C for one hour and then placed for 30 minutes at room temperature in a formalin buffer solution with 10% (w/v) sucrose and 4% (v/v) formalin. Finally, the tissues were dehydrated by successive baths in increasing concentrations of ethanol (70% to 100% v/v). Finally, the sections were stored for further processing. Quantification of CCO activity was performed by densitometry. To quantify enzymatic activity and control staining variability across different baths, sets of tissue homogenate standards from rat brains (Poremba, Jones, & Gonzalez-Lima, 1998) were cut at different thicknesses (10, 30, 40, and 60 μm), and included in each COx staining bath together with the experimental brain sections. The batch standards of brain homogenate were previously analyzed by spectrophotometrical methods to measure mean COx activity and were used to generate a single regression equation between COx activity and the optical density of the experimental sections, as reference for the comparison of the different tissues.

Calibration of OD measures for COx activity units was performed using the stained homogenate standards for each staining batch. For each staining batch, the software calculated a linear regression between optical density and COx activity, using the measured OD attributed to each section. Average relative OD measured in each brain region was converted into COx activity units (1 unit: 1 μmol of cytochrome c oxidized/min/g tissue wet weight at 23 °C), using the calculated regression curve in each homogenate standard. The linear regression equations calculated to estimate COx activity from OD measures in the brain sections were also used to assess inter-batch variability.

The brain regions selected were defined according to the coordinates of the Paxinos and Watson (2013) atlas. These regions were: the anterior cingulate cortex (ACC), the prelimbic cortex (PrL), the infralimbic cortex (IL), the nucleus accumbens (NAc), the dorsal hippocampal region (dCA1, dCA3d, and dGD), the Amygdalae complex (AMG), and the Habenula (Hab). These brain regions correspond to the following atlas coordinates (Bregma): 3.24 mm (ACC, PrL, IL), 2.52 mm NAc; −3.36 mm (dCA1, dCA3 and dGD); −2.28 mm (AMG) and −2.76 (Hab) (Fig. 2).

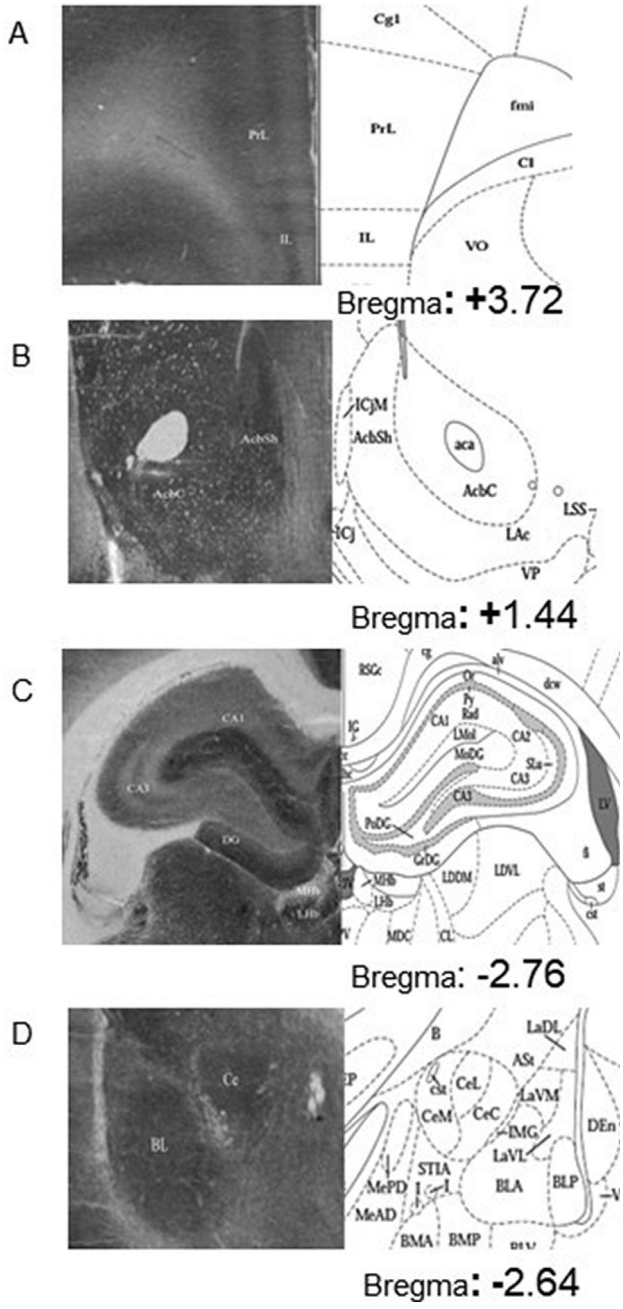


Fig. 2. Representative photographs of the different regions that were stained for the cytochrome c oxidase technique (CCO). (**A**) Prefrontal regions, (**B**) Accumbens nuclei, (**C**) dorsal Hippocampus and (**D**) Amygdalae complex.

Statistical analysis

Behavioral and histological data were analyzed with SPSS 23 (SPSS Inc. Chicago, USA). The results were presented graphically with SigmaPlot 12.0 (Systat, Richmond, EEUU) and Microsof Excel program. The results were presented with box-and-whiskers plot, with min–max showing all points. Median values are indicated by horizontal lines with mean(x) in the center. The horizontal boundaries of the boxes represent the interquartile range (first and third quartiles).

For the anhedonia protocol, sucrose consumption was measured by weighing the bottles before and after each experimental session. For the analysis of mean lick cluster size, a cluster was defined as a series of licks

separated by pauses of no more than a 0.5-s interval, a criterion used in our previous studies examining flavor aversion by licking analysis (Dwyer, 2012). Data from consumption, total licks, and lick cluster size during Pre (3 days) and Post (2 days) phases were analyzed by mixed analyses of variance (ANOVAs) with Group as a between-subject factor and a within-subject factor of Days. Multivariate analyses of variance (MANOVA) were performed to analyze data from the zero maze and cat odor tests. The Bonferroni test was used for post hoc analyses.

Brain Networks

Network analysis was performed to study the functional brain connections of the different groups using JASP 16.01 software (University of Amsterdam). Network models can be used to analyse the dynamic interactions between several brain regions. In our research, brain regions are represented by nodes, while edges are their functional interactions. We used partial correlations to estimate the networks. We decided to use partial correlations instead of the classical Pearson correlation because they allow us to remove the common variance in the relationship between two variables due to their relationship with the rest of the variables. Thus, the influence of the rest of the variables is blocked when calculating the relationship between two specific variables (Epskamp and Fried, 2018). In addition to the weight matrix and the graphical representation of the network, this model calculates several indexes that describe the network. Here, these indexes are a way of operationalizing the importance of a node based on the connections with other nodes (Costantini et al., 2015). In our study, we consider four different measures (degree, closeness, betweenness, and expected influences). Closeness shows the capacity of a region to influence and be influenced by other regions. Having a high closeness index depends on the number of connections a node has and how strong they are. These nodes are represented in the middle of the network graphic. Betweenness reveals the importance of a node as a mediator. A high betweenness index indicates many paths uniting nodes passing through a concrete node. Expected influences indicate the influence of a node considering not only the number of connections and how strong they are (as the closeness index does) but also whether these connections are positive or negative. In some cases (when there are several negative connections in our network), the influence of a node can be compensated because some of its connections are positive and others negative, both having similar strengths. When this happens, even when the closeness index is high, the real influence of a node could be limited.

RESULTS

Behavioral data: Anhedonia test

The mixed ANOVA performed with the consumption data from the Pre phase (Pre 1–3) and Post phase (4–5 day) revealed no significant effects of group, amounted of sucrose consumed ($F_{2,42} = 2.07$, $p = 0.13$; $\eta^2 = 0.09$), total number of licks ($F_{2,42} = 1.76$, $p = 0.18$; $\eta^2 = 0.07$), or lick cluster size ($F_{2,42} = 0.572$, $p = 0.56$; $\eta^2 = 0.02$), but there were statistically significant differences between the days ($F_{12,31} = 6.14$, $p = 0.001$ $\eta^2 = 0.70$) and the Days $\times$ Group interaction ($F_{24,64} = 1.83$, $p = 0.029$ $\eta^2 = 0.40$). So, after exposure to 4 weeks of chronic stress, there were statistically significant differences between groups on the first post-day ($F_{6,82} = 2.33$, $p = 0.03$; $\eta^2 = 0.14$; with lower consumption in the case of the UCMS group compared to the control group ($p = 0.019$) and the EE + UCMS group ($p = 0.05$). The same effect was observed in the case of the total number of licks, $F_{2,42} = 4.56$, $p = 0{,}016$; $\eta^2 = 0.17$, where the UCMS group showed a statistically significant reduction in the total number of licks on two days (post phase) compared to the control group ($p = 0.024$) and the EE + UCMS group ($p = 0.019$). The second post day, the group EE + UCMS showed a statistically significant higher total number of licks than UCMS group ($p = 0.002$), but no statistically significant difference compared to the control group ($p = 1$). These results indicate that the stress treatment (group UCMS) reduced the hedonic evaluation of the sucrose solution and that group EE + UCMS attenuated the negative effects of the stress manipulation. The data from the anhedonia test are shown in Fig. 3.

Anxiety: Elevated Zero Maze

We found significant differences between the groups in the EZM in the number of entries into the open arm ($F_{2,40} = 12{,}77$, $p = 0.001$; $\eta^2 = 0.40$). The group CONT showed a higher number of entries into the open zone compared to UCMS ($p = 0.001$) and EE + UCMS ($p = 0.001$). However, no differences were found between the groups submitted to stress, UCMS, and EE + UCMS. Regarding the latency of entry into the open arm, ($F_{2,40} = 37{,}48$, $p = 0.001$; $\eta^2 = 0.65$). There was no significant difference between CONT and EE + UCMS ($p = 0.118$), but UCMS presented higher latency than the rest of the groups ($p = 0.001$, $p = 0.001$). In the time by entry index, there are statistically between groups ($F_{2,40} = 16{,}54$, $p = 0.001$; $\eta^2 = 0.45$). There were no differences between the groups UCMS and EE + UCMS in the time by entry index ($p = 0.186$). However, CONT showed significant differences in this index compared to UCMS ($p = 0.002$) and EE + UCMs ($p = 0.001$). Data from this test are shown in Fig. 4.

Unconditioned fear: Cat odor test

As for the unconditioned fear data, there were significant differences between the groups in the time they spent in the cat odor zone ($F_{2,41} = 7.16$, $p = 0.002$; $\eta^2 = 0.26$). The group EE + UCMS spent less time in this zone than the group CONT ($p = 0.002$) and the group UCMS ($p = 0.014$). In the central zone, there were significant differences between groups ($F_{2,41} = 5.268$, p: 0.009; $\eta^2 = 0.20$). The group

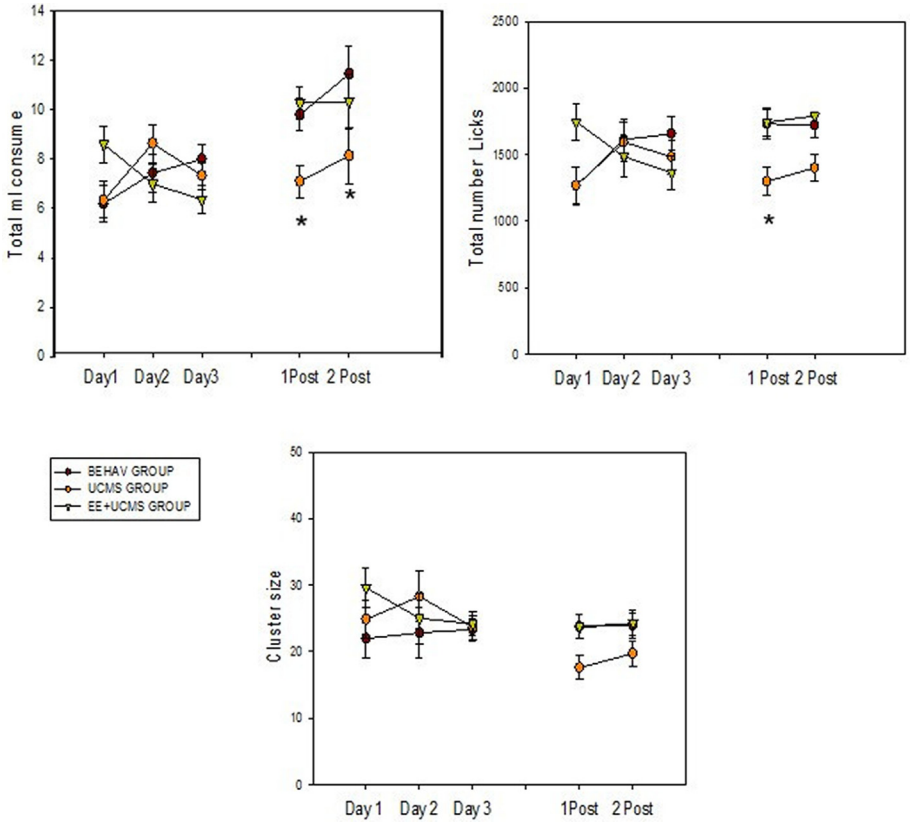


Fig. 3. Behavioral results in the anhedonia task. In the post phase, the UCMS group shows a lower consumption of sucrose than the other groups, $p \leq 0.05$ and a lower number of licks than the EE + UCMS group and control group $p < 0.05$; * represent the statistically significant differences between the UCMS and the Control and EE-UCMS groups. The cluster size of the two phases over the days shown no statistically significant group differences.

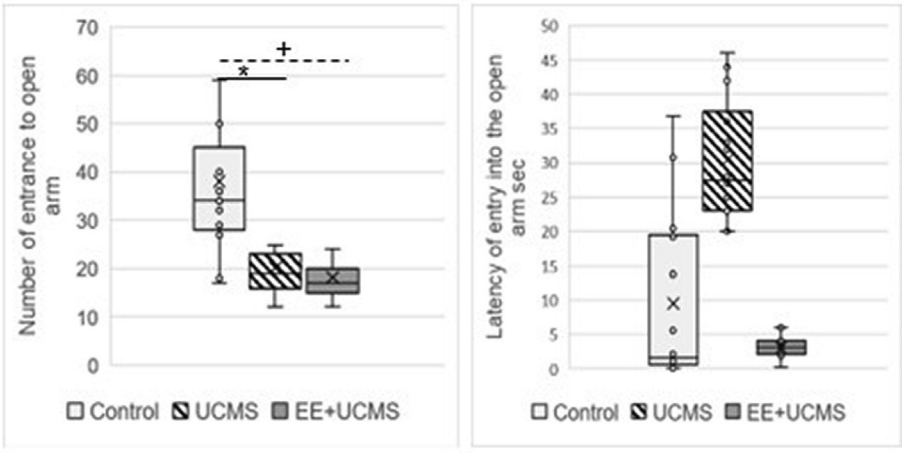


Fig. 4. Total number of visits (frequency)and latency to enter the open arm in the zero maze. The Control group showed a higher number of entries into the open zone*p = 0.001. But the UCMS group showed a higher latency to enter the open arm than the rest of the groups*p = 0.001. Median values are indicated by horizontal lines with mean (x) in the center.

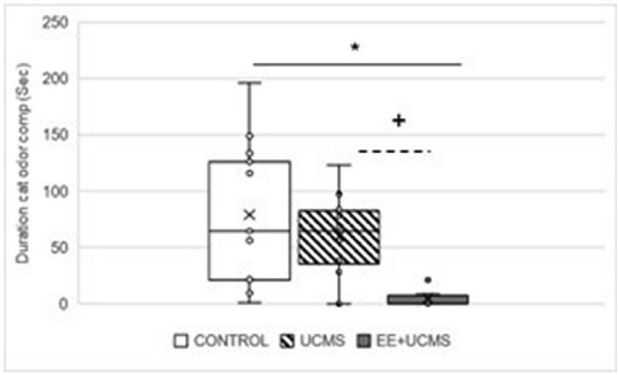
EE + UCMS spent more time in the central zone (close to the dangerous zone) than the CONT (p = 0.049) and UCMS (p = 0.012). The group EE + UCMS could quickly detect the cat odor and remained vigilant in the central compartment. There were no differences between the groups in the time spent in the neutral zone, where no cat odor was located ($F_{2, 41} = 1.26$, p = 0.29, $\eta^2 = 0.05$). Data from this test shown Fig. 5.

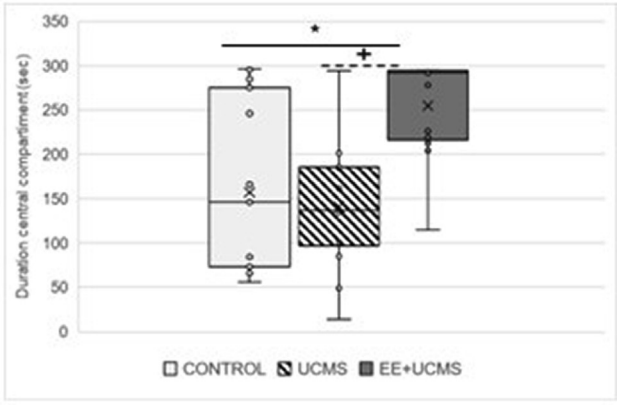
There were differences between groups in their body posture during this task ($F_{8,70} = 4.15$, p = 0.001; $\eta^2 = 0.32$). These differences were found in the normal ($F_{2, 41} = 9.79$; p = 0.001, $\eta 2 = 0.35$, contrated ($F_{2, 41} = 4.78$; p = 0.014, $\eta^2 = 0.20$; and stretched behaviors during cat odor exposure ($F_{2, 41} = 7.04$p = 0.003 $\eta^2 = 0.27$, respectively). The EE group showed a normal posture more frequently and for more time than CONT (p = 0.016) and UCMS(p = 0.001). These differences were not shown during habituation (rearing, p = 0.13; normal, p = 0.10; stretched, p = 0.19). The stretched-attend posture, a defensive behavior, was shown for more time by the group CONT and UCMS compared to the EE + UCMS (p = 0.004 and p = 0.006, respectively). This could be due to greater awareness of a natural aversive stimulus and a higher level of anxiety in the groups CONT and UCMS. In the case of the EE + UCMS group, this pattern was not shown so intensely. Fig. 6 shown the data from this test.

Brain Networks

The quantification of cytochrome c oxidase activity in brain regions revealed statistically significant differences between the groups ($F_{36,160} = 4.27$, p = 0.001 $\eta^2 = 0.49$). The group differences in each brain region are shown in Table 1. In this case, the EE group showed lower cytochrome c oxidase activity than the rest of the groups in all cerebral regions. Furthermore, in the case of the UCMS group, there was always greater brain activity in general. It should be noted that in the case of the UCMS group, this greater activity was statistically significant compared to the group previously subjected to EE.

Regarding the descriptive analysis of brain networks, the JASP program has been applied to assess the





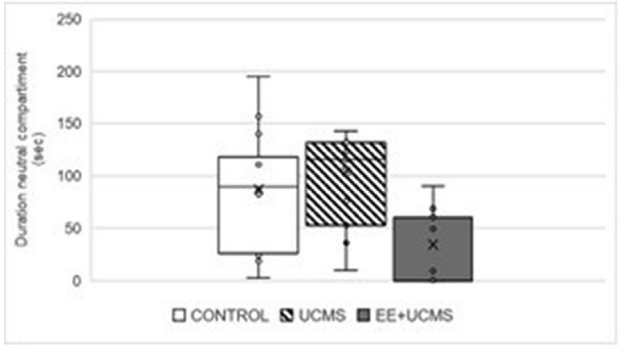
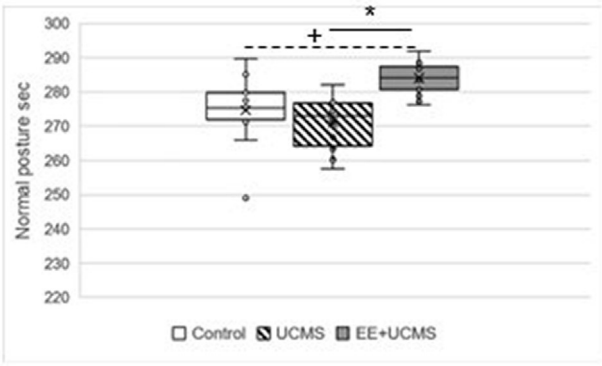
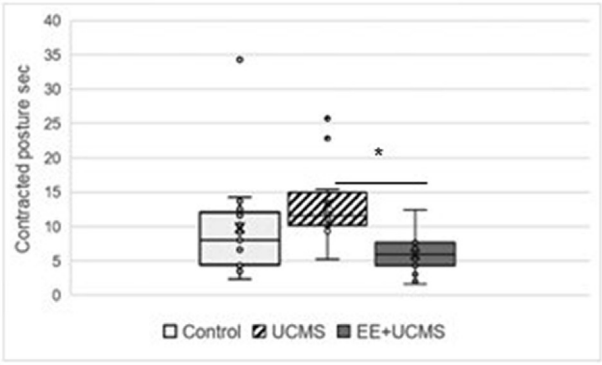


Fig. 5. Time spent in the compartments during the cat odor phase. The EE + UCMS spent less time during the cat odor compartment than the other groups. The group EE + UCMS spent more time in the central zone than the CONT *p = 0.002 and UCMS + p = 0.014. The EE + UCMS group could quickly detect the cat odor and remained vigilant in the central compartment. Median values are indicated by horizontal lines with mean (x) in the center.





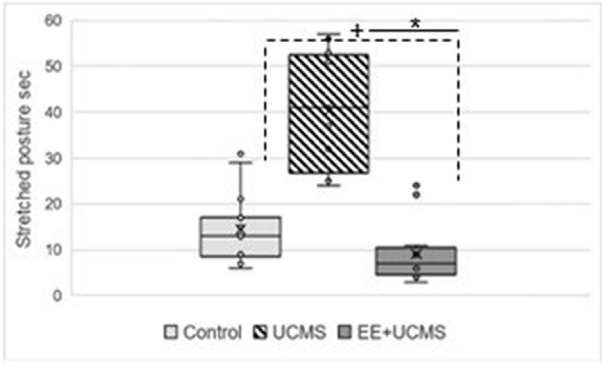


Fig. 6. The box-whisker represents the duration of the body posture during the cat odor phase. Median values are indicated by horizontal lines with mean (x) in the center. In these graphs, the atypical values are also represented, outliers. In this case, the stretching-attend posture, a defensive behavior, was shown for more time by the CONT and UCMS group compared to the EE + UCMS group (*p = 0.004 and + p = 0.006, respectively). This could reflect a greater awareness of a natural aversive stimulus and a higher level of anxiety in the CONT and UCMS groups. The EE group showed a normal posture more frequently and for more time than CONT *p = 0.016 and UCMS, +p = 0.001.

different brain networks related to stress resilience. This analysis shows the neurobiological profiles of the groups, allowing us to compare the effect of UCMS and EE. The importance and contribution of different brain regions is established, reflecting their functional implication in the control of resilience versus vulnerability. The betweenness, closeness, and expected influences were calculated in each experimental group Table 2. The partial correlation analyses are shown in Figs. 7 and 8.

DISCUSSION

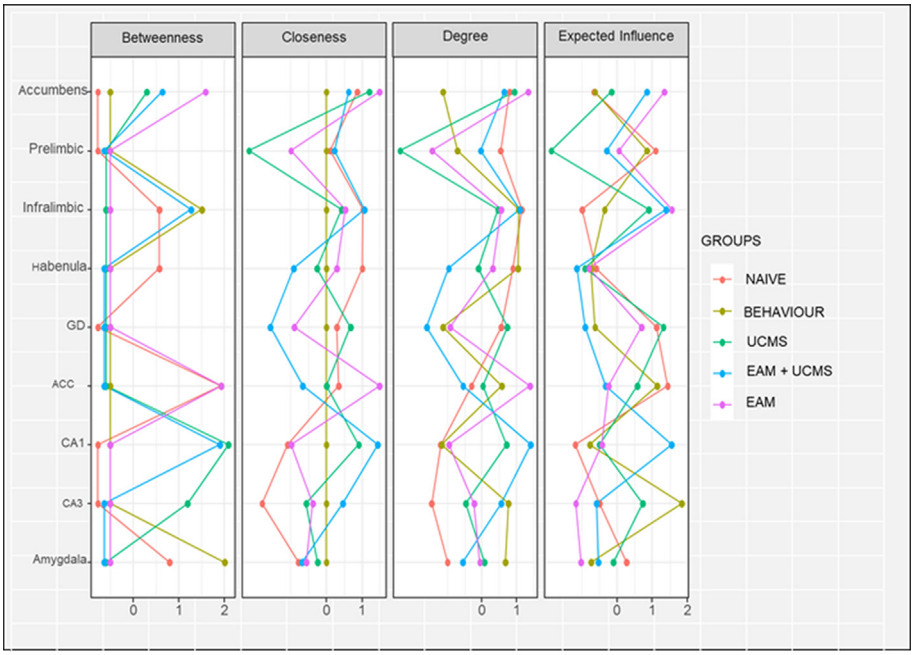
The main objective of our work was to evaluate the effects of environmental enrichment (EE) on cerebral activity and resilience behavior in rats. The results obtained in this study show that chronic stress induces anhedonia, anxiety and predator avoidance behaviors, and EE exposure protects subjects from these effects. We explored the neuronal activity of the brain regions

Table 1. Quantification of cytochrome c-oxidase on the different cerebral regions. Mean and ± SEM of the CCO values. *Regions that show statistically significant differences ($p \leq 0.05$) between UCMS and the rest of the groups. **In the case of the EE + UCMS group, there is a reduction of the activity compared with the UCMS group in the Hab region ($p = 0{,}006$). Therefore, the EE protocol could favor the basal activity of this region

Cerebral Regions	Naive Group	Behaviour Group	EE Group	UCS Group	EE + UCMS Group
Amygdala	28,15(4,82)$^{+}$	31,95(7,62) +	19,06(4,28)	33,31(8,60) +	29,17(3,43) +
dCA3	21,91 (3,78)*	28,53 (4,62) +	16,39(3,64)	33,70 (8,72) +	26,78 (4,55) +
dCA1	18,35 (3,07)*$^{+}$	23,6 (3,62) +	11,64(2,89)	24,59 (8,72) +	20,73 (1,90) +
dGD	31,96 (3,98)$^{+}$	34,32 (3,83) +	20,94(5,08)	37,55 (9,03) +	35,77 (3) +
ACC	23,54 (4,11)$^{*+}$	29,9 (2,74) +	17,84 (2,34)*	33,22 (3,42) +	33,78 (2,91) +
Prelimbic	26,35 (4,26)*	28,70 (3) +	20,73 (3,01)*	30,91 (2,86) +	31,16 (2,68) +
Infralimbic	25,17 (4,81)$^{+}$	27 (2,99) +	18,30(1,99)	28,63 (2,56) +	29,85 (4,02) +#
Accumbens Nucleus	36,40 (4,63)*	41,18 (10,22) +	26,86(3,52)	50 (10,25) +	43,77 (5,23) +
Habenula Nucleus	25,46 (4,32)* +	28,56 (4,60) +	15,85 (3,08)*	31,56 (6,07) +	**24,50 (2,03)* +**

+ Statistically significant differences between EE with the rest of the groups ($p \leq 0.05$) except for dCA3 in the naive group. The group EE shows lower cytochrome c oxidase activity in most of the regions.
# Statistically significant differences between the EE + UCMS group and the naive group ($p \leq 0.05$).

Table 2. Centrality analysis: The values on the X axis represent the score in the centrality indices of each group in each brain region. Zero values indicate a lower centrality index and a value of 1 shows a higher centrality. In this case of degree index (density of connections of each group), the nucleus accumbens maintains a smaller number of connections in the behavior group compared to the rest of the groups. In addition, in the UCMS group, the DG maintains greater connectivity than the EE + UCMS group. The closeness index, which shows the capacity of a region to influence and be influenced by other regions (mediator), highlights the DG in the UCMS group compared to the EE + UCMS group. This higher rate reflects that the DG maintains functional connections with more distant regions. Taking into account all these indices, the DG could act as an intermediary between networks implicated not only in the control of the HPA axis but also in the control of affective symptomatology and cognitive control



Anterior cingulate cortex (ACC), the prelimbic cortex (PrL), the infralimbic cortex (IL), the nucleus accumbens (NAc), the dorsal hippocampal region (dCA1, dCA3d, and dGD), the Amygdalae complex (AMG), and the Habenula (Hab).

involved in depressive-like symptoms. In this sense, we included brain regions involved both in cognitive and emotional behaviors. The analysis of the neuronal activity showed that chronic stress induces hyperactivity of the Hab. However, EE reduces this activity, maintaining basal levels. This hyperactivity has been related to the persistence of depressive symptoms. Therefore, EE exerts a protective effect against stress, facilitating greater resilience in the subjects. The anhedonic behavior was evaluated through the analysis of microstructure of licking behavior in addition to sucrose consumption. As before noted, lick cluster size shown a positive monotonic relationship to the concentration of palatable sweet solutions, reflecting an increase in the positive hedonic evaluation on the fluid (Dwyer, 2012). However, a reduction in consumption is not necessarily an indicator of a reduced hedonic response to the fluid; it can also be associated with a reduced expectation of reward. Studies in humans with MDD have shown that anhedonia often persists despite antidepressant treatment and these patients also show an inability to feel pleasure (Höflich et al., 2018). Our

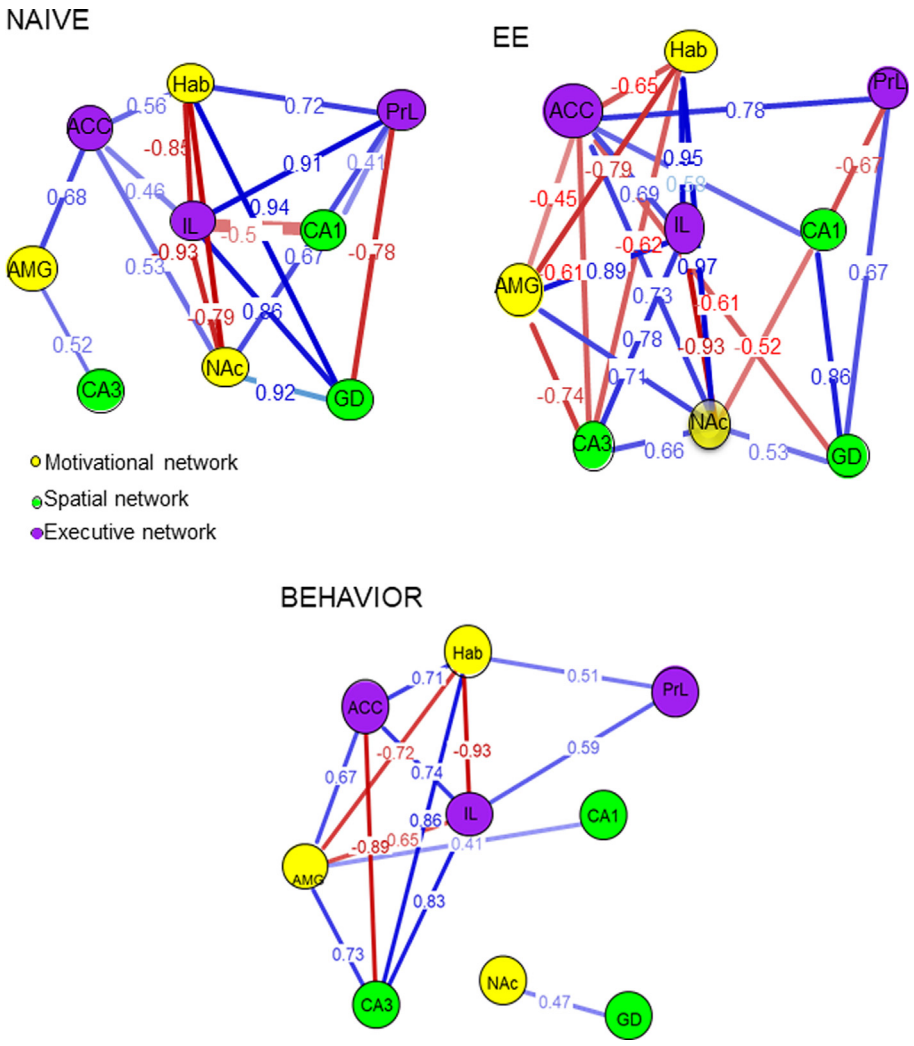


Fig. 7. Graphic representation of the partial correlation (≥0.4) in the naïve, EE, and behavior groups. The red lines represent the negative correlations between two cerebral regions. The blue lines represent positive correlations.

study also demonstrated that EE protects subjects from anhedonia, as the EE + UCMS group showed a similar sucrose consumption to the control group. Thus, a stimulating environment could reduce the presence of depressive symptoms, such as anhedonia and anxiety. This effect has been observed in male SERT knockout mice (SERT-/-), a model of depression, after 1 month of EE exposure (Sbrini et al., 2020). Similarly, Shilpa et al. (2017) showed that EE decreases anxiety responses and increases neurotropic and vascular endothelial growth factors after a period of restraint in Wistar rats. All these results show that EE is a stress inoculator that can reduce the harmful effects of stress by inducing greater resilience. Studies have suggested that the effects of EE exposure follow an inverted U-shape (Smail et al., 2020). Short EE protocols, of approximately-four weeks, induce anxiolytic effects compared to long-lasting EE exposure, which increases anxiety. The UCMS group in the present study preferred to stay in the closed zone of the zero maze, showing a long latency to explore the open zone. When UCMS is preceded by EE exposure, latency to explore open zones is shortened, and the subjects quickly explore the open zones. Therefore, our EE protocol induced an anxiolytic effect (Crofton et al., 2015). In addition, the analysis of the stretching behavior during the cat odor test showed that the UCMS group maintained the stretch-attend posture for a longer time than the rest of the groups. This body posture is considered an index of anxiety and risk assessment. Previous exposure to EE prevents this posture and facilitates normal behavior.

We explored the neuronal activity of the brain regions involved in depressive-like symptoms. In this sense, we included brain regions involved both in cognitive and emotional behaviors. In our study, the group exposed to chronic stress showed higher CCO activity than the rest of the groups. This increase of CCO could challenge mitochondrial homeostasis, exceeding mitochondrial reserves and leading to abnormally increased or decreased mitochondrial biogenesis, respiratory chain dysfunction, decreased ATP production, increased ROS generation, lipid peroxidation, mitochondrial and nuclear DNA damage, and increased cell apoptosis, and/or necrosis. MDD shows an increase in oxidative stress markers, a decrease in antioxidant capacity, and low levels of ATP (Manoli et al., 2007; Giménez-Palomo et al., 2021). This mitochondrial dysfunction affects the oxidative phosphorylation process, decreasing the activity of mitochondrial complexes I and IV. Chronic stress alters oxidative control mechanisms in the hippocampus and PFC. This functional alteration could decrease hippocampal neurogenesis. In fact, neurogenesis disruption produces more difficulty both in controlling the HPA axis and releasing adequate corticosterone levels prior to stress (Peng and Bonaguidi, 2018; Park, 2019). These changes are associated with greater severity of MDD and worse treatment response (Belleau et al., 2019). Our results reveal that EE exposure before chronic stress induces low CCO activity in the brain regions assessed. This reduction could have a beneficial effect because previous studies have shown increased CCO activity in the limbic regions in hippocampal atrophy related to a high level of excitatory amino acid input from the amygdala (Mällo et al., 2009). CCO activity decrease in the EE group could be related

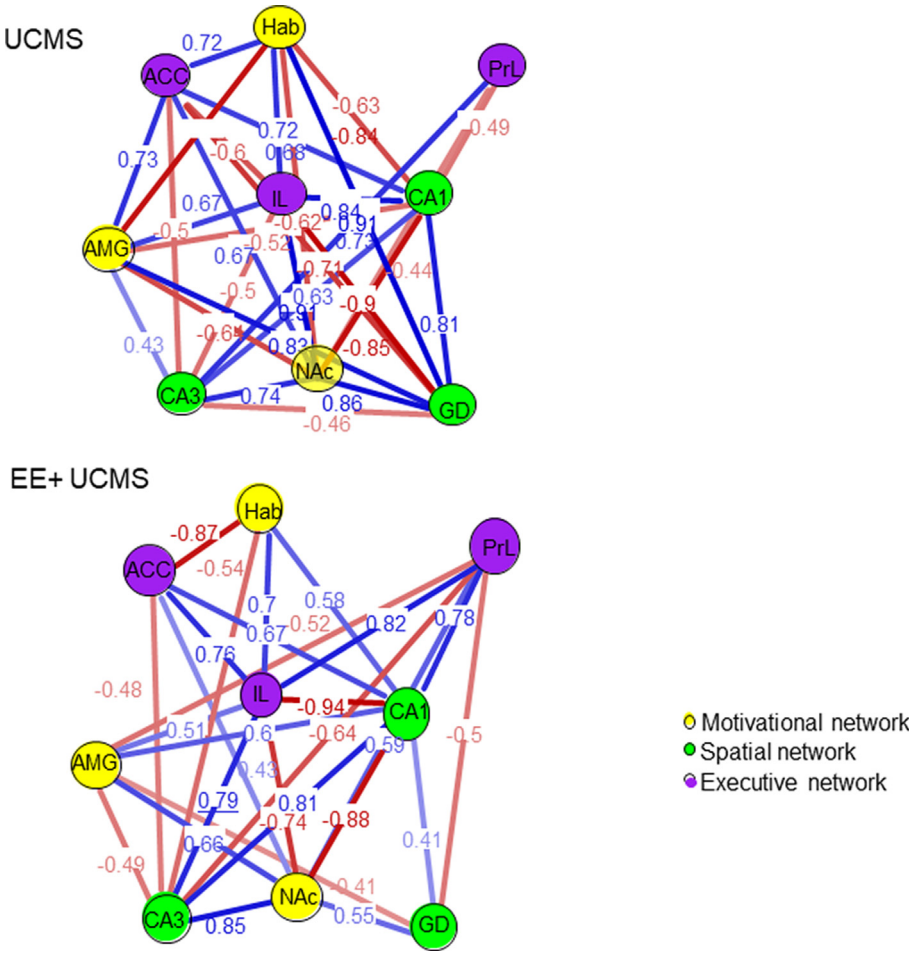


Fig. 8. Graphic representation of the partial correlations (≥0.4) in the UCMS and EE + UCMS groups.

to an increase in GABAergic activity in the hippocampus and PFC (Sampedro-Piquero and Begega, 2017). Recent findings report parvalbumin neuron (PV^{++}) dysfunctions in these regions in MDD and bipolar disorder (Perlman et al., 2021). In depressed subjects, a high level of oxidative stress is associated with PV^{++} neuron loss. In this sense, GABAergic neuron damage in the NAc nucleus is associated with depressive disorder and a reduction of the resilient response (Zhu et al., 2017). These consequences of chronic stress are ameliorated by the effects of EE exposure. This effect has been shown in aged rats in which low anxiety and an increase of PV^{++} neurons in ACC and PL regions were observed after EE exposure for 2 months (Sampedro-Piquero et al, 2016). Therefore, resilience could be related to GABAergic activity (Zou et al., 2021). In our study, the analysis of CCO activity revealed higher inverse connectivity in the EE + UCMS group compared with the rest, which could be related to the fundamental role of GABA in resilience. Thus, EE could induce greater inhibitory control and favor resilience mechanisms.

The results of CCO in the different regions have shown higher CCO activity in the Hab of the UCMS group compared with the EE + UCMS group. This Hab hyperactivity has also been observed in studies of humans with depressive disorder (Yang et al., 2018; Aizawa and Zhu, 2019). In fact, the rapid antidepressant and antianhedonic effect of ketamine, a pharmacological treatment for depression, are believed to be mediated by the inhibition of NMDAR-burst activity in Lhab (Gold and Kadriu 2019). Increased CCO activity in the Hab could be related to an inhibitory effect on the dopaminergic VTA and serotonergic system via the rostrotegmental nucleus (RTMg). The Hab sends glutamatergic efferences to the RMTg, which increases GABAergic inhibition of the dopaminergic VTA. In our study, the increased CCO activity of the Hab could induce a dopaminergic reduction of the VTA in the UCMS group, increasing this group's anhedonia response. This hyperactivity produces a drastic reduction of reward-associated dopaminergic activity. Conversely, Hab inactivation produces remission of MDD (Coccurello, 2019). In our EE + UCMS group, the lower activity of the Hab could be related to the reduction of the anhedonia response.

In our study, brain network analysis is complementary to the comparison of the mean CCO activity levels. This analysis shows a more densely interconnected network of brain regions in the UCMS group compared with the other groups. We can deduce that stress affects more brain regions than those merely involved in the stress response. Brain regions linked to motivation and context and/or stimulus assessment also play a relevant role in the brain network of the UCMS group. This could explain the complex symptomatology of depressive disorders. The groups exposed to EE (EE and EE + UCMS) also showed a more densely interconnected brain network compared with basal and control groups. Currently, the effect of EE is understood to be mainly focused on those connections between brain regions involved in the stress response, as opposed to those not directly participating in this response (Smail et al., 2020). However, the effect of EE exposure could be broader because most of the brain regions directly involved in stress regulation are also linked to cognitive-affective functioning. Therefore, it is difficult to understand how these networks interact.

The brain function analysis in depressive disorder is currently approached from network models (Francis and Lobo, 2016). These models not only analyze the relationship between different brain regions involved in depressive symptoms but also the interaction between different

networks and their modifications (inter-network analysis) (Constantini et al., 2015). Functional neuroimaging also points to aberrant connectivity within the affective network, which may underlie emotion dysregulation, a hallmark of depression (Bao-Li, et al, 2017; Davey et al., 2015). Recent research examining the default network has revealed striking differences between MDDs and Healthy subject. MDDs show increased default-network connectivity (compared with healthy subjects) with the subgenual-cingulate cortex (SCC), a region located in the mPFC, which is positively correlated with the length of MDDs' current depressive episodes (Nixon et al., 2014). In our case, the chronic stress group manifests greater connectivity (greater density) between brain regions not only related to the control of the HPA axis but also to anxiety response, anhedonia, attention, and concentration. These analyzes are commonly addressed using Pearson correlations. However, we chose partial correlations. In view of the brain regions' multitude of relationships, partial correlations offer information about the influence of one region on another, blocking the influence of other regions. The JASP program is very useful and intuitive for this type of analysis. In the present study, the regions were distributed in three networks according to their role in the affective-cognitive processes involved in depressive symptoms (Grahek et al., 2018). Thus, three brain networks were differentiated: ACC-PL and IL as a cognitive control network, Amyg-NAc-Hab as an affective-motivational network, and, finally, DG-CA1 - CA3 (dorsal hippocampus) as a spatial-contextual network.

Within the prefrontal circuit, the mPFc-NAc connections were highlighted as being involved in situations of positive valence. The IL region (area 25 in humans) has been related to positive experience processing through its connection with NAc (Francis and Lobo, 2016). In the UCMS group, there was a positive relationship between IL-NAc; both regions showed high neuronal activity in this group. Considering that most NAc neurons are GABAergic, the increased activity in this nucleus could reflect a reduction in GABAergic action due to the effect of chronic stress. Electrophysiological studies on NAc activity after UCMS for 3 weeks show impaired GABAergic activity and decreased spontaneous inhibitory postsynaptic current (sIPSc) frequency in NAc neurons. In addition, greater hyperexcitability of the NAc-mPFc connections induces suppression of reward-motivated behaviors in male rats (Bittar and Labonté, 2021; Zhu et al., 2017). However, in the case of the groups subjected to EE, the functional relationship between the two regions was negative. Thus, a higher glutamatergic activity of the IL induced a higher GABAergic activity in the NAc. This increased GABAergic activity is linked to resilience, and EE favors the enhancement of GABAergic activity (Zhu et al, 2017; Sbrini et al., 2020). Recently Zou et al. (2021) analyzed the GABAergic activity in the IL in mice exposed to a UCMS protocol. This group showed decreased expression of GABA1 receptors in the IL compared with the resilient group (Zou et al., 2021). The IL-Amygdala functional connection is present in both the UCMS and EE + UCMS groups. Considering that this relationship is involved in aversive information processing, it seems plausible for the UCMS conditions to present this result. Besides, chronic stress in rats produces shrinkage and spine loss in the PL and IL regions, thus altering the mPFC mesolimbic system (Stanton et al., 2019). Consequently, a downregulated dopaminergic mesolimbic pathway and anhedonic behaviors emerge. The greater neuronal activity in the PL and IL in the EE + UCMS group found in our results could indicate greater dopaminergic facilitation with anhedonia reduction compared with the rest of the studied groups. This effect could be mediated by the connections of the prefrontal cortex with VTA. Within this circuit, the Hab plays a notable role. Neuroimaging studies in humans have shown greater activity in this region when anticipating negative consequences due to exposure to negative or aversive stimuli. Indeed, the Hab indirectly interacts with dopaminergic activity in the prefrontal cortex through the LhB-VTA connection (Baker et al., 2016; Yang et al., 2018).

The spatial-contextual network (dorsal DG, CA3, and CA1) was altered in the stressed groups. Both the UCMS group and the EE + UCMS group maintained direct connections between DG-CA1 and CA3-CA1, but the DG-CA3 connection was inverse in the UCMS group. This could be due to the effect of stress on the neurogenesis of the DG. In depression, the impact of stress on this region reduces hippocampal neurogenesis (Lucassen et al., 2016). In the case of the naïve and behavior groups, the connections between hippocampal subregions were not functionally relevant. This could be due to the fact that none of the behavioral tasks requires contextual information processing for proper execution. The role of DG, CA3, and dorsal CA1 has been related to context processing. Several studies have confirmed the relevant role of these regions in spatial memory tasks, as well as the participation of the DG in the pattern separation process (Wosiski-Kuhn and Stranahan, 2013). The cat odor test does not require relevant spatial clue processing; the odor is the only key stimulus. The participation of these regions is not necessary, and, therefore, their neuronal activity would remain without important variations between the groups.

It is necessary to highlight the amygdala-dCA3 connections found in the groups exposed to EE. In both EE and EE + UCMS groups, we found an inverse connection between the two regions, while the rest of the studied groups presented a positive direct amygdala-dCA3 connection. The inverse relationship between these brain regions in the EE exposed groups could reflect an inhibitory control mechanism. Both the hippocampus and the prefrontal cortex exert an inhibitory-homeostatic effect on the HPA axis against an overactivation of the amygdala. In the groups exposed to EE, this functional change could enhance the inhibitory response in an attempt to maintain homeostasis, reducing the negative impact of stress. In the case of the NAc, its connection with dorsal hippocampal subregions remains similar in both the UCMS and EE + UCMS groups.

Finally, we conducted a descriptive analysis to determine each group's functional restructuring of brain networks. Using the JASP program, the functional importance of the brain regions in each group was analyzed. For this purpose, several centrality indices (betweenness, degree, closeness) and their strength (expected influence index) were reported. We performed a previous analysis of partial correlations, and only the connections that exceeded 0.40 are represented graphically. Hence, we only evaluated those connections between brain regions that clearly participate in the brain functioning of the studied groups. When examining both closeness and expected influence index, we found differences between EE + UCMS and UCMS groups in the closeness index. This index shows the capacity of a region to influence and be influenced by other brain regions. A high closeness index reveals a greater influence of this region on the group's network. When comparing the UCMS group with EE + UCMS and Basal groups, the PL region did not show a relevant functional role in this stressed group. This brain region has been involved in facilitating an adaptation to the context. Its relevant role in the EE + UCMS group could explain a reduction of the effect of chronic stress by EE and its positive impact on cognitive functioning. In the UCMS group, the DG is the brain region with the most significant influence on the network. The DG shows a higher closeness index in this group compared with the EE + UCMS group. The involvement of the DG in the stressed group could be related to the action of glucocorticoids. Stress situations elevate circulating levels of glucocorticoids and stimulate hippocampal glutamate release, inhibiting the proliferation of granule cell precursors. A high level of glucocorticoids induces neurotoxicity and causes hippocampal atrophy (McEwen and Akil, 2020). This could affect hippocampal neurogenesis and DG functioning. In the case of the EE + UCMS group, these effects could be attenuated by EE and, therefore, the DG did not show a relevant role in the network. The same pattern of results was shown in CA3 when comparing the UCMS and EE + UCMS groups. Dorsal hippocampal regions are more sensitive to the effect of stress. This is evident in dCA3, as this region shows synaptic alterations after prolonged exposure to glucocorticoids.

In conclusion, by analyzing the effects of chronic stress on emotional-cognitive processes and the activity of brain regions supporting these processes, we have demonstrated that EE during adulthood protects individuals from chronic stress, supporting the notion that the development and reorganization of brain networks across the human lifespan are active and continuous processes, which allow the emergence of resilience mechanisms across the entire lifespan. The present results have provided behavioral and brain functional evidence on the role of EE treatment during adulthood in facilitating resilience. Therefore, EE could be proposed to promote resilience and as a supportive non-pharmacological approach to treating mood disorders.

ACKNOWLEDGMENTS

This study was supported by grants from the Spanish Ministry of Science and Innovation (PID2019-104177GB-I00) to AB, and the Regional Government of Asturias (FICYT-PCTI, FC-GRUPIN-IDI/2018/000182) to ML. We thank Begoña Diaz Valdes for her technical assistance during brain processing and the care of the experimental animals.

AUTHOR CONTRIBUTIONS

AB: Conceptualization, Methodology, Formal analysis, Validation, Behavioral and brain protocols; Manuscript preparation, Funding; ML: Conceptualization, Behavioral protocol, Data Analysis, Data collection, Manuscript preparation, Funding; IC and MC: Methodology, Data curation, Visualization; CJ and RM: Behavioral protocols, Data collection. All authors have approved the final version of the manuscript.

REFERENCES

Aizawa H, Zhu M (2019) Toward an understanding of the habenula's various roles in human depression. Psychiatry Clin Neurosci 73 (10):607–612. https://doi.org/10.1111/pcn.12892.

Allen J, Romay-Tallon R, Brymer KJ, Caruncho H, Kalynchuk L (2018) Mitochondria and mood: Mitochondrial dysfunction as a key player in the manifestation of depression. Front Neurosci 12:1–13. https://doi.org/10.3389/fnins.2018.00386.

Antoniuk S, Bijata M, Ponimaskin E, et al. (2019) Chronic unpredictable mild stress for modeling depression in rodents: Meta-analysis of model reliability. Neurosci Biobehav Rev 99:101–116. https://doi.org/10.1016/j.neubiorev.2018.12.002.

Ashokan A, Sivasubramanian M, Mitra R (2016) Seeding Stress Resilience through Inoculation. Neural Plast. https://doi.org/10.1155/2016/4928081.

Baker PM, Jhou T, Li B, et al. (2016) The lateral habenula circuitry: Reward processing and cognitive control. J Neurosci 36 (45):11482–11488. https://doi.org/10.1523/JNEUROSCI.2350-16.2016.

Baker SL, Kentner AC, Konkle ATM, Barbagallo L, Bielajew C (2006) Behavioral and physiological effects of chronic mild stress in female rats. Physiol Behav 87(2):314–322. Available from: 10.1016/j.physbeh.2005.10.019.

Bechar AR, Cacodcar N, King MA, Lewis MH (2016) How does environmental enrichment reduce repetitive motor behaviors? Neuronal activation and dendritic morphology in the indirect basal ganglia pathway of mouse model. Behav Brain Res 299:122–131. https://doi.org/10.1016/j.bbr.2015.11.029.

Belleau EL, Treadway MT, Pizzagalli DA (2019) The Impact of Stress and Major Depressive Disorder on Hippocampal and Medial Prefrontal Cortex Morphology. Biol Psychiat 85(6):443–453. https://doi.org/10.1016/j.biopsych.2018.09.031.

Bittar TP, Labonté B (2021) Functional Contribution of the Medial Prefrontal Circuitry in Major Depressive Disorder and Stress-Induced Depressive-Like Behaviors. Front Behav Neurosci 15. https://doi.org/10.3389/fnbeh.2021.699592.

Bortolato B, Miskowiak KW, Köhler CA, Maes FB, Berk M, Carlvaho A (2016) Cognitive remission: A novel objective for the treatment of major depression? BMC Med 14(1). https://doi.org/10.1186/s12916-016-0560-3.

Burke NN, Coppinger J, Deaver DR, Roche M, Finn DP, Kelly J (2016) Sex differences and similarities in depressive-and anxiety-like behaviour in the Wistar-Kyoto rat. Physiol Behav 167:28–34. https://doi.org/10.1016/j.physbeh.2016.08.031.

Coccurello R (2019) Anhedonia in depression symptomatology: Appetite dysregulation and defective brain reward processing. Behav Brain Res 372. https://doi.org/10.1016/j.bbr.2019.112041 112041.

Costantini G, Epskamp S, Borsboom D, Perugini M, Mottus R, Waldorp L, Cramer A (2015) State of the aRt personality research: A tutorial on network analysis of personality data in R. J Res Personality 54:13–29. https://doi.org/10.1016/j.jrp.2014.07.003.

Crofton EJ, Zhang Y, Green TA (2015) Inoculation stress hypothesis of environmental enrichment. Neurosci Biobehav Rev 49:19–113. https://doi.org/10.1016/j.neubiorev.2014.11.017.

Czéh B, Fuchs E, Wiborg O, Simon M (2016) Animal models of major depression and their clinical implications. Prog Neuro-Psychopharmacol Biol Psychiaty 64:293–310. https://doi.org/10.1016/j.pnpbp.2015.04.004.

Davey CG, Whittle S, Harrison BJ, Simmons JG, Byrne ML, Schwartz OS, Allen NB (2015) Functional brain-imaging correlates of negative affectivity and the onset of first-episode depression. Psychol Med 45:1001–1009. https://doi.org/10.1017/S0033291714002000.

Davis JD, Smith GP (1992) Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behav Neurosci 1:217–218.

Dutcher JM, Creswell JD (2018) The role of brain reward pathways in stress resilience and health. Neurosci Biobehav Rev 95:559–567. https://doi.org/10.1016/j.neubiorev.2018.10.014.

Dwyer DM (2012) Licking and liking: The assessment of hedonic responses in rodents. In Quarterly J Exp, Psycholvol 65 (3):371–394. https://doi.org/10.1080/17470218.2011.652969.

Epskamp S, Fried EI (2018) A tutorial on regularized partial correlation networks. Psychol Methods 23(4):617–634. https://doi.org/10.1037/met0000167.

Fornaguera J, Brenes JC (2019) Behavioural characterization of chronic unpredictable stress based on ethologically relevant paradigms in rats. Sci Rep. https://doi.org/10.1038/s41598-019-53624-1.

Francis TC, Lobo MK (2016) Emerging Role for Nucleus Accumbens Medium Spiny Neuron Subtypes in Depression. Biol Psychiat 81 (8):645–653. https://doi.org/10.1016/j.biopsych.2016.09.007.

Giménez-Palomo A, Dodd S, Anmella G, Carlvaho A, Scaini G, Quevedo J, Pacchiarotti I, Vieta E, Beck M (2021) The Role of Mitochondria in Mood Disorders: From Physiology to Pathophysiology and to Treatment. Front Psychiat 12:1–26. https://doi.org/10.3389/fpsyt.2021.546801.

Girotti M, Adler SM, Bulin SE, Fucich EA, Paredes D, Morilack DA (2017) Prefrontal cortex executive processes affected by stress in health and disease. Prog Neuro-Psychopharmacol Biol Psychiat. https://doi.org/10.1016/j.pnpbp.2017.07.004.

Gold PW, Kadriu B (2019) A major role for the lateral habenula in depressive illness: Physiologic and molecular mechanisms. Front Psychiat 10. https://doi.org/10.3389/fpsyt.2019.00320.

Gonzalez-Lima F, Barksdale BR, Rojas JC (2014) Mitochondrial respiration as a target for neuroprotection and cognitive enhancement. Biochem Pharmacol 88(4):584–593. https://doi.org/10.1016/j.bcp.2013.11.010.

Gonzalez-Lima F, Cada A (1994) Cytochrome Oxidase Activity in the Auditory System of the Mouse: A Qualitative and Quantitative Histochemical Study. Neuroscience 63:559–578. https://doi.org/10.1016/0306-4522(94)90550-9.

Grahek I, Shenhav A, Musslick S (2018) Motivation and cognitive control in depression. BioRxiv 32:371–381. https://doi.org/10.1101/500561.

Grigson PS, Spector AC, Norgren R (1993) Microstructural analysis of successive negative contrast in free-feeding and deprived rats. Physiol Behav 54(5):909–916. https://doi.org/10.1016/0031-9384(93)90301-U.

Harro J, Kanarik M, Matrov D, Pankppse J (2011) Mapping patterns of depression-related brain regions with cytochrome oxidase histochemistry: Relevance of animal affective systems to human disorders, with a focus on resilience to adverse events. Neurosci Biobehav Rev 35(9):1876–1889. https://doi.org/10.1016/j.neubiorev.2011.02.016.

Hattori S, Hashimoto R, Miyakawa T, Yamanaka H, Maeno H, Wada K, Kunugi H (2007) Enriched environments influence depression-related behavior in adult mice and the survival of newborn cells in their hippocampi. Behav Brain Res 180(1):69–76. https://doi.org/10.1016/j.bbr.2007.02.036.

Hearing CM, Chang WC, Szuhany KL, Deckerbasch T, Nierenberg A, Sylvia LG (2016) Physical Exercise for Treatment of Mood Disorders: A Critical Review. Curr Behav Neurosci Rep 3 (4):350–359. https://doi.org/10.1007/s40473-016-0089.

Höflich A, Michenthaler P, Kasper S (2018) Circuit mechanisms of reward, anhedonia, and depression. Int J Neuropsychopharmacol 22(2):105–118. https://doi.org/10.1093/ijnp/pyy081.

Jha S, Dong B, Sakata K (2011) Enriched environment treatment reverses depression-like behavior and restores reduced hippocampal neurogenesis and protein levels of brain-derived neurotrophic factor in mice lacking its expression through promoter IV. Transl Psychiatry 1(9):e40. https://doi.org/10.1038/tp.2011.33.

Konkle AT, Baker SL, Kentner AC, Barbagallo LS, Merali Z, Bielajew C (2003) Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: sex and strain compared. Brain Res 992(2):227–238. https://doi.org/10.1016/j.brainres.2003.08.047.

Lambert K, Hunter RG, Bartlett A, Lapp H, Kent M (2020) In search of optimal resilience ratios: Differential influences of neurobehavioral factors contributing to stress-resilience spectra. Front Neuroendocrinol 56. https://doi.org/10.1016/j.yfrne.2019.100802.

Lucassen PJ, Oomen CA, Schouten M, Encinas JM, Fitzsimons CP (2016) Adult Neurogenesis, Chronic Stress and Depression. In: Canales JJ, editor. Adult neurogenesis in the hippocampus: health, psychopathology, and brain disease. Academic Press. p. 177–206. https://doi.org/10.1016/B978-0-12-801977-1.00008-8.

Mahati K, Bhagya V, Christofer T, Shena A, Shankaranarayana BS (2016) Enriched environment ameliorates depression-induced cognitive deficits and restores abnormal hippocampal synaptic plasticity. Neurobiol Learn Mem 134:379–391. https://doi.org/10.1016/j.nlm.2016.08.017.

Mällo T, Matrov D, Kõiv K, Harro J (2009) Effect of chronic stress on behavior and cerebral oxidative metabolism in rats with high or low positive affect. Neuroscience 164(3):963–974. https://doi.org/10.1016/j.neuroscience.2009.08.041.

Manoli I, Alesci S, Blackman MR, Su A, Rennert DM, Chrousos GP (2007) Mitochondria as key components of the stress response. Trends Endocrinol Metabol 18(5):190–198. https://doi.org/10.1016/j.tem.2007.04.004.

McCreary JK, Metz GAS (2016) Environmental enrichment as an intervention for adverse health outcomes of prenatal stress. Environ Epigenet 1–12. https://doi.org/10.1038/tp.2011.33.

McEwen BS (2016) Stress-induced remodeling of hippocampal CA3 pyramidal neurons. Brain Res 1645:50–54. https://doi.org/10.1016/j.brainres.2015.12.043.

McEwen BS, Akil H (2020) Revisiting the stress concept: Implications for affective disorders. J Neurosci 40(1):12–21. https://doi.org/10.1523/JNEUROSCI.0733-19.2019.

Menke A (2019) Is the HPA Axis as Target for Depression Outdated, or Is There a New Hope? Front. Psychiatry 10:1–8. https://doi.org/10.3389/fpsyt.2019.00101.

Nixon NL, Liddle PF, Nixon E, Worwood G (2014) Liotti M and Palaniyappan L (2014) Biological vulnerability to depression: linked structural and functional brain network findings. Br J Psychiatry 204:283–289. https://doi.org/10.1192/bjp.bp.113.129965.

Ortiz JB, Conrad CD (2018) The impact from the aftermath of chronic stress on hippocampal structure and function: Is there a recovery? Front. Neuroendocrinol 49:114–123. https://doi.org/10.1016/j.yfrne.2018.02.005.

Pang TYC, Hannan AJ (2013) Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology 64:515–528. https://doi.org/10.1016/j.neuropharm.2012.06.029.

Park SC (2019) Neurogenesis and antidepressant action. Cell Tissue Res 377(1):95–106. https://doi.org/10.1007/s00441-019-03043-5.
Paxinos G, Watson Ch (2013) The Rat Brain coordinates. 7th ed. Academic Press.
Peng L, Bonaguidi MA (2018) Function and Dysfunction of Adult Hippocampal Neurogenesis in Regeneration and Disease. Am J Pathology 188(1):23–28. https://doi.org/10.1016/j.ajpath.2017.09.004.
Perlman G, Tanti A, Mechawar N (2021) Parvalbumin interneuron alterations in stress-related mood disorders: A systematic review. Neurobiol Stress 15. https://doi.org/10.1016/j.ynstr.2021.100380 100380.
Poremba A, Jones D, Gonzalez-Lima F (1998) Classical conditioning modifies cytochrome oxidase activity in the auditory system. Eur J Neurosci 10:3035–3043. https://doi.org/10.1046/j.1460-9568.1998.00304.
Queen NJ, Hassan QN, Cao L (2020) Improvements to Health span Through Environmental Enrichment and Lifestyle Interventions: Where Are We Now? Front Neurosci 14:1–17. https://doi.org/10.3389/fnins.2020.00605.
Roca M, Vives M, Gili M (2016) Executive functions in depression. Psiquiatria. Biologica 23:23–28. https://doi.org/10.1016/S1134-5934(17)30050-7.
Sampedro-Piquero P, Begega A (2017) Environmental Enrichment as a Positive Behavioral Intervention across the Lifespan. Curr Neuropharmacol 15(4):459–470. https://doi.org/10.2174/1570159X14666160325115909.
Sampedro-Piquero P, Begega A, Zancada-Menendez C, Cuesta M, Arias JL (2013) Age-dependent effects of environmental enrichment on brain networks and spatial memory in Wistar rats. Neuroscience 248:43–53. https://doi.org/10.1016/j.neuroscience.2013.06.003.
Sampedro-Piquero P, Castilla-Ortega E, Zancada-Menendez C, Santín LJ, Begega A (2016) Environmental enrichment as a therapeutic avenue for anxiety in aged Wistar rats: Effect on cat odor exposition and GABAergic interneurons. Neuroscience 330:17–25. https://doi.org/10.1016/j.neuroscience.2016.05.032.
Sbrini G, Brivio P, Bosch K, Homberg J, Calabrese F (2020) Enrichment Environment Positively Influences Depression- and Anxiety-Like Behavior in Serotonin Transporter Knockout Rats through the Modulation of Neuroplasticity, Spine, and GABAergic Markers. Genes 11(1248):1–14.
Scheggi S, de Montis MG, Gambarana C (2018) Making Sense of Rodent Models of Anhedonia. Int J Neuropsychopharmacology 21 (11):1049–1065. https://doi.org/10.1093/ijnp/pyy083.
Schloesser RJ, Lehmann M, Martinowich K, Manji HK (2010) HerkenhamM (2010) Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. Mol Psychiatry 15(12):1152–1163. https://doi.org/10.1038/mp.2010.34.
Schulz D (2020) Depression development: From lifestyle changes to motivational deficits. Behav Brain Res 395. https://doi.org/10.1016/j.bbr.2020.112845.
Shilpa B, Bhagya V, Hrish G, Srinivas MM, Sahnkaranarayana BS (2017) Environmental enrichment ameliorates chronic immobilization stress-induced spatial learning deficits and restores the expression of BDNF, VEGF, GFAP and glucocorticoids receptors. Prog Neuropharmacol Biol Psychiatry 2(76):88–100. Available from: 101016/j.pnpbp.2017.02.025.
Smail MA, Smith BL, Nawreen N, Hearman JP (2020) Differential impact of stress and environmental enrichment on corticolimbic circuits. Pharmacol Biochem Behav 197:145. https://doi.org/10.1016/j.pbb.2020.172993.
Smith PJ (2019) Pathways of Prevention: A Scoping Review of Dietary and Exercise Interventions for Neurocognition. Brain Plast 5(1):3–38. https://doi.org/10.3233/bpl-190083.
Spector AC, Klumpp PA, Kaplan JM (1998) Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. Behav Neurosci 112(3):678–694. https://doi.org/10.1037//0735-7044.112.3.678.
Stanton CH, Holmes AJ, Chang SWC, Joormann J (2019) From Stress to Anhedonia: Molecular Processes through Functional Circuits. Trends Neurosci 42(1):23–42. https://doi.org/10.1016/j.tins.2018.09.008.
Strickland JA, Austen JM, Sanderson D (2018) A biphasic reduction in a measure of palatability following sucrose consumption in mice. Physiol Behav 129–134. https://doi.org/10.1016/j.physbeh.2017.11.019.
Umschweif G, Greengard P, Sagi Y (2021) The dentate gyrus in depression. Eur J Neurosci 53(1):39–64. https://doi.org/10.1111/ejn.14640.
Willner P (2017a) Reliability of the chronic mild stress model of depression: A user survey. Neurobiol Stress 6:68–77. https://doi.org/10.1016/j.ynstr.2016.08.001.
Willner P (2017b) The chronic mild stress (CMS) model of depression: History, evaluation and usage. Neurobiol Stress 6:78–93. https://doi.org/10.1016/j.ynstr.2016.08.002.
World Health Organization WHO (2017) Depression and Other Common Mental Disorders, Global Health Estimate.
Wosiski-Kuhn M, Stranahan AM (2013) From pattern separation to mood regulation: Multiple roles for developmental signals in the adult dentate gyrus. Front Cell Neurosci 7:1–4. https://doi.org/10.3389/fncel.2013.00096.
Yang Y, Wang H, Hu J, Hu H (2018) Lateral habenula in the pathophysiology of depression. Curr Opinion Neurobiol 48:90–96. https://doi.org/10.1016/j.conb.2017.10.024.
Zhu Z, Wang G, Ma K, Cui S, Wang JH (2017) GABAergic neurons in nucleus accumbens are correlated to resilience and vulnerability to chronic stress for major depression. Oncotarget 8 (22):35933–35945. Available from: https://doi.org/10.18632/onco.
Zou HW, Jin XY, Wang Y, Liu YJ, Li LF (2021) The GABA(B1) receptor within the infralimbic cortex is implicated in stress resilience and vulnerability in mice. Behav Brain Res 406:11324. https://doi.org/10.1016/j.bbr.2021.113240.

(Received 21 July 2022, Accepted 14 January 2023)
(Available online 17 February 2023)