https://doi.org/10.36315/2023inpact094
Psychological Applications and Trends 2023

PRO-RESILIENT EFFECTS OF ENVIRONMENTAL ENRICHMENT ON GABAergic AND GR ACTIVITY IN DORSAL HIPPOCAMPUS: AN ANALYSIS IN WISTAR ADULT RATS

Azucena Begega[1], Claudia Jove[2], & Matías López[1]
[1]*Department Psychology, INEUROPA, Faculty of Psychology, University of Oviedo (Spain)*
[2]*Laboratory of Neuroscience, Faculty of Psychology, University of Oviedo (Spain)*

Abstract

Our work aims to evaluate the impact that environmental enrichment has on male Wistar rats in an animal model for depression. For this purpose, before applying an unpredictable chronic stress model, one group was exposed to environmental enrichment for four weeks in order to test if this procedure has protective effects against chronic stress. The selected groups were three: control group (n=8), unpredictable chronic stress group (n=8) (UCM) and environmental enrichment+chronic stress group (n=8) (EE+UCM). In all of them, the following behavioral tests were evaluated: sucrose preference (anhedonia test), zero maze (anxiety test) and forced swimming (passive coping test). This was followed by the evaluation of GR receptors activation and GABAergic activity in dorsal and ventral hippocampus. These receptors have been related to the inhibitory control of the hypothalamic-pituitary-adrenal axis after chronic stress. Moreover, GABAergic activity has been reduced in hippocampus in a chronic model stress. For data analysis, a mixed factorial ANOVA was used in the anhedonia test and a one-way ANOVA in the other tests. Results showed that environmental enrichment reduced the effects of the chronic stress, promoting a greater resilience. There were statistically significant differences between UCM group and the others. UCM group showed an anhedonia response and more anxiety than either the control group or the EE+UCM group. Results of the GR receptors activity and GABAergic neurons in hippocampal regions showed again statistically significant differences in the UCM group. There was a reduction in GABAergic activity in dorsal hippocampus coupled with an increased activation of GR in this group; for ventral hippocampus there were not differences between groups. These results suggest environmental enrichment could enhance greater resilience, reducing the vulnerability of the subjects to develop disorders such as depression and anxiety.

Keywords: *Resilience, environmental enrichment, GABAergic neurons, glucocorticoids receptors, rat*.

1. Introduction

Chronic stress is a key factor in the development of disorders such as anxiety and depression. But not every subject who experiences adverse situations develops depressive symptoms: those subjects are presented as resilient. Resilience is defined as a dynamic process that allows the subject to face adverse situations with less impact on their overall health. The neurobiological studies are currently focused on understanding the mechanisms that favor this resilience (Pascual-Leone & Bartres-Faz, 2021; Yao & Hsieh, 2019).

The Hypothalamic-Pituitary-Adrenal (HPA) axis plays a central role pathophysiology of depression (Gold, 2015). The hippocampus exert negative feedback control over the HPA axis, reducing cortisol release. Neuroimaging studies in humans confirm that there is a reduction in the hippocampal volume in chronically depressed patients, mainly in the dentate gyrus of the hippocampus (Malhi & Mann, 2018).

At the brain level, cortisol acts through mineralocorticoid (MR) and glucocorticoid receptors (GR) (Groeneweg et al., 2012; Meijer et al., 2019). MR act in the presence of cortisol basal levels, while the GR play a role when the cortisol level is high, and its elevated levels are maintained continuously. Therefore, this receptor could be related to greater vulnerability or resilience to affective disorders.

The GABAergic system is also involved in the regulation of the HPA axis activation under conditions of chronic stress. Studies using proton magnetic resonance spectroscopy reveal significant reduction in the GABAergic activity of the prefrontal cortex of MDD patients, showing deficit concentration in brain, alteration of the GABAergic interneurons and dysfunction of GABAA receptors (Luscher & Fuchs, 2015). Interventions such as environmental enrichment (EE) and physical exercise could act on GABAergic activity favoring greater resilience.

2. Objectives

We hypothesize that UCMS-exposed subjects would show changes in the GR and lower hippocampal GABAergic activity compared to those UCMS-exposed subjects previously submitted to EE. So that, EE application could induce a resilient process.

For this, we evaluated the impact of UCMS alone and the UCMS preceded by EE exposure on anhedonia, anxiety and despair, as well as on GABAergic activity and GR density in the dorsal hippocampus. In this way, we assessed whether EE favors greater resilience not only at a behavioral level, but also by affecting the GR and GABAergic activity of relevant brain regions involved in MDD.

3. Design

For evaluate that, twenty-six adult male rats supplied by the vivarium of the University of Seville (Spain) were used. 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 animals were randomly assigned to Control group (CONT, n:8), housed under normal conditions without any intervention and submitted to behavioral tests; Unpredictable Chronic Mild Stress group (UCMS, n:8), submitted to a UCMS protocol and the behavioral tasks; Environmental Enrichment in Unpredictable Chronic Mild Stress group (EE+UCMS, n:10), exposed to EE 4 weeks before experiencing chronic stress.

The UCMS model consisted of exposing the rats for 4 consecutive weeks to different stressors such as abnormal cage inclination (45º for 6 h), wet sawdust (6 h), food deprivation (for 24 h) among others. This protocol has proven to be a powerful model of animal depression (Atrooz et al., 2021). For the EE model, it consisted of applying for 4 weeks the presence of novel cues and different types of objects every week in their house cage, favoring interaction with each other and with the environment.

The anhedonia protocol involves analyzing the microstructure of licking behavior during voluntary consumption (Dwyer et al., 2012). A contact sensitive lickometer registered the licks made by rats, and MED-PC software controlled the equipment and recorded total consumption (mL) and total number of licks. This procedure allows to stablish the hedonic evaluation of the sucrose consumed before UCMS is applied and the effects after.

Elevated zero maze is a variant of the elevated plus maze (Czech et al., 2016). The session was carried out one week after the anhedonia test. Each session was recorded with a video camera connected to a computer equipped with a Ethovision Pro, video tracking program. The following variables were recorded: number of entries into the open area, time spent in each area and latency of entry into the open arm, all of them considered anxiety rates.

A single session of 5 min duration of forced swim task was carried out. The recorded variables were: duration and frequency of immobile behavior versus high and normal mobility.

For immunohistochemistry analyses and its quantification, we studied dorsal hippocampus subregions (dentate gyrus (DG), CA3 and CA1). For GABA, quantification was realized with Leica LAS X Software using magnification 10x and measuring manually the number of positive cells. For GR quantification of density was measured using ImageJ software.

4. Discussion

Results showed that environmental enrichment reduced the effects of chronic stress: in licking test the UCM group showed a clear anhedonia response whereas EE+UCMS not since it does not differ significantly with the control group.

In the zero-maze test, the EE+UCMS group spent longer time in the open zone compared to the rest of the groups. Furthermore, EE+UCMS group showed shorter latency to enter this area than the rest of groups ($F(2,24) = 5{,}519$; $p{:}0.011$; $\eta 2 : 0.31$) proving less anxiety and more exploration behavior than the UCMS group.

Quantification of GABA PV+ neurons and GR in dorsal hippocampus showed that UCMS group presented higher density of GR in all dorsal hippocampal regions compared to control and EE+UCMS group. Moreover, GABAergic PV+ neurons quantification revealed statistically significant differences between the groups in all dorsal hippocampal subregions (dDG (F2,21=12.01; p=0,001); dCA1 (F2,21=16.58; p: 0,001) and dCA3 (F2,21= 31.56; p: 0.001). In this case, UCMS groups showed lower PV+ density than the rest of groups.

5. Conclusions

In general, our results show a previous EE experience buffers the effects on anhedonia and anxiety behaviors associated with stress. These effects are accompanied of the lower density of GR and greater PV activity, especially in the dorsal hippocampus in the EE+UCMS group. Therefore, probably EE would act increasing resilience and inducing high GABAergic activity.

In conclusion an enriched environment could buffer the effects of chronic stress, reducing the possibility of the onset of depressive disorder. In humans, activities such as meditation, mildness and cognitive reappraisal and aerobic exercise exert their adaptive effects on emotion regulation by acting on limbic and brainstem systems. Both preclinical and clinical studies support the idea that these interventions could complement pharmacological treatments.

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