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Research report

Social defeat leads to changes in the endocannabinoid system: An overexpression of calreticulin and motor impairment in mice

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HIGHLIGHTS

- Motor coordination and motor learning are compromised in social defeat mice.
- After three weeks of social stress, the cerebellum of stressed mice compared to control non-stressed mice showed higher 2-AG concentration and lower CB1 mRNA and protein expression.
- Next Generation Sequencing revealed an increase in calreticulin expression after 3 weeks of chronic psychosocial stress.

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ABSTRACT

Prolonged and sustained stimulation of the hypothalamo–pituitary–adrenal axis have adverse effects on numerous brain regions, including the cerebellum. Motor coordination and motor learning are essential for animal and require the regulation of cerebellar neurons. The G-protein-coupled cannabinoid CB1 receptor coordinates synaptic transmission throughout the CNS and is of highest abundance in the cerebellum. Accordingly, the aim of this study was to investigate the long-lasting effects of chronic psychosocial stress on motor coordination and motor learning, CB1 receptor expression, endogenous cannabinoid ligands and gene expression in the cerebellum.

After chronic psychosocial stress, motor coordination and motor learning were impaired as indicated the righting reflex and the rota-rod. The amount of the endocannabinoid 2-AG increased while CB1 mRNA and protein expression were downregulated after chronic stress. Transcriptome analysis revealed 319 genes differentially expressed by chronic psychosocial stress in the cerebellum; mainly involved in synaptic transmission, transmission of nerve impulse, and cell-cell signaling. Calreticulin was validated

Abbreviations: HPA, hypothalamo–pituitary–adrenal; eCB, endocannabinoid; CB1, cannabinoid receptor type 1; THC, Δ^9-tetrahydrocannabinol; AEA, *N*-arachidonylethanolamine; 2-AG, 2-arachidonoylglycerol; PEA, palmitoylethanolamide; OEA, oleoylethanolamide; ER, endoplasmic reticulum; UPR, unfolded protein response; PTSD, post-traumatic stress disorder; Calr, calreticulin; Zfp488, zinc finger protein 488; Hyou1, hypoxia up-regulated 1; Slc2a1, solute carrier family 2 (facilitated glucose transporter) member 1; Ptprn, protein tyrosine phosphatase, receptor type, N; Xbp1, X-box binding protein 1; Tnr, tenascin R; Peg3, paternally expressed 3.

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as a stress candidate gene. The present study provides evidence that chronic stress activates calreticulin and might be one of the pathological mechanisms underlying the motor coordination and motor learning dysfunctions seen in social defeat mice.

1. Introduction

Study of the physiological consequences of acute and chronic stress for a range of organ systems was launched more than 70 years ago in pioneering work on the "general adaptation syndrome" by Hans Selye [1]. Since then, an ever-burgeoning body of research has explored the interfaces among the central and autonomic nervous, endocrine, and immune systems. When an organism is exposed to a stressor, several mechanisms are activated to restore homeostasis [2], which include a series of physiological reactions such as endocrine activation (especially of the hypothalamo–pituitary–adrenal–HPA axis) and cardiovascular changes, which, per se, do not produce pathological changes. It is only when a prolonged and sustained stimulation exceeds the body capacity to maintain homeostasis that stress can have persistent sequelae. Repeated social defeat was chosen here as a stress model because it has been shown to have excellent etiological [3,4], predictive, discriminative and face validity [4,5], producing a variety of molecular, physiological and behavioral changes [6]. Chronic stress negatively affects motor coordination [7].

Numerous studies have established the importance of the cerebellum in motor coordination and motor learning [8–12]. Motor coordination and motor learning are essential for animal survival and involve the complex coordination of various functions, including sensory inputs, integration in the central nervous system, and outputs to peripheral skeletal muscles. Conversely there is evidence that the cerebellum also participates in emotional reactions [13,14], constant states of activity [15,16], and experience of rage, anger of fear [17,18].

The endocannabinoid (eCB) system interacts with the stress response at several brain areas to influence behavior [19]. In fact, the G-protein-coupled CB1 cannabinoid receptor is highly abundant in the brain [20,21] and densest in the cerebellum [22]. These receptors are the main target for endocannabinoids lipid signaling molecules and mediate the pharmacological actions of Δ^9-tetrahydrocannabinol (THC) and synthetic cannabinoid drugs [23–26]. The main endogenous ligands for CB1 receptors are *N*-arachidonylethanolamine (AEA) [27] and 2-arachidonoylglycerol (2-AG) [28].

Accordingly, we direct the present study in order to investigate the interplay between motor behavior, the endocannabinoid system, and gene expression in the cerebellum of chronic psychosocial stressed mice.

2. Materials and methods

All the procedures performed on mice were approved by Göttingen University Institutional Animal Care and Use Committee and were in accordance with NIH guidelines for the use of animals in research and the European Communities Council Directive (86/609/EEC).

2.1. Animals

A total number of 30C57Bl6/J male mice at age of 7–8 week were obtained from Charles River Laboratories (Sulzfeld, Germany). After arrival the animals were housed 5 mice per cage and maintained under standard conditions (12 h light/dark cycle with 6:00/18:00 lights on/off, room temperature of $21 \pm 2\,^{\circ}C$ and food and water ad libitum). One week after the habituation period mice were subjected to the experiment. FVB/N male mice (Charles River Laboratories, Sulzfeld, Germany) at age 1 year old were housed individually and served as residents in the resident–intruder paradigm. FVB/N mice were preferentially selected as residents because they display much active offensive behavior than C57Bl/6 strain [29]. The FVB/N colony was maintained under the same conditions that C57Bl/6J mice but housed in a different room to avoid habituation of C57Bl/6J mice to the odor of residents.

2.2. Experiment design and experimental groups

C57Bl6/J mice were divided into 2 groups: mice exposed daily for 1 h to psychosocial stress (stress) or left undisturbed (control). The social stress paradigm was performed daily for three weeks. On the day 21, the animals were evaluated by behavioral testing and finally sacrificed.

2.3. Social stress procedure

The resident–intruder paradigm was characterized elsewhere for rats [29–32] and mice [33–35]. In brief, a C57Bl/6J mouse (intruder) was placed into a home cage of a FVB/N mouse (resident) where they were allowed to interact freely until the first signs of an aggression occurred. After this first sign of an aggression, the intruder mouse was protected by a small plastic wire-mesh cage within the resident's cage. Accordingly, intruder mice were protected against direct attacks and injuries but still exposed to an unfamiliar environment where they were subjected to olfactory, visual and to some vibrissae physical contact with residents. After 1 h, the intruder mouse was put back into its home cage. Every day intruders were encountered with different residents to avoid habituation. The resident–intruder protocol was performed daily at similar daytime to enhance the stress factor induced by prediction of social defeat in intruders. In contrast, controls were placed daily for 1 h in an empty cage. Thus, controls were subjected to the same experimental protocol considering handling and a new environment (novel cage) aside from psychosocial stress as compared to stressed mice.

2.4. The righting reflex

Each mouse was placed on its back and tested for the ability to right itself. The mouse was determined to have impaired righting reflex if it could not right itself within the following seconds and regained the righting reflex if it could fully right itself immediately after it was placed on its back. Righting reflex was scored from 1 (normal) to 3 (very slow). Score 1 if the mouse rights within 1 s (immediate), score 2 if the mouse rights within 2 s, score three if the mouse does not right or it takes longer than 2 s as described [36].

2.5. Rota-rod

Motor coordination and balance were evaluated in a rota-rod apparatus (TSE RotaRod System, Germany) which consisted of a