Effects of uniform rocking exercise on anxiety and its related nuclei in acutely stressed rats

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Abstract

Rocking can make us feel relaxed and reduce anxiety. Now it has been proved that uniform rocking exercise can promote sleep in rodents, but there are less studies on whether it affects anxiety. The objective of this experiment is to explain the effect of uniform rocking exercise on anxiety level in rats under acute stress, using plantar electrical stimulation can induce acute stress model, which was verified by the open field test and elevated plus-mest. In addition, the levels of anxiety-related hormones adrenocorticotropic hormone (ACTH) and corticosterone (CORT) were examined by serum Enzyme-Linked Immuno Sorbent Assay (ELISA), and it was found that the anxiety level of rats, as well as the levels of ACTH and CORT, were significantly reduced after 1 hour of rocking. In addition, we examined the anxiety-related nuclei by C-fos and found that uniform rocking motion decreased neural activity in the hippocampus (HIP) and amygdala (AMY) and increased neural activity in the vestibular nucleus in rats under acute stress. In addition, we examined the expression of Iba1, a marker of microglia. We found that uniform rocking exercise alleviated anxiety levels in acutely stressed rats, which may be related to the activation of microglia in the hippocampus, medial prefrontal cortex, and vestibular nucleus. Our study reveals a significant correlation between the ability of rocking to alleviate anxiety, activation of neural nuclei, and microglia in acutely stressed rats.

Introduction

Stress is a non-specific systemic response of the body to various internal and external environmental stimuli, and this system is mainly composed of the hypothalamic-pituitary-adrenal axis (HPA) and the blue spot norepinephrine-autonomic nerves[1–3] The main endocrine hormones are glucocorticoid (GC) and adrenocorticotropic hormones, which play an important role in the homeostasis of the body under resting and stressful conditions. A moderate amount of stress can mobilize the body's non-specific systems and improve the body's ability to cope with external challenges, which is positive for the survival of the organism. If the stress response is dysregulated in terms of intensity and duration, it will adversely affect important physiological functions such as growth, development, metabolism, circulation, reproduction, immune response, cognition and behavior. Rocking has long been used to promote sleep and aid digestion in infants [4], and although some studies have argued against it, in recent years it has been shown that uniform rocking at 0.25 Hz promotes sleep and enhances the consolidation of nighttime memory in humans [5], and it has also been shown that uniform rocking at 1.0 Hz promotes non-rapid eye It has also been shown that rocking at 1.0 Hz can promote non-rapid eye movement sleep (NREM) and reduce rapid eye movement sleep (REM) sleep and wakefulness, as well as reduce sleep latency in mice [6], and it has been experimentally found that rocking can bring a relaxing experience [7]. Whether uniform rocking movement can reduce the anxiety level of the body has not been demonstrated in animal experiments, and whether it affects anxiety-related nuclei such as HIP, amygdala (AMY), basal lateral amygdala (BLA), and mPFC is unclear.

The hypothalamic-pituitary-adrenocortical axis is an important part of the stress response. Activation of the HPA axis causes secretion of GC, which acts on multiple organ systems to redistribute energy
resources to meet the needs of different organs. The expression of HPA axis activation depends on the duration, intensity, frequency and mode of stress. It is essential to terminate the activation of the HPA axis by activating the glucocorticoid receptors (GR), which perform a negative feedback mechanism to properly terminate the stress response. Proper control of the stress response is critical because inappropriate or prolonged HPA axis activation is extremely energetically draining and is associated with many physical and psychological disease states.

There are many associations between anxiety and inflammation, and experiments are often conducted on rodents injected with lipopolysaccharide to induce models of anxiety and depression, as evidenced by studies such as major depressive disorder (MDD) and bipolar disorder (BD) and anxiety-related disorders such as generalized anxiety disorder (GAD), generalized anxiety disorder (GAD), panic disorder, and post-traumatic stress disorder (PTSD)) patients exhibit chronic low-grade inflammation, as measured by increased peripheral and central inflammatory cytokines, inflammatory mediators, and acute phase reactants to measure \([8-10]\). Numerous studies have reported increased circulating inflammatory cytokines such as IL-1, IL-6, TNF and C-reactive protein in patients with mood and anxiety related disorders \([11, 12]\). Anxiolytic effects have been reported in rats lacking inflammatory TNF pathway receptors \([13]\), as well as reports demonstrating a crucial role of TNF-\(\alpha\) in anxiety associated with neuroinflammation \([14]\). This suggests that inflammation is capable of modulating anxiety behavior. Microglia are resident immune cells of the central nervous system (CNS) \([15]\) and constantly monitor the surrounding microenvironment. They are activated in response to stress or pathological conditions and are involved in inflammation, protection, recovery and immune clearance, processes that may have deleterious effects on neurons and microglia \([16]\). Activation of microglia is accompanied by the release of cytokines. Inhibition of microglia activation may ameliorate neuroinflammation and neuro behavioral abnormalities such as disruption of spatial memory or depression \([17, 18]\).

**Material and methods**

**Animals**

- Male SD rats of about 200 g were selected, and all rats were individually housed before and after surgery at a temperature of 22 ± 3°C and relative humidity of 56 ± 5% in a suitable ventilated environment with adequate water and food, unrestricted activity, and light and dark conditions, provided by the Experimental Animal Center of Yanbian University. Experimental procedures were carried out in accordance with the Animal Protection and Utilization Committee of Yanbian University.

**Experimental procedure**

- The rats were subjected to 5 minutes of touch and grasping each day for 5 days to prevent additional stress reactions due to grasping, and were divided into 3 groups according to whether they
were subjected to electrical stimulation and rocking stimulation: control group (normal control, NC; normal feeding), model group (model control, MC; plantar electrical stimulation) and rocking group (Rocking; plantar electrical stimulation + rocking). The rats in the NC group were placed in a shock cage without electric shock stimulation and subjected to an elevated cross maze or open field experiment after an interval of 1 h. The rats in the MC group were placed in a shock cage with electric shock stimulation (1.0 Hz, 10 V, 5 min) and subjected to an open field experiment with an elevated or elevated cross maze experiment after an interval of 1 h. The rats in the Rocking group were placed in a shock cage with electric shock stimulation (1.0 Hz, 10 V, 5 min) and subjected to an elevated cross maze experiment after an interval of 1 h. The rats in the Rocking group were placed in an electric shock cage and given electric shock stimulation (1.0 Hz, 10 V, 5 min), rocking for 1 hour and then subjected to open-field experiments or elevated cross maze. Blood was taken at different time intervals for serum ELISA to detect the content of ACTH and CORT, and brain was taken by perfusion after anesthesia, and brain tissue immunofluorescence was performed to detect C-fos and Iba1 in hippocampus, prefrontal cortex, amygdala, and vestibular nucleus.

**Behavioral experiments**

**Open-field experiment**

- The rats were placed in a 100cm×100cm×50cm all-black open field, kept quiet, with appropriate light intensity and a camera above the open field. The time that the rats were in the central area was calculated. At the beginning of the test, each rat was placed in a corner and recorded by a camera above the box for 6 min. The equipment was cleaned with 75% alcohol after each trial to eliminate any olfactory traces. The behavioral system automatically recorded the number of entries into the central area, the time spent in the central area and the total distance moved.

**Elevated cross-maze experiment**

- The EPM was a platform with a shape of “+” consisting of 2 open arms (50 cm × 10 cm) and 2 closed arms (50 cm × 10 cm × 15 cm) extending from the central platform (10 cm × 10 cm). At the beginning, the rats were placed on the central platform facing the open arms. After each 6 min test, the maze was cleaned to avoid any olfactory traces. The TM-Vision behavioral experimental system recorded and measured the time of opening of the arms and the total number of entries into the arms to assess anxiety-related behaviors.

**Immunofluorescence technique**

- Rats were anesthetized with isoflurane at the end of behavioral studies and perfused transcardially with 100 ml of 0.01 M phosphate buffer (PBS, pH 7.4). Then 40 ml of 4% (w/v) paraformaldehyde was perfused in 0.2 M phosphate buffer (pH 7.4). The brains were quickly removed and fixed with
the same fixative at 4° C for 24 h. After being dehydrated by 30% (w/v) sucrose solution at 4° C for 48 h at low temperature, they were embedded with OCT and stored in the refrigerator at -20 degrees C. Sections were performed using a frozen sectioning machine with a thickness of 5 µm selected for continuous sectioning, and 4 nuclei of mPFC, AMY, HIP, and VN were found for continuous sectioning according to the Paxinos and Waston rat brain atlas. The slides were dried at room temperature for 15 min, soaked in PBS for 10 min to remove OCT, placed in citric acid repair solution (PH 6.0), microwaved for 5 min, cooled naturally, washed 3 times with PBS for 5 min each time, and the sections were closed at room temperature for 1 h in PBS with 10% goat serum, followed by the addition of C-fos primary antibody (1:200, CST) and IBa1 primary antibody ( The sections were incubated with C-fos primary antibody (1:200, CST) and IBa1 primary antibody (1:500, Huaan) overnight at 4°C. The primary antibody was washed off in PBS three times for 10 min each time, the fluorescent secondary antibody was added and incubated at room temperature for 2 h. The secondary antibody was washed off in PBS three times for 10 min each time, and the sections were sealed by adding anti-fluorescence quencher (containing DEPI) dropwise, and the results were observed by fluorescence microscopy and photographed.

Western Blot

- Two hours after the end of the behavioral experiments, mPFC, HIP, and VN nuclei were separated and homogenized. A total of 18 rats (6 rats in each group), protein concentration was determined using the ratio BCA protein assay kit (R0010,Solarbio) 20 µg protein samples were taken from each group and separated by SDS-PAGE. The separated proteins were transferred to PVDF membranes, blocked with 5% skim milk in TBST for 1 h at room temperature, and incubated with primary antibody overnight at 4°C. Goat anti-rabbit IgG fluorescent secondary antibody was added after washing 3 times for 5 min each using TBST, and after incubation for 1 h, the membrane was developed after washing 3 times for 5 min each using TBST and adding high-sensitivity ECL reflecting solution (monad). The primary antibodies include anti-C-fos (#2250, 1:1000, Cell Signaling Technology), β-actin (#3700, 1:1000, Cell Signaling Technology), and goat anti-rabbit IgG fluorescent secondary antibody (1:10000, licor).

Statistical analysis

- The WB bands were analyzed for grayscale values using image J software, and the immunofluorescence was analyzed for grayscale values of the mean fluorescence intensity, and the data were processed using SPSS25.0 software for independent sample t-test or one-way ANOVA. If the variance was not equal, Tamhane's T2 test was used. All data were expressed as mean ± standard error (mean ± SEM), and the significance test level was α = 0.05, with P < 0.05 being statistically significant. GrapPad prism 7.0 was used for graphing.

Result
Effect of rocking on OFT in acute stress rat model

The anxiety level of rats was assessed by OFT, and the experimental data showed a significant decrease in the intermediate dwell time in the MC group compared to the NC group (F=0.029, P<0.05), and a significant increase in the intermediate dwell time in the Rocking group compared to the MC group (P<0.05). The distance of movement in the open field was significantly decreased in MC compared to NC group (F=0.081, P<0.05), while there was no significant change for Rocking group (P>0.05, post hoc test LSD). n=12 for NC group; n=12 for MC group; n=11 for Rocking group. (see Fig 1 C,D,E)

Effect of rocking on EPM in acute stress rat model

The level of anxiety in rats was assessed by EPM, and the experimental results showed that the MC group had significantly less retention time (F = 0.226, P < 0.05) and number of entries (F = 0.459, P < 0.05) in the open arm compared to the NC group, while the Rocking group was able to increase the retention time and number of entries in the open wall (P < 0.05). n = 8 in the NC group; n = 8 in the MC group; Rocking group n = 9, post hoc test LSD. (See Fig. 1F,G,H)

Effect of rocking on serum ACTH in acute stress rat model

ELISA detected serum ACTH levels in rats without stress stimulation, 1h, 2h, 3h after stress stimulation, and the ACTH levels in Rocking group ACTH levels were significantly lower than those in MC group (P < 0.05, MC group n = 6; Rocking group n = 6). At 2h and 3h after acute stress stimulation in rats, ACTH levels did not change significantly in the MC group compared to the Rocking group (P > 0.05, independent samples t-test). (See Fig. 2)

Effective of Rocking on serum CORT in acute stress rat model

ELISA detected serum CORT levels in rats without stress stimulation, 1h, 2h and 3h after stress stimulation, the CORT levels in the Rocking group were significantly lower than those in the MC group after 1h of acute stress stimulation in rats (P < 0.05, n = 6 in MC group; n = 8 in Rocking group). CORT levels were not significantly changed in the MC group compared to the Rocking group at 2h versus 3h after acute stress stimulation in rats (P > 0.05, MC group n = 6; Rocking group n = 6, independent samples t-test). (see Fig. 3)

Effect of Rocking on the expression of C-fos and Iba1 in HIP of acute stress rat model

Using immunofluorescence to detect the expression of C-fos and Iba1 in each group of HIP, the experimental data showed that the C-fos fluorescence intensity was significantly higher in the Rocking group compared to the MC group (F = 0.013, P < 0.001). the C-fos content was significantly higher in the MC compared to the NC group in the CA3 region (F = 0.052, P < 0.001) the mean fluorescence intensity of C-fos was significantly up, and C-fos content was also significantly higher in MC group compared to
Rocking group (P < 0.0001). In the DG region of hippocampus, the mean fluorescence intensity of C-fos was significantly lower in the Rocking group compared with the MC group (F = 0.063, P < 0.0001), and the C-fos content was also significantly lower in the NC group (P < 0.0001) compared with the MC group. In the DG area, the mean fluorescence intensity of Iba1-positive microglia was significantly lower in the Rocking group compared to the MC group (F = 0.223, P < 0.01). n = 3 in the NC group; n = 3 in the MC group; n = 3 in the Rocking group. (see Fig. 4)

Effect of Rocking on the expression of C-fos and Iba1 in mPFC and BLA in acute stress rat models

Using immunofluorescence to detect C-fos as well as Iba1 expression in mPFC and BLA in each group, the experimental data showed that the mean fluorescence intensity of C-fos was significantly decreased in MC group compared with NC group (F = 0.126, P < 0.001) in mPFC. And the mean fluorescence intensity of Iba1 positive microglia was significantly increased in Rocking compared to MC group (F = 0.212, P < 0.001). in BLA the mean fluorescence intensity of C-fos and Iba1. the mean fluorescence intensity of C-fos was significantly decreased in Rocking group compared to MC group (F = 0.33, P < 0.001). the mean fluorescence intensity of C-fos was significantly increased in NC group (P < 0.001) compared to MC group. the mean fluorescence intensity of Iba1 positive microglia was significantly increased in BLA. fos content increased significantly. n = 3 in NC group; n = 3 in MC group; n = 3 in Rocking group. (see Fig. 5)

Effect of rocking on the expression of C-fos and Iba1 in the acute stress rat model VN

Using immunofluorescence to detect C-fos as well as Iba1 expression in MVe and LVe in each group, the experimental data showed that the mean fluorescence intensity of C-fos was significantly higher in the Rocking group compared to the NC group (F = 0.026, P < 0.05) in MVe. The mean fluorescence intensity of Iba1-positive microglia was significantly higher in the NC group (F = 0.640, P < 0.05) compared to the MC group. The mean fluorescence intensity of Iba1 positive microglia was significantly higher in the NC group (F = 0.640, P < 0.05) compared to the MC group (P < 0.0001). In LVe, the mean fluorescence intensity of C-fos was significantly higher in the Rocking group compared with the MC group (F = 0.946, P < 0.0001), while the mean fluorescence intensity of C-fos was significantly higher in the MC group compared with the NC group (P < 0.0001). n = 3 in NC group; n = 3 in MC group; n = 3 in Rocking group. (see Fig. 6)

Immunoblotting of C-fos protein in HIP, mPFC, and VN in acute stress rat model by Rocking

The expression of C-fos in HIP, mPFC, and VN in each group was detected using WB, and the experimental data showed that the expression of C-fos was significantly higher in the MC group compared to the Rocking group in HIP (F=0.655, **P<0.01), and in mPFC, the C-fos content was significantly lower in both the MC group (P<0.05) compared to the NC group. the Rocking group was significantly lower in VN compared to the C-fos content was significantly increased in the Rocking group compared to the NC group (F=0.667, P<0.05). n=3 in the NC group; n=3 in the MC group; n=3 in the Rocking group. (see Fig 7)

Discussion
Rocking can improve anxiety-like behavior in a rat model of acute stress

- Anxiety disorder is a psychiatric disorder, and with the accelerated pace of life, the incidence of anxiety disorders is increasing year by year. The current mainstream view is that anxiety disorders are based on the neurotransmitter hypothesis and the endocrine disruption hypothesis as well as abnormal immune function, implicating the regulatory disorder of multiple systems. Acute stress in experimental animals is often performed using plantar electrical stimulation, plantar heating, forced swimming, restraint stress, social frustration, early life stress, and tail suspension. Homogeneous rocking movements have been experimentally demonstrated to affect sleep in rats via peripheral vestibular receptors otoliths [6], and the ability of rocking beds to relax has also been reported [7]. The effect on anxiety specifically in animal experiments is less reported in the literature. In this experiment, the anxiety level of rats can be observed indirectly through OFT to observe the autonomous behavior of acutely stressed rats in a novel environment, and according to the experimental data, it is suggested that the residence time in the center of the MC group rats in the open field decreased significantly and the total distance of movement decreased significantly after 1 h of electric shock stress stimulation compared with the NC group rats, indicating that the rats had less spontaneous movement, less exploratory behavior, and anxiety at 1 h after electric shock stress stimulation level significantly increased. The central area dwell time increased significantly after the rocking stimulation compared to the MC group, indicating that the 1h rocking exercise significantly reduced the anxiety-depression-like behavior of the rats. In EPM, the open arm dwell time as well as the number of open arm entries decreased significantly in the MC group rats compared to the NC group rats, indicating a significant increase in anxiety level after electroshock stimulation, while the open arm dwell time as well as the number of open arm entries increased significantly in the Rocking group rats compared to the MC group rats, suggesting an improvement in anxiety in the rats.

Rocking improves HPA axis hyperresponsiveness in the rat acute stress model

- The HPA axis is an important component of acute stress and plays an important role in the stress process, where the most important hormone is GC, which signals through at least two receptor subtypes, the salt corticosteroid receptor and GR. Both GR and the salt corticosteroid receptor are ligand-gated transcription factors that can alter the expression of a large number of genes [19], the GR receptor has a weaker affinity than the salt corticosteroid receptor, and higher High levels of GC activate low-affinity GR, promote the expression of a variety of genes, and are thought to mediate the mobilization of GC on energy storage, inflammation, and neurological function [20], and are generally thought to act as anti-inflammatory, but GC has also been reported to act as pro-inflammatory under specific conditions [21]. Among them, increased secretion of ACTH and CORT is one of the indicators of HPA axis hyperactivity [22], and GC secretion depends on the magnitude duration of the stress.
response, and it has been reported in the literature that under restraint stress conditions, the early peak of ACTH in rodents occurs at 15 to 20 min, while CORT begins to appear at 30 to 60 min and ends within 2 to 3 h after the end of the stress stimulus [23]. In the present experiment, ACTH and CORT serum levels were monitored at different time periods of plantar electrical stimulation, and the experimental data suggest that when acute stress in rats leads to increased secretion of ACTH and CORT, suggesting hyperactivity of the HPA axis and increased secretion of GC and CORT, ACTH and CORT levels decreased after rocking treatment, suggesting that hyperactivity of the HPA axis was alleviated and that uniform rocking exercise was able to attenuate the expression of anxiety hormones. ACTH and CORT levels returned to normal after 3h, indicating that the HPA axis returned to normal after 3h.

**rocking and plantar electrical stimulation altered the activation of microglia in some brain regions**

- Microglia are resident immune cells in the CNS [15] and constantly monitor the surrounding microenvironment. Activated microglia have two phenotypes, M1 and M2. M1 type, also known as pro-inflammatory or neurotoxic phenotype, produces pro-inflammatory factors such as IL-6, IL-1β and TNF-α, which induce the production of iNOS and NO and lead to cellular damage. M2 type is anti-inflammatory or neuroprotective phenotype, an anti-inflammatory phenotype that produces anti-inflammatory cytokines such as IL-4 and IL-10, which are involved in tissue repair and reconstruction [24]. They are activated in response to stress or pathological conditions and are involved in various inflammatory, protective, restorative and toxic processes that may have deleterious effects on neurons and glial cells [16]. Iba1 is a calcium-binding protein with a molecular weight of approximately 17 kDa. Since Iba1 is specifically expressed in microglia of CNS, it is used as a microglial cell marker [25]. Its expression is increased in activated microglia [26], and is often accompanied by microglia activation and cytokine release during anxiety states resulting from sleep deprivation periods and anxiety-like states induced by plantar electroshock [27]. Inhibition of microglia activation ameliorates neuroinflammation and neurobehavioral abnormalities, such as depression [28]. mPFC plays an important role in anxiety. The number of Iba1-positive microglia is increased in mPFC of animals that have undergone stressful stimulation [29]. However, the mechanisms of microglia activation and anxiety-like behavior in mPFC are still far from being understood. The experimental data suggest that Iba1 + microglia activation is reduced in the CA3 region of HIP in response to stress stimulation, but increased in the DG region, similar in this respect to Andrea Du Preez and their findings [30], which showed differential microglia expression in various regions of HIP, with a reduction in Iba1 in the DG region in the Rocking group compared to the MC group, able to indicate microglia activation decreased and reduced cellular inflammation, and it is possible that the DG region is an important nucleus for rocking to reduce acute stress anxiety. Rocking stimulation was able to increase mPFC microglia activation, but behavioral and serological did not bring about anxiety-depression-like behavior and HPA axis activation, probably for increased microglia M2 phenotype, which plays a neuroprotective and anti-inflammatory role. Further
experimental evidence is needed. It has been reported that the lipopolysaccharide-anxiety-depression model induces AMY microglia activation [31], but in the present experiments, no microglia overexpression was found. Decreased microglia activation in the medial vestibular nucleus after MVe stress stimulation, while rocking was able to increase the activation of microglia in the MVe area and LVe, and peripheral vestibular injury could lead to an increase in VN inflammatory factors TNF-α and NFκB expression and the expression of the anti-inflammatory factor superoxide dismutase, which promote vestibular compensation and protect the neuronal internal environment of the VN [32]. Prolonged swaying requires the vestibule to adapt to the swaying stimulus and reduce swaying-induced vertigo, and may activate some microglia used to adjust, the homeostasis of the neuronal internal environment altered by prolonged swaying stimulation.

**rocking and plantar electrical stimulation alter neuronal activity in some brain regions**

- In the field of neuroscience, C-fos protein is a marker of neuronal activity, encoded by proto-oncogene and overexpressed in a variety of tumor cells. Due to its rapid increase in proto-oncogene levels and immediate response, it can produce rapid responses to various cellular stimuli, including electrical stimulation, growth factors and neurotransmitters.

- HIP is the area of the brain mainly responsible for learning and memory, which is related to memory and stress regulation, and stress stimulation in the present experiment resulted in increased expression of C-fos in CA3 and DG regions in HIP, which is similar to what they reported by Nae Saito [33], and the same results of WB experiments for C-fos in HIP. This suggests that stressful stimuli may affect the contextual fear conditioning reflex of recent memory. And rocking movement was able to reduce the expression of C-fos in the DG area of CA3 region, CA1, brought about by stress. It may reduce the contextual fear conditioning reflex of recent memory, resulting in a decrease in anxiety levels.

- Stress stimulation reduces C-fos expression in the mPFC, possibly because stress reduces glutamatergic transmission and related signaling pathways [34, 35], decreases neuronal activity, and leads to dendritic atrophy and axonal loss in the mPFC, resulting in persistent depressive behavior [36]. However, rocking did not alleviate the decrease in mPFC neuronal activity but rather exacerbated it, suggesting that rocking may not produce anxiolytic effects by decreasing mPFC glutamatergic neuronal activity. However, it has also been reported that the administration of benzodiazepines to rats stimulated by plantar electroshock was able to reduce C-fos expression in the mPFC [37]. So further evidence is needed on what type of neurons these reduced activities are.

- AMY are a group of nuclei located in the medial temporal lobe and are key limbic structures involved in emotion regulation, associative learning and regulation of cognitive functions [38]. They consist of 80% glutamatergic neurons and 20% GABAergic interneurons, where the most important nucleus associated with stress is the BLA, and stress stimulation increases the expression of C-fos in the BLA and rocking decreases C-fos expression, suggesting that acute stress stimulation enhances AMY
neural activity, which may be related to anxiety-emotion regulation, with results similar to those of Prager who lipopolysaccharide induced anxiety-depression-like changes in rats similarly [39]. In contrast, rocking reduced AMY activity, which may control for reduced activity of anxiety-related neurons, thus regulating the level of anxiety.

- VN neurons contain multiple neurotransmitters, among which glutamate plays a key role [40]. VN glutamatergic neurons are also directly connected to other functional areas, such as sleep-wake state (blue spot, dorsal nucleus of the middle suture, dorsolateral periaqueductal nucleus, giant reticular nucleus, lateral paracellular nucleus, periaqueductal gray matter of the midbrain, subnucleus of the blue spot, small cell reticular nucleus, paracellular median nucleus of the middle suture) and emotion regulation (blue spot and dorsal nucleus) [41]. This indirectly suggests that the vestibule is directly or indirectly associated with sleep-wake and emotion regulation C-fos expression is increased in both MVe and LVe, but C-fos levels are not very low in the MC group. Considering that plantar electrical stimulation may cause bouncing in rats, this inevitably stimulates the peripheral vestibule, allowing the rats to adjust their posture and their own balance under electroshock conditions. Possible stimulation to the activity of neurons of MVe and LVe.

Conclusion and outlook

- Uniform rocking exercise can improve anxiety levels and reduce ACTH, CORT in acutely stressed rats. Uniform rocking exercise can reduce the neural activity of hippocampus and amygdala and increase the neural activity of vestibular nucleus in rats under acute stress. The reduction of anxiety level in rats under acute stress by uniform rocking may be related to the activation of microglia in hippocampus, medial prefrontal cortex, and vestibular nucleus.
- The present study suggests that rocking can reduce anxiety levels in rats, which provides a new idea for non-pharmacological treatment of patients with anxiety disorders and identifies relevant neural clusters that may be affected, and these results can be expected to bring value in combination with clinical applications.

Declarations

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Author contributions

Xian Jiang is in charge of experimental design

Guangyao Luo and Yaowen Zhang were responsible for the experimental procedure

Yulian Jin is in charge of thesis revision
Bin Wen and Tongtong Guo are in charge of thesis writing

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**Availability of data and materials**

The authors confirm that the data supporting the findings of this study are available within the article (and its Additional File).

**Ethics approval and consent to participate**

This experiment was approved and reviewed by the Animal Conservation and Utilization Committee of Yanbian University.

**Competing interests**

The authors declare that they have no competing interests.

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**References**


Figures

Figure 1
(A): Rocking device; (B): Experimental flow chart; (C): typical path diagram of each group of OFT; (D): dwell time in the central region of each group of OFT; (E): total distance statistics of OFT. *P < 0.05, compared with NC group; #P < 0.05, compared with MC group. (F): typical thermogram for each group; (G): open arm dwell time; (H): statistical plot of the number of entries into the open arm. *P < 0.05, compared with NC group; #P < 0.05, compared with MC group.

**Figure 2**

Effect of rocking on serum ACTH in acute stress rat model *P < 0.05, compared with the MC group.

**Figure 3**

Effect of rocking on serum CORT in acute stress rat model #P < 0.05, compared with the MC group.
Figure 4

Effect of rocking on the expression of C-fos and Iba1 in various regions of HIP in acute stress rat model (A): positive expression of C-fos as well as Iba1 microglia in each region of HIP; (B): mean fluorescence intensity of C-fos in each region of HIP; (C) mean fluorescence intensity of C-fos in each region of HIP in the Iba1 region. Scale bar = 100 μm. **P < 0.01, ***P < 0.001, ****P < 0.0001, compared with the NC group; ##P < 0.01, ##P < 0.001, ###P < 0.0001, compared with the MC group.

Figure 5
Effect of rocking on the expression of C-fos and Iba1 in mPFC and BLA (A): positive expression of C-fos as well as Iba1 microglia in mPFC; (B): positive expression of C-fos as well as Iba1 microglia in BLA in AMY; (C): mean fluorescence intensity of C-fos and Iba1 in mPFC. (D): mean fluorescence intensity of C-fos and Iba1 of BLA. Scale bar = 100 μm. **P < 0.01, ***P < 0.001, compared with NC group; ####P < 0.001, compared with MC group.

Figure 6

Immunofluorescence results of the medial vestibular nucleus and lateral vestibular nucleus (A) Positive expression of C-fos as well as Iba1 microglia in VN; (B) is the mean fluorescence intensity of C-fos and Iba1 of MVE. (C) Mean fluorescence intensity of C-fos and Iba1 for LVE. Scale bar = 100 μm. *P < 0.05, ****P < 0.0001, compared with the NC group; ##P < 0.01, ###P < 0.001, ####P < 0.0001, compared with the MC group.
Figure 7

Immunoblotting of C-fos protein in HIP, mPFC, VN. (A): Immunoblotting of C-fos protein in HIP, mPFC, and VN; (B): Relative ratio of protein C-fos to β-actin. *$P<0.05$, compared with NC group; ##$P<0.01$, compared with MC group.