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ORIGINAL ARTICLE

Effect of Local Vibration and Passive Exercise on the Hormones and Neurotransmitters of Hypothalamic–Pituitary–Adrenal Axis in Hindlimb Unloading Rats

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Abstract

Astronauts are severely affected by spaceflight-induced bone loss. Mechanical stimulation through exercise inhibits bone resorption and improves bone formation. Exercise and vibration can prevent the degeneration of the musculoskeletal system in tail-suspended rats, and long-term exercise stress will affect endocrine and immune systems that are prone to fatigue. However, the mechanisms through which exercise and vibration affect the endocrine system remain unknown. This study mainly aimed to investigate the changes in the contents of endocrine axis-related hormones and the effects of local vibration and passive exercise on hypothalamic–pituitary–adrenal (HPA) axis-related hormones in tail-suspended rats. A total of 32 Sprague–Dawley rats were randomly distributed into four groups (n=8 per group): tail suspension (TS), TS+35Hz vibration, TS+passive exercise, and control. The rats were placed on a passive exercise and local vibration regimen for 21 days. On day 22 of the experiment, the contents of corticotrophin-releasing hormone, adrenocorticotropic hormone, cortisol, and 5-hydroxytryptamine in the rats were quantified with kits in accordance with the manufacturer's instructions. Histomorphometry was applied to evaluate histological changes in the hypothalamus. Results showed that 35Hz local vibration cannot cause rats to remain in a stressed state and that it might not inhibit the function of the HPA axis. Therefore, we speculate that this local vibration intensity can protect the function of the HPA axis and helps tail-suspended rats to transition from stressed to adaptive state.

Keywords Passive exercise · Vibration · Hypothalamic–pituitary–adrenal axis · Hormone · Hindlimb unloading

Introduction

Space flight affects the immune responses and other biological systems of humans (Sonnenfeld 1994; Strollo 1999; Sonnenfeld 1998; Li et al. 2017). In the process of space flight, astronauts are affected by stressors that are completely different from those on the ground. Space-flight stressors, which include weightlessness, noise, circadian rhythm changes, space radiation, physical factors, and other special environmental factors, easily affect the body's metabolic balance and subsequently cause neuroendocrine–immune system dysfunction (Esposito et al. 2001; Sonnenfeld 1998; Kaur et al. 2004). The neuroendocrine–immune system maintains the stability of the environment in the body and its dysfunction is closely related to the occurrence and development of many diseases (Clement et al. 2015; Macho L1 et al. 1991; Riviere 2009). Space flight and simulated weightlessness can change the inherent neuroendocrine–immune function of astronauts, particularly in astronauts who are susceptible to colds, rhinitis, gastroenteritis, and other diseases (Martinez et al. 2015; Si et al. 2016; Caren et al. 1980; Ritzmann et al. 2017). Thus, the neuroendocrine–immune systemhas received special attention in aerospace medicine.

Space flight and simulated microgravity decrease bone mass and strength and cause muscle atrophy (Baldwin 1996; Fitts et al. 2000; Almeida-Silveira et al. 2000; Reeves et al. 2005). Exercise training and vibration are applied to counter bone loss and muscle atrophy during simulated unloading

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(Ji et al. 2015; Sun et al. 2014). Physical exercise have been shown to play a positive effect on the treatment degeneration of musculoskeletal system, but they have only limited success (Dudley-Javoroski et al. 2016; Musumeci et al. 2013; Musumeci 2016). For example, the effect of resistive exercise, swimming and jumping on improving bone loss was different. In addition to exercise trainings, whole body vibration (WBV) could enhance bone formation, reduce the osteoclast activity, inhibit bone loss, and counteract muscle atrophy (Musumeci 2017; Cardinale and Bosco 2003; Cerciello et al. 2016; Castrogiovanni et al. 2016; Pichler et al. 2013). However, some other studies showed that WBV did not preclude the increase in bone resorption (Murfee et al. 2005) and might damage the peripheral vessel of animals (Baecker et al. 2012). Recently, local vibration has been used either alone as a countermeasure or coupled with other methods to enhance their effects (Sun et al. 2014; Huang et al. 2017).

The effect of exercise training and vibration is biologically based on exercise stress, which can induce hormonal changes. Serum cortisol levels increase in response to high-intensity exercise or submaximal (Fry et al. 1991; Gong et al. 2015; VanBruggen et al. 2011; del Corral et al. 1994). Long-term exercise stress will affect the endocrine and immune systems, which are prone to fatigue. Exercise and vibration can prevent the degeneration of the musculoskeletal system in tail-suspended rats. The mechanism through which exercise and vibration affect the endocrine system, however, remains unknown. The present study mainly aimed to investigate the changes in the concentrations of endocrine axis-related hormonesin tail-suspended rats and the effects of local vibration and passive exercise on related hormones. The results of this study will be helpful in studying the changes in the neuroendocrine–immune system under simulated microgravity and provide insight into the inhibitory mechanism of vibration and exercise on the deterioration of the musculoskeletal system, as well as help improve exercise training efficiency. Therefore, this study has considerable significance for the study of stress response in a weightless environment and in the development of interventions for spaceflight-induced stress.

Methods and Materials

Experimental Animals and Animal Care

32 female 8-week-old Sprague Dawley rats were recruited from the Experimental Animal Center of Beijing University and were adapted for 7 days. All animal treatments were conducted in accordance with the Regulation of Administration of Affairs Concerning Experimental Animals of State Science and Technology Commission of China and were approved by the Animal Care Committee of Beihang University. All rats were housed in the same cages with rationed lab chow and enough water. The room was controlled at 25±2°C with a 12/12h light/dark cycle. Animals were randomly divided into four groups (n=8, each group): 1) tail-suspension (TS), 2) TS plus 35Hz vibration (TSV), 3) TS plus passive exercise (TSP), and 4) control (CON) (Fig. 1a).

Hindlimb Unloading

In TS, TSV and TSP groups, rats' tails were suspended and hindlimbs were unloaded for 3 weeks according to Morey's methods (Morey-Holton and Globus 2002). Briefly, the body was maintained at approximately 30° angle from the cage floor to ensure that the feet did not touch the cage floor. The animal was able to reach food and water easily.

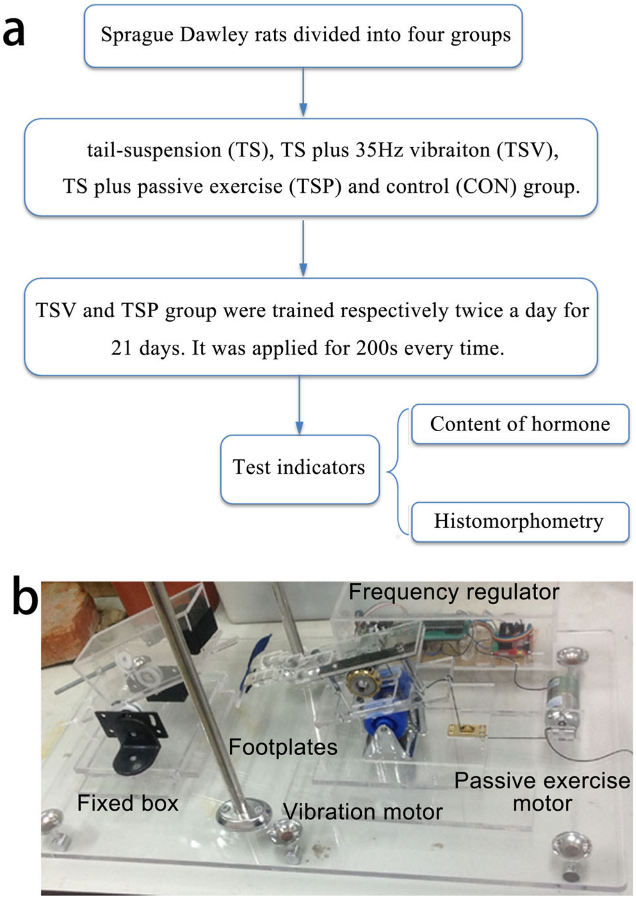

Fig. 1 Flow chart and device of passive exercise and local vibration for tail-suspended rats. (a) Flow chart of experiment. (b) A novel designed device of passive exercise and local vibration for tail-suspended rats. The passive exercise or/and local vibration was generated by two motors. The rat's trunk was placed in the fixed box and its feet were taped on the footplates

Passive Exercise and Vibration

Animals in TSV and TSP group were trained respectively in local vibration, passive exercise and twice a day (at 8 a.m. and 5 p.m.) for 21 days. We developed a novel training device for passive exercise and local vibration on hindlimbs as showed in Fig. 1b. During training, the rat's body was maintained 30° angle in a fixed box. Its feet were both immobilized on the footplates of the device with medical adhesive tape. Passive exercise was performed by a lifting motor drove the footplates overcoming a 4N load generated by the gravity of footplates that caused passive contraction of rat's hindlimbs. The hindlimbs were from fully extended to fully bended, then back to be fully extended as one bout. Each bout lasted 2 seconds with 8-second interval. 20 bouts were applied every time. Another motor connected to an eccentric bearing generated the vibration (35Hz, 1mm amplitude). It was applied for 200s every time.

Kit Detection

On day 22 of the experiment, the rats were sacrificed through narcotic overdose with 1% pentobarbital sodium (18ml/kg, i.p.). The brain tissues and serum of the rats were harvested and preserved at −20 °C for further examination. Afterward, the contents of hypothalamic–pituitary–adrenal (HPA) axis-related hormones in the collected brain tissues and serum were evaluated. Briefly, hypothalamic tissue homogenate was prepared in accordance with the instructions included with the corticotrophin-releasing hormone (CRH) and 5-hydroxytryptamine (5-HT) assay kits. The serum contents of corticotrophin (ACTH), cortisol (CORT), and 5-HT were measured in accordance with the kits' instructions.

Histomorphometry

The hypothalamus was immersed in 10% formalin for 3 days, dehydrated in a gradient ethanol series, and embedded in paraffin. The tissue was then longitudinally sectioned to a thickness of 5μm using a Leica SM2500 heavy-duty sectioning system (Leica, Germany). The sections were stained with hematoxylin–eosin (HE). Digital images of each section were obtained by an Olympus microscope (BX 51, Japan).

Statistical Analyzes

All values are expressed as means ±standard deviation. SPSS 20.0 was used to perform statistical analyzes with univariate analysis. The level of statistical significance was set at $P<0.05$.

Results

Kit Detection of HPA Hormones

CRH content decreased significantly in the TSV group relative to that in the control (CON) and the TSP groups,

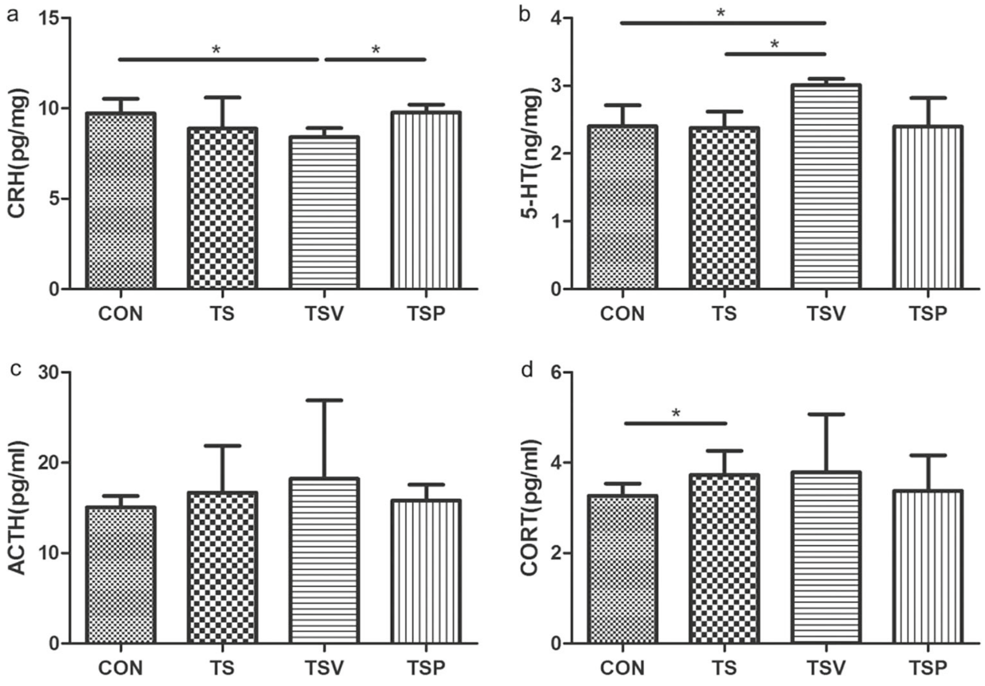

Fig. 2 Kit detection of HPA hormones. Local vibration has a significant effect on the content of CRH and 5-HT in hypothalamus. Local vibration exercise can protect the function of the HPA axis in tail-suspended rats. Passive exercise was inefficient. **a** The content of CRH in hypothalamus. **b** The content of 5-HT in hypothalamus. **c** The content of ACTH in serum. **d** The content of cortisol in serum. Values are mean ±SD. * $p<0.05$

but there was no significantly difference between the TS and TSV groups. CRH content in the TS, CON, and TSP groups were not significantly different (Fig. 2a). The 5-HT content significantly increased in the TSV group relative to that in the TS group but was not significantly different among CON, TSV, and TSP groups (Fig. 2b). Moreover, ACTH content increased in the TSV group compared with that in the CON, TS, and TSP groups. Nevertheless, no significant difference in ACTH content existed among the four groups (Fig. 2c). CORT content in TS group markedly increased compared with that in CON group. CORT content was not significantly different among the CON, TSV, and TSP groups (Fig. 2d).

Histomorphometry of the Hypothalamus

The hypothalamus sections stained with HE are shown in Fig. 3. No severe surface irregularities were observed in any of the samples. The cells were integral. The nuclei were stained, and the cells were dispersed. The neurocyte was rich in cytoplasm, lightly stained. Although the number of neurocytes decreased in TS groups, no obvious difference existed among the four groups. There was no significant differences in the number of non-neurocytes among four groups. Three area of interest (each square 200 μm in length) were chosen to count the number of cells (Table 1).

Table 1 Quantification of cells in the Hypothalamus

	CON	TS	TSV	TSP
Neurocyte	16.7±7.5	11.0±2.6	14.7±6.8	12.3±4.5
Non- neurocyte	23.3±8.4	19.7±2.5	21.3±7.7	24.3±7.6

Discussion

Changes in hormonal levels are associated with exercise load; hormone levels significantly change when the exercise load reaches a certain intensity (Xie Minhao and Zhang 2008). Our previous studies demonstrated that local vibration exerts a positive effect on rat bone loss, whereas passive exercise has a poor effect on rat bone loss (Huang et al. 2017). Local vibration and passive exercise may

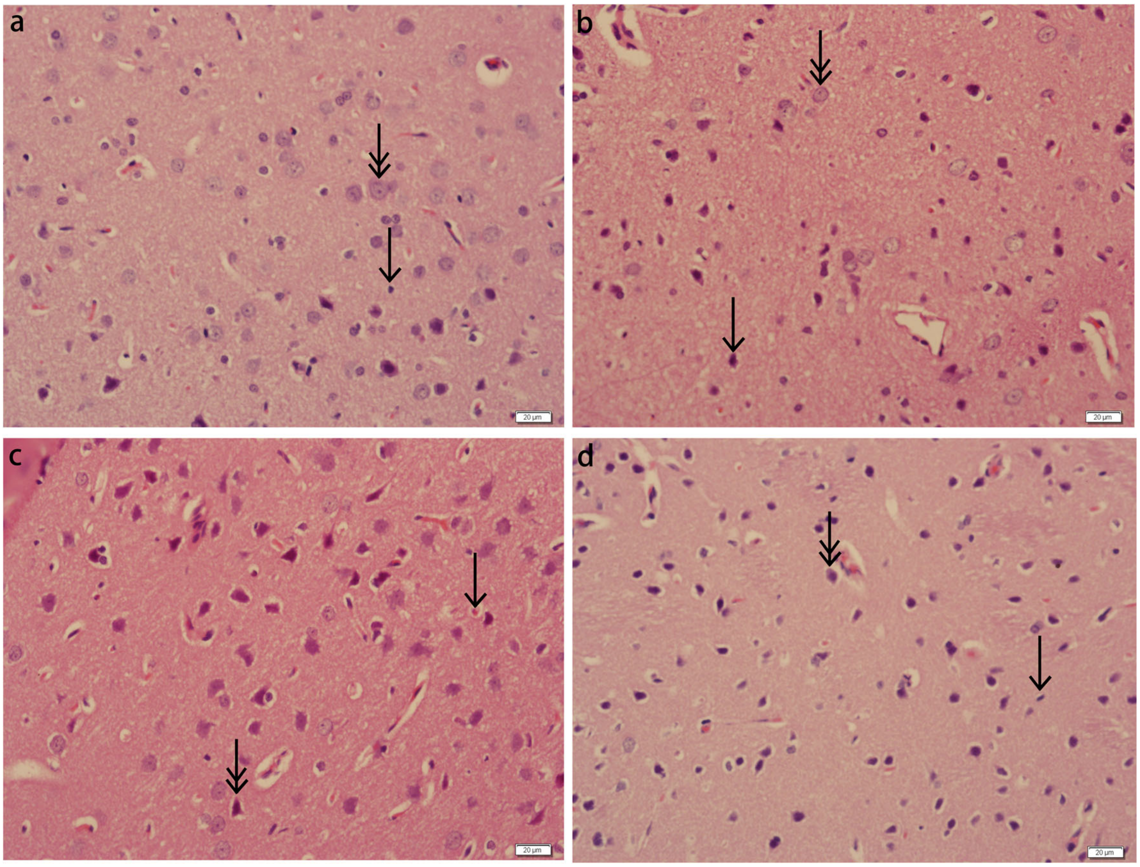

Fig. 3 HE staining of hypothalamic. Tail suspension might be no significant effect on cell number and morphology of the hypothalamus in this study. **a** CON group. **b** TS group. **c** TSV group. **d** TSP group. Single arrow showed non-neurocyte; Double arrow showed neurocyte. Values are mean ±SD. Bar= 20μm

explain why different training methods affect hormonal levels differently.

Aerospace flight or simulated flight can induce the development of multiple stresses in the body (Strewe et al. 2012; Schneider et al. 2009). The levels of HPA axis-related hormones will change accordingly when the body is stimulated by various acute or chronic stresses. After spaceflight, CORT serum levels significantly increased, whereas ACTH serum levels showed no evident increase (Macho et al. 1996; Macho L1 et al. 1991). Consistent with the results of previous studies, the present study showed that CORT serum levels significantly increased in the TS group, whereas ACTH serum levels showed no significant increase. Under the normal physiological state, the adrenal secretion of CORT regulates the pituitary and hypothalamus through negative feedback. ACTH also has a negative feedback on the hypothalamus. In this study, the CRH content decreased in the TS group. This response may be regulated by the negative feedback of CORT or ACTH.

Astronauts will experience a stress–adaptation–recession process after entering space (Lackner and DiZio 1991). The CORT level in the TS group significantly increased, whereas the CRH level showed no obvious change. These results indicated that tail suspension causes rats to remain in a stressed state. Therefore, the HPA axis levels of some hormones remained at a high level, and the HPA axis remained active. The function of the HPA axis might have been inhibited in the TS group. In addition, the TSV group exhibited significantly decreased CRH content and unchanged CORT content. These results suggested that local vibration training could weaken the excitability of the HPA axis in tail-suspended rats. The above results indicated that 35Hz local vibration cannot cause rats to remain in a stressed state and might not inhibit the function of the HPA axis. Therefore, we speculated that this local vibration intensity could protect the function of the HPA axis and helps tail-suspended rats to transition from the stressed to the adaptive state.

5-HT is an important neurotransmitter and immunomodulatory factor. Chronic stress can decrease the brain content of 5-HT. Local vibration can obviously improve the 5-HT content of the hypothalamus of tail-suspended rats. The local vibration effect is remarkably evident in the regulation of hypothalamic brain neurotransmitters.

The lack specific staining with specific antibodies of histology was one of our limitations in this study. We only show overviews with no difference between neuronal cells and non-neuronal cells. Moreover, the identification of the expression of some proteins related to HPA axis-related hormones, which may play a crucial role in regulating hormones secretion induced by mechanical loading, should be investigated in future studies. Finally, we did not consider the effect of exercise or vibration on normal control animals. It could be helpful to explore the mechanism of how the exercise or vibration works on relief stressed state. It will be considered in our future studies.

In summary, local vibration exercise can inhibit the hyperexcitation and protect the function of the HPA axis in tail-suspended rats. Local vibration exercise might delay the recession of tail-suspended rats. Furthermore, local vibration might be a promising countermeasure or adjunct to exercise for stress relief during long space flight or immobilization in normal people who are unsuitable to use active exercise or WBV. Therefore, more attention should be given to improve the efficiency and convenience of countermeasure protocols in the future.

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Compliance with Ethical Standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethical approval All animal treatments were conducted in accordance with the Regulation of Administration of Affairs Concerning Experimental Animals of State Science and Technology Commission of China and were approved by the Animal Care Committee of Beihang University.

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