

Electroacupuncture attenuates working memory impairment after chronic stress exposure in rat

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Abstract

Chronic stress (CS) exposure causes cytogenic and structural changes in the hippocampus which lead to cognitive impairment. Acupuncture has been shown to reduce cortisol secretion and protects hippocampus from stress-related damage. We hypothesize that acupuncture can alleviate stress-induced working memory deficit. This study aimed to investigate whether electroacupuncture (EA) can improve memory in chronic stress model in rat. This was an experimental study with pretest and posttest control group design. Rats were divided randomly into four groups: control group (C), CS group (T1), EA group (T2), and EA plus CS group (T3). Working memory was assessed using Eight-arm radial maze. A marked decrease in working memory was observed in the CS group demonstrated by more working memory correct errors ($p=0.020$) and incorrect errors ($p=0.024$) than control group. The CS plus EA rats made fewer working memory correct error ($p=0.001$) and incorrect error ($p=0.000$) compared to the CS only group. EA treatment in non-stressed rats did not significantly improve working memory compared to control. Chronic stress decreases working memory in rats. EA treatment at Baihui (DU 20) and Zusanli (ST 36) significantly attenuates stress induced working memory deficits in rats.

Keywords: electroacupuncture, working memory, chronic stress.

ABSTRAK

Paparan stress kronik dapat menyebabkan perubahan struktural dan sitogenik di hippocampus sehingga timbul gangguan kognisi. Akupunktur dapat menurunkan sekresi kortisol dan melindungi hippocampus dari kerusakan akibat stres. Penelitian ini bertujuan untuk mengetahui pengaruh akupunktur terhadap gangguan memori akibat paparan stress kronik. Penelitian ini merupakan penelitian eksperimental dengan pretest and posttest control group design. Tikus putih dibagi menjadi 4 kelompok yaitu kontrol (C), CS (T1), EA (T2), dan EA plus CS (T3). Memori kerja diuji menggunakan Eight-arm radial maze. Penurunan memori kerja secara signifikan tampak pada kelompok CS dibanding kontrol. Akupunktur dapat memperbaiki penurunan memori kerja akibat stress secara signifikan. Stress kronik mampu menurunkan memori kerja dan pemberian EA di titik Baihui (DU 20) dan Zusanli (ST 36) secara signifikan mampu mengurangi penurunan memori kerja akibat paparan stress tersebut.

Kata kunci: elektroakupunktur, memori kerja, stress kronik.

1. Introduction

One of the major predisposing factors for the development of psychiatric disorder such as depression and anxiety is stressful events.¹ Not surprisingly, exposure to stress has become one of the most widely used approach to create animal model of depression in the laboratory.²

The body's response to any stressful physical and psychological stimulus gives rise to the disruption of the ongoing homeostasis.³ Stress exposure leads to the development of stress syndrome ranging from emotional disorders, behavioral disorders, reproductive dysfunction, growth and immunity up to cognitive impairment.⁴ Previous studies showed that exposure to stress impaired

cognitive function demonstrated with several behavioral paradigms such as impairment in spatial learning memory assessed with Morris water maze test^{5,6} and recognition memory deficit in the novel object recognition task^{5,7} Working memory reflects the cognitive function required for the representation of objects or places during goal directed learning.⁸ Working memory plays a crucial role in temporary holding, processing and manipulation of information and represents a core executive function.^{9,10} It is not a single phenomenon but rather a combination of cognitive processes¹¹ consisting of short term memory and attentional control.¹² Hippocampus and medial prefrontal cortex are two main areas in the brain associated with working memory.¹³ Chronic stress gives rise to decreased hippocampal volume and number of neurons.^{14,15}

Various kind of therapeutic modalities have been used to alleviate the impact of stress on human working memory. Liu et al. (2007) reported that 30% of patients do not respond chemical drugs. Moreover, the occurrence of serious side effects also limits the use of these drugs.¹⁶ Thus, there is an urge to find an alternative therapy that is safe and effective to treat the disorders arising from chronic stress exposure.

Acupuncture alone has long been used as a treatment of physical and mental disorders in traditional Chinese medicine with minimal side.¹⁷ Previous studies have shown that stimulation of acupuncture points can stimulate the brain to secrete endorphine¹⁷, reduces level of stress hormone, cortisol¹⁸, increases levels of antioxidants¹⁹, inhibits apoptosis in hippocampus to regulate gene expression of apoptosis²⁰, and increases neurogenesis in the hippocampus.¹⁶ Considering the facts that acupuncture can regulate stress response and protects hippocampus against stress, we hypothesize that electroacupuncture treatment can restore working memory deficit caused by chronic stress exposure.

2. Material And Methods

Twenty four adult (aged about 8 weeks) male rats (*Rattus norvegicus*) were used in this study. They were housed with 12 h light/dark cycle and ad libitum access to food and water. Rats were exposed to chronic restrain stress for 21 days. Each rat was placed in a transparent acrylic tube sized 15 x 5,5 cm for 2 hours everyday from 11:00 a.m to 01:00 pm

Electroacupuncture was performed bilaterally at two points namely the Baihui (GV 20) located at the intersection of the sagittal midline and the line linking the two ears and Zusanli (ST 36) located 5 mm below lateral anterior tibial tubercle. Disposable acupuncture needles (sized 0.20 x 13 mm, Huanqiu, China) were inserted perpendicularly 5 mm deep into the acupoints. An electrostimulator was connected and electrical current was delivered to the needles. EA was performed with constant frequency at 2 Hz for 15 minutes. This treatment was carried out for 14 days, starting from the 2nd week of stress exposure up to the end of the 3rd week.

Working memory was assessed using 8-arms radial maze. Everyday during the experiments, the rats had to learn about the location of food in all arms of the maze. Arms that did not contain food were used as the reference and remained constant to extra maze cues throughout the experiment. Thus, entries to these arms indicated poor reference memory and impairment of long term learning. The remaining arms all contained foods at the start of the experiment. However, no food would be in the arm once the rat successfully entered the arm and ate the pellet. Therefore, the rat had to remember which arms that had been entered previously over the successive testing. Working memory was examined by counting re-entries into arms containing food previously visited within the same testing day or re-entries into reference memory arms. Repeated entries into any arm that previously contained food were considered the working memory correct errors while repeated entries into reference arms were the working memory

incorrect errors. Working memory assessment was performed twice before and after stress exposure and EA treatment.

This was an experimental laboratory study with pre- and posttest control group design conducted in the Laboratory of Biochemistry Faculty of Medicine, Sebelas Maret University, Surakarta. Rats were divided into 4 groups randomly: the control group (C), chronic stress group (CS), electroacupuncture group (EA) and chronic stress plus electroacupuncture group (CS+EA). Data were analyzed using Anova followed by Post Hoc Tukey HSD Test with SPSS for Windows Release 22.0. The significance level was set at $P < 0.05$.

3. Results

Prior to stress exposure and EA treatment, working memory was assessed using 8-arms radial maze. There was no significant difference in the number of working memory correct error ($p=0.769$) and working memory incorrect error ($p=0.804$) between groups. This indicates that the cognitive function of all rats at the beginning of the experiment were homogenous. However, after stress exposure, a marked decrease in working memory was observed in the CMS group demonstrated by more working memory correct errors ($p=0.020$) and incorrect errors ($p=0.024$). On the other hand, EA significantly restored the working memory impairment due to stress exposure. The CMS plus EA rats made fewer working memory correct error ($p=0.001$) and incorrect error ($p=0.000$) compared to the CMS only group. EA group performance did not differ significantly when compared to control ($p=0.971$ and $p=0.814$ for working memory correct and incorrect errors respectively) (See Figure 2 and 3).

4. Discussion

Varying degrees and forms of stressor can be found in everyday life. Stress leads to the activation of the fast acting sympathetic

nervous system (SNS) and the slow acting hypothalamic–pituitary–adrenal (HPA) axis. SNS activation causes the adrenal medulla to release the catecholamines adrenaline and noradrenaline which prepare the organism for a ‘fight-or flight’ response. HPA-axis activation leads to the release of glucocorticoid hormones from the adrenal cortex (mainly cortisol in humans, corticosterone in rats). Stress-related effects on health, emotion, and cognition are mediated by these catecholamines and glucocorticoids.^{21,22} Glucocorticoids secreted during stressful events can readily cross the blood–brain barrier and bind to their receptors in limbic brain areas and modulate memory processes in the hippocampus and prefrontal cortex.²³

Previous findings demonstrated that stress exposure affects learning and memory.^{24,25} Exposure to stress impaired cognitive function such as impairment in spatial learning memory^{5,26} and recognition memory.^{5,27} In our experiment, stress exposure in the form of chronic restrain stress significantly impaired the working memory in rats compared to control group and EA treatment successfully restored the memory decline after stress exposure. This finding is consistent with previous report showing that acupuncture can attenuate stress-induced memory loss.^{28,29}

We used two acupoints in this study: Baihui (DU 20) and Zusanli (ST 36). Acupuncture at point Zusanli (ST-36) was reported to cause deactivation of the limbic system including the amygdala and hypothalamus.^{23,30} The limbic system constitutes a group of limbic, paralimbic and neocortical brain regions that regulate cognition, affect, biological behavior, as well as endocrine and autonomic nervous functions. These structures are activated by stress and pain.²³

We argue that, in our study, improvement of working memory in the CS plus EA group was, in part, mediated by the central effect of acupuncture on the limbic system resulting in the suppression of

stress-induced HPA-axis hyperactivity. As a consequence, glucocorticoids level decreased and thus, glucocorticoid-mediated downstream signaling associated with memory deficit such as decreased neurogenesis, neuronal atrophy and apoptosis in the hippocampus is inhibited. Baihui (GV20) is considered one of the most important acupoints of the entire meridian system. It has stimulatory effects on the central nervous system leading to the release of neurotransmitters and neuromodulators in the brain and spinal cord and has been widely used to treat psychiatric disorder.³¹ Acupuncture at this point has been shown to ameliorate cognitive impairment, inhibited neuronal apoptosis and increased the level of BDNF in the hippocampus of the mouse of model Alzheimer's disease.³² Considering the central effect of acupuncture on the limbic system^{23,31}, we believe that acupuncture has a potential significance for therapeutic use in cognitive disease states.

5. Conclusion

Electroacupuncture at Baihui (DU 20) and Zusanli (ST 36) significantly alleviate the working memory impairment induced by chronic restrain stress in rat.

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