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Basic Study

Effects of acupuncture on neuro-electrophysiological activities in hippocampal CA1 and CA3 areas of rats with post-traumatic stress disorder

针刺对创伤后应激障碍模型大鼠海马CA1和CA3区神经电生理活动的影响

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Abstract

Objective: To observe the effects of acupuncture on the characteristics of neuro-electrophysiological activity in hippocampal CA1 and CA3 areas of rats with post-traumatic stress disorder (PTSD).

Methods: Fifty Sprague-Dawley (SD) rats were randomly divided into a blank group, a model group, a grasping group, a Western medicine group and an acupuncture group, with 10 rats in each group. Except for the blank group, rats in the other 4 groups all received the combined stress modeling method. Rats in the Western medicine group were intragastrically administrated with paroxetine hydrochloride, those in the acupuncture group received acupuncture intervention, those in the grasping group received grasping fixation, and those in the model group and the blank group did not receive any interventions. After 14 d of intervention, the interspike interval (ISI) and power spectral densities (PSD) were analyzed and mapped by *in vivo* multiple channels to record the neuron clusters discharge in the hippocampal CA1 and CA3 areas.

Results: Compared with the blank group, ISI was prolonged in the CA1 and CA3 areas of the model group and the grasping group, and the concentrated PSD distribution area moved down ($P<0.05$ or $P<0.01$). Compared with the grasping group, the ISI of the CA1 and CA3 areas in the Western medicine group and the acupuncture group was shortened, and the concentrated PSD distribution area moved up ($P<0.05$ or $P<0.01$). The ISI and PSD distributions in the CA1 and CA3 areas of the acupuncture group were not statistically different from those in the Western medicine group (both $P>0.05$).

Conclusion: Both acupuncture and paroxetine hydrochloride can significantly regulate the neuro-electrophysiology activity of hippocampal CA1 and CA3 areas in PTSD rats, which may be one of the mechanisms of acupuncture intervention to promote PTSD recovery.

Keywords: Acupuncture Therapy; Point, Baihui (GV 20); Point, Neiguan (PC 6); Point, Shenmen (HT 7); Point, Taichong (LR 3); Stress Disorders, Post-traumatic; Electrophysiology; Rats

【摘要】目的: 观察毫针针刺对创伤后应激障碍(PTSD)模型大鼠海马 CA1 和 CA3 区神经电生理活动特征量的影响。**方法:** 将 50 只 Sprague-Dawley (SD)大鼠随机分为空白组、模型组、抓取组、西药组和针刺组, 每组 10 只。除空白组外, 其他 4 组大鼠以复合应激法造模。造模同时西药组予以盐酸帕罗西汀灌胃, 针刺组接受针刺干预, 抓取组接受抓取固定, 模型组和空白组不接受任何干预。干预 14 d 后, 通过在体多通道记录海马 CA1 和 CA3 区神经元集群放电, 分析峰-峰间期(ISI)和功率谱密度(PSD)并绘图。**结果:** 与空白组比较, 模型组和抓取组 CA1 和 CA3 区 ISI 延长, PSD 集中分布区域下移($P<0.05$ 或 $P<0.01$); 与抓取组比较, 西药组和针刺组 CA1 和 CA3 区 ISI 缩短, PSD 集中分布区域上移($P<0.05$ 或 $P<0.01$); 针刺组 CA1 和 CA3 区的 ISI 和 PSD 集中分布区域与西药组无统计学差异(均 $P>0.05$)。**结论:** 毫针针刺与盐酸帕罗西汀干预均可显著调节 PTSD 模型大鼠海马 CA1 和 CA3 区神经电生理活动特征量发放模式, 可能是针灸干预促进 PTSD 恢复的机制之一。

【关键词】 针刺疗法; 穴, 百会; 穴, 内关; 穴, 神门; 穴, 太冲; 应激障碍, 创伤后; 电生理学; 大鼠

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Post-traumatic stress disorder (PTSD) refers to the stress reactions following exposure to the threatening or catastrophic psychological trauma. The reactions may gradually aggravate over a period of time. It is a mental and psychological syndrome[1]. Social events such as natural disasters, traffic and workplace accidents have increased the incidence of PTSD. Drugs and psychological methods commonly used to treat PTSD

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nowadays all have obvious adverse reactions or limitations[2-3]. Our group members once participated in the post-disaster treatment and found that acupuncture treatment of PTSD showed significant advantages, and it has attracted attention rapidly and widely because it is convenient and free of adverse reactions and side effects.

Hippocampus is the high-level regulation center of stress response, and it directly participates in the development of PTSD[4]. Hippocampal damage can lead to the disappearance of its regulation on negative stress feedback, while the decrease in neuronal activity can further aggravate the stress response[5]. Because the CA1 and CA3 areas in hippocampus are most closely related to PTSD[6], our research group obtained the interspike interval (ISI) and power spectral densities (PSD) of the neuron cluster discharge in the hippocampal CA1 and CA3 areas of PTSD model, to initially explore the neurological mechanism of acupuncture intervention on PTSD.

1 Experimental Animals and Materials

1.1 Experimental animal

Fifty SPF healthy Sprague-Dawley (SD) rats, with 2 month old and weighing (200±20) g [provided by Research Center of Gansu University of Chinese Medicine, certificate number: 62001000000091, license number: SCXK (Gan) 2011-0001] were adaptively fed for 7 d with free access to food and drink with the ambient temperature (23±2) ℃, relative humidity (60±10)%, 12 h/12 h light shifting (8:00-20:00 light), and daily stroke for 3 min to avoid fright. Animal treatment strictly followed the instructive notions with respect to caring for laboratory animals.

1.2 Laboratory instruments and apparatuses

Electric shock plus incarceration modeling box, power supply, and circuit (made by our laboratory); brain stereo locator (Shenzhen RWD Life Science Co., Ltd., China); Cerebus multi-channel data acquisition system (including microelectrode array, preamplifier, processor, independent constant voltage power supply, PC workstation, etc.) (Blackrock Microsystem Inc., USA); MEA 16 channel (4×4) acute microarray electrode (MicroProbes, USA); Hwato Brand disposable sterile acupuncture needle (0.25 mm in diameter, 25 mm in length, Suzhou Medical Products Factory Co., Ltd., China).

1.3 Experimental drugs and reagents

A 10% chloral hydrate (batch number: 20121106, Shanghai Zhanyun Chemical Co., Ltd., China) and 25% urethane (batch number: 20130609, Sinopharm Chemical Reagent Co., Ltd., China) were mixed at 1:1 for the anesthetic; solution of paroxetine hydrochloride (batch number: 150104, 2 mg/mL, Zhejiang Jianfeng Pharmaceutical Co., Ltd., China).

2 Experimental Methods

2.1 Grouping and intervention

Using random number table method, 50 rats were divided into a blank group, a model group, a grasping group, a Western medicine group and an acupuncture group, with 10 rats in each group, and each rat was kept in a single cage. Since up to 6 rats can be modeled at the same time, all the rats are randomly divided into batches after numbered, 6 rats/batch, and experiments were performed in batches until the *in vivo* multi-channel signal recording was completed. The intervention methods of each group are as follows.

Blank group: No modeling, no grasping and fixing, no treatment.

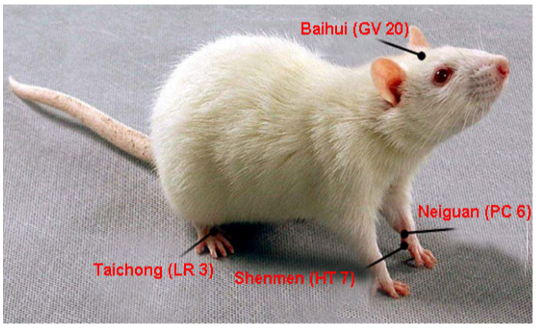
Model group: PTSD model was prepared without grasping, fixing and treatment.

Grasping group: PTSD model was prepared with grasping and fixing, without treatment. Grasping time was the same as that in the acupuncture group and the Western medicine group.

Western medicine group: After grasping and fixing, the PTSD model rats received administration of the paroxetine hydrochloride solution with conventional gavage method at a dose of 5 mL/(kg·bw) each time. The drug solution was evenly injected within 1 min, and the gavage needle was then withdrawn and the rats were given continuous grasping for 4 min.

Acupuncture group: PTSD rat model was prepared. After grasping and fixing, Baihui (GV 20) (locates at the center of the parietal bone), Neiguan (PC 6) (locates at the medial of the forelimb, about 3 mm away from the wrist joint between the ulna and the radius), Shenmen (HT 7) (locates at the medial of the forelimb, along the lateral edge of ulna at the wrist rasceta) and Taichong (LR 3) (locates at the depression between the 1st and 2nd metatarsal bones of the hind-paw dorsum) were selected for acupuncture by referring to *Experimental Acupuncture Science* (Figure 1)[7]. After routine disinfection, Baihui (GV 20) was first punctured backward for 4-5 mm by subcutaneously insertion and holding and lifting the soft tissues; and then Neiguan (PC 6), Shenmen (HT 7) and Taichong (LR 3) received oblique needling for 2-3 mm. The needles were retained. The basic needling manipulation was conducted for one minute per needle. All needles were removed after 4 min and the needle holes were pressed with dry cotton swab to stop bleeding. Rats were then returned back to the cages for feeding. Bilateral acupoints of the extremities were alternately used every other day for acupuncture.

The above grasping, fixation or treatment was performed once a day for 14 d.



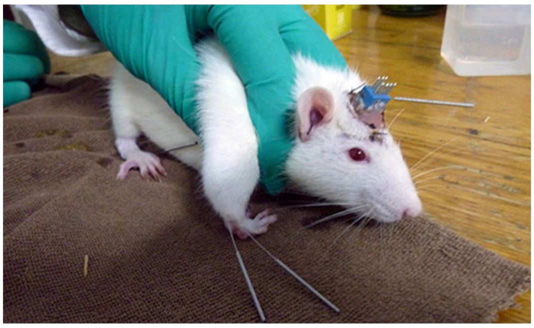


Figure 1. Locations of acupoints in rats

2.2 PTSD model replication

All rats other than in the blank group were used to prepare PTSD models by a combined stress modeling method. Each batch of 6 rats was simultaneously modeled according to the reference[8]. The modeling box was cleaned to set AC voltage at 60 V and current at 48 mA (8 mA per box). Each single rat was put into a box to avoid light for 30 min; during the period, the power was randomly turned on, and 4 s electrical stimulation was applied to the rat paw bottom for 30 times with random interval in between. The above electric shock and claustrophobia were performed once a day for 6 d.

On the 7th day, the rat was tied with a 70 g load around the neck and placed in a water tank [temperature: (28±2) ℃] to swim. The rats were immediately taken out, dried in a warm and dry place, and returned to the cage for feeding when the rats were exhausted, gave up struggle and started to sink to the bottom. Rats in the blank group did not receive any treatment and were fed routinely.

2.3 Stereotactic positioning and craniotomy

Rats were anesthetized by intraperitoneal injection with a mixed anesthetic at a dose of 5 mL/(kg·bw). It's indicated that the anesthesia was successful when the rats had fallen, with relaxed muscles and stable breathing, and the paw bottom losing response to stimulation. After successful anesthesia, the rats were placed on the brain stereotaxic instrument, and the skull was fixed with a nose clip to ensure normal ventilation; the height was adjusted so that the frontal fontanelle and the herringbone seams remained at the same level; the scale of the bilateral ear rods was adjusted so that the rat's head was in the middle and fixed, and then the local hair was shaved for skin preparation and disinfection. Opened the skull skin as '–' shape with a handle scalpel along the sagittal suture from the midpoint of the line connecting the two eyes to the midpoint of the line connecting the leading edges between the ears. The subcutaneous tissue was separated by blunt dissection with straight tweezers and the periosteum was removed. The residual on the skull surface was removed by hydrogen peroxide and infiltrated in the saline to fully expose the markers of the anterior fontanelle, the midline and the herringbone.

Referring to the *Rat Brain in Stereotaxic Coordinates*[9] and the literature[10], the hippocampal CA1 and CA3 areas were located on the left and right sides of the midline, respectively. Under the microscope, a projection region of about 3 mm×3 mm was delineated from the skull surface corresponding to the transmission point of the hippocampal CA1 and CA3 areas. The edge of the region was drilled with multiple points of craniotomy, and the depth was suitable for penetrating the skull without damaging the cerebral dura mater. After the drilling, the skull was taken with ophthalmology tweezers and the cerebral dura mater was removed. The cerebral cortex was exposed and infiltrated with saline for electrode embedding.

2.4 Electrode implantation and signal acquisition

The microelectrode pusher was installed on the stereo locator, the MEA was fixed, and the cerebral dura mater was set to the vertical axis '0' position. According to the reading of the digital display module, the MEA was slowly pushed into the hippocampal CA1 and CA3 areas by the microelectrode pusher. The electrode ground wire was embedded in the surrounding subcutaneous tissues.

The multi-channel neural signal recording system was turned on. The Central software was set with the sampling frequency at 20 kHz, the filtering range in 0.3-7.5 kHz, and the effective threshold potential at –63 μV. The real-time data were recorded *in vivo* for 180 s for ensuring that the signal was not disturbed and the electricity activity was rich and stable. The collected electrical signals were converted into ISI by Neuro Explorer software, and the average ISI in 180 s was obtained to generate a scatter plot. The spectrum signal was synchronously recorded, and the fast Fourier transform (FFT) of the signal autocorrelation function was calculated for the PSD, and the spectrogram was exported. After the signal recording was completed, the animals were sacrificed.

2.5 Statistical processing

Statistical processing was performed using SPSS version 19.0 software. When setting each group of data, the group distance was equal. The measurement data were expressed as mean ± standard deviation ($\bar{x}$ ±*s*). One-way ANOVA was used for between-group comparison. The least significant difference (LSD) was used for homogeneity of variance. Tamhane's T2 test was used for heterogeneity of variance. The difference was considered statistically significant when $P \le 0.05$.

3 Experimental Results

3.1 General condition and behavioral investigation

After modeling, compared with the blank group, the activity and food intake of rats in the model group were significantly reduced with irritation, contraction, and messy hair; the behavioral test found that the number of crossing lines was reduced in the open field test, and the refraction reflex score was reduced, confirming the successful modeling. After treatment, compared with the grasping group, the rats in the Western medicine group and the acupuncture group showed neat hair, normal movement, high agility, good mental condition, mild temper, not easy to be provoked, and normal amount of food intake, but some rats still showed higher alertness. Some rats in the Western medicine group appeared lethargy.

3.2 ISI

3.2.1 Distribution

Compared with the blank group, the discharge interval sequence of the model group was significantly prolonged, and the pulse number was reduced, but the grasping group did not change significantly compared with the model group. Compared with the grasping group, the Western medicine group and the acupuncture group showed that the discharge interval sequence was significantly shortened and the pulse number was increased, but there was no significant difference between the two treatment groups, suggesting that both treatments could regulate the ISI distribution, but there was no significant difference between them (Table 1).

Table 1. ISI distribution in hippocampal CA1 and CA3 areas of rats in each group (s)

Group	*n*	CA1 area		CA3 area	
		Centralized distribution range	Distribution shape	Centralized distribution range	Distribution shape
Blank	10	0.0-2.0	Divergence	0.0-2.5	Divergence
Model	10	0.0-15.0	Divergence	0.0-20.0	Divergence
Grasping	10	0.0-15.0	Divergence	0.0-15.0	Divergence
Western medicine	10	0.0-2.0	Divergence	0.0-3.0	Divergence
Acupuncture	10	0.0-2.5	Divergence	0.0-2.5	Divergence

3.2.2 Data results

After modeling, compared with the blank group, the ISI was prolonged in the model group ($P<0.05$ in CA1 area and $P<0.01$ in CA3 area), suggesting that combined stress prolonged the pulse interval of the brain area to cause ISI abnormality, which was more significant in CA3 area. There was no statistical difference between the grasping group and the model group ($P>0.05$ in both areas), suggesting that grasping would not affect ISI. Compared with the grasping group, the ISI was shortened in the Western medicine group and the acupuncture group after treatment ($P<0.05$ in the CA1 area and $P<0.01$ in the CA3 area), suggesting that both treatments could regulate ISI, which was more obvious in the CA3 area; there was no significant difference between the Western medicine group and the acupuncture group ($P>0.05$ in both areas), suggesting that the two therapies were equally effective in regulating ISI. The detail is shown in Figure 2.

3.3 PSD

3.3.1 Distribution

Compared with the blank group, the PSD concentrated distribution area of the model group moved down, but the grasping group did not change significantly compared with the model group. Compared with the grasping group, the PSD concentration area after treatment moved up in the Western medicine group and the acupuncture group, suggesting that both therapies could positively regulate PSD, but there was no significant difference between the Western medicine group and the acupuncture group. Both therapies could adjust the PSD distribution range (Table 2).

3.3.2 Data results

After modeling, compared with the blank group, the PSD of the model group was decreased ($P<0.05$ in CA1 area and $P<0.01$ in CA3 area), suggesting that compound stress could decrease PSD and produce a greater effect on CA3 area. There was no significant difference between the grasping group and the model group (P>0.05 in both areas), suggesting that grasping would not affect PSD. Compared with the grasping group, the PSD was increased after treatment in the Western medicine group and the acupuncture group (P<0.05 in both CA1 areas, P<0.05 in the Western medicine group CA3 area, and P<0.01 in the acupuncture group CA3 area), suggesting that both therapies could promote PSD recovery, and the impact of acupuncture on CA3 area was more significant; there was no statistically significant difference between the Western medicine group and the acupuncture group (P>0.05 in both areas), suggesting that there was no significant difference in the regulation of PSD between the two treatments. The detail is shown in Figure 3.

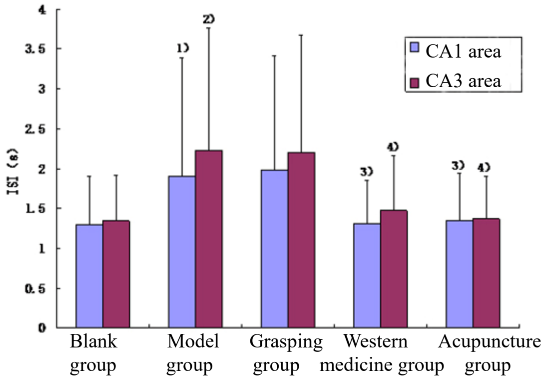


Figure 2. ISI histogram of hippocampal CA1 and CA3 areas of rats in each group

Note: Compared with the blank group in hippocampus, 1) P<0.05, 2) P<0.01; compared with the grasping group in hippocampus, 3) P<0.05, 4) P<0.01

Table 2. PSD distribution in hippocampal CA1 and CA3 areas of rats in each group (dB)

Group	n	Centralized distribution range of CA1 area	Centralized distribution range of CA3 area
Blank	10	−(105-111)	−(105-109)
Model	10	−(116-121)	−(121-125)
Grasping	10	−(117-122)	−(122-127)
Western medicine	10	−(108-114)	−(106-111)
Acupuncture	10	−(110-106)	−(105-110)

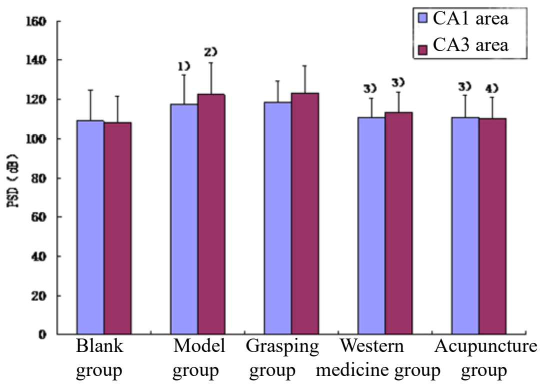


Figure 3. PSD histogram of hippocampal CA1 and CA3 areas in each group

Note: Compared with the blank group in hippocampus, 1) P<0.05, 2) P<0.01; compared with the grasping group in hippocampus, 3) P<0.05, 4) P<0.01

4 Discussion

Hippocampus is one of the brain regions that regulate stress and most significantly affected by stress. During the stress response, the sympathetic adrenal medulla system can increase the long-term concentration of norepinephrine[11], promote the supernormal release of excitatory amino acids in the hippocampus, cause intracellular calcium overload, and then lead to the overload and increased release of calcium ion in mitochondria, endoplasmic reticulum and other organelles. Interact with calmodulin, resulting in decreased activity and dysfunction of hippocampal neurons[12]. Increased corticotropin-releasing hormone (CRH) is a key link in the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamus increases CRH secretion to stimulate secretion of adrenocorticotropic and glucocorticoids hormones. The abnormal activation of the HPA axis causes PTSD-related symptoms. Some studies have found that CRH is highly expressed in the hippocampal CA1 and CA3 areas of PTSD rats, indicating that CA1 and CA3 areas are most closely related to PTSD in hippocampal structures[6]. Sousa N, *et al*[13] found that unpredictable chronic stress stimulation could not only shorten the dendritic length of the hippocampal CA3 area by 28%, but also shorten the dendritic cell length of the hippocampal CA1 area by 13%. Although not as significant as the CA3 area, the hippocampal CA1 was also proved to present obvious changes during the stress response. Hippocampus is an important part of brain information processing[14]. Neuro-electrophysiology believes that neurons are connected by synapses. Information exchange is realized by discharge time and spatial sequence of many action potentials. Due to the complex network structure of many cerebral cortex neurons forms the information coding characteristics of the nerve center, the research based on the characteristics of neural bioelectric signals is an innovative field of brain function research[15-16].

Neuron cluster discharge *in vivo* synchronous multi-channel recording technology is a key technology for brain neural network research[17]. Applying this technology, the traditional activity record of a single neuron is evolved into a synchronous recording of the neuron cluster discharge activity, which can accurately analyze the relationship between time, frequency and space of the neural electrical signal. Recording the high-resolution time and space information during the local area transmission provides new ideas for studying the relationship between different brain regions and nuclear groups in time and space. At present, *in vivo* multi-channel technology has been applied to the study of life phenomena and disease changes closely related to the nervous system such as higher animal learning, memory, sensation, and exercise[18], and has become an important tool to reveal the synergistic effects of different levels of neural electrical signals[19]. The information coding characteristics of the neuron group are not only reflected in the discharge frequency, since the main features of the action potential are shown by the peak potential, which has become synonymous with the action potential. Therefore, ISI has become the object of many neuro-electrophysiological studies[20]. Some scholars believe that the ISI of electrophysiological activity in living organisms is the sequence coding of the information transmitted by organisms. Sequence analysis is recognized as the main element carrying biological information and plays an important role in the coding of neural information[21-22]. Treatment of the cluster discharge signal in the mouse hippocampal CA1 area revealed that the pyramidal cell discharge wave in the hippocampal CA1 showed short and sharp peaks, and decreasing peaks and interval peaks. ISI suggested the periodic characteristics of hippocampal discharge[23]. In the estimation of the field potential power spectrum, the PSD of the random signal can describe the power characteristics of the signal as a function of the discharge frequency[14]. It is a frequency domain coherence analysis and also extremely important information for electrical signal processing[21].

The results of this study showed that acupuncture could shorten the abnormal action potential ISI in the hippocampal CA1 and CA3 areas of PTSD model rats, and increase the pulse number, promote the upward shifting of the PSD concentrated distribution area and restore the abnormal PSD. Among them, the effect on the hippocampal CA3 area was obvious, and there was no significant difference between acupuncture and paroxetine hydrochloride. It's indicated that acupuncture could effectively regulate the basic formation of abnormal neuro-electrophysiological neural network coding, which may be related to the mechanism of acupuncture in promoting PTSD recovery, and the study also provided a neuro-electrophysiological experimental basis for the effectiveness of acupuncture treatment of PTSD.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria.

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