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Basic Study

Effect of liver-soothing and mind-regulating acupuncture on resting-state electroencephalographic signals in rats with post-traumatic stress disorder

疏肝调神针法对创伤后应激障碍模型大鼠静息态脑电信号的影响

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Abstract

Objective: To observe the effect of liver-soothing and mind-regulating acupuncture method on the resting-state electroencephalography (EEG) in rats with post-traumatic stress disorder (PTSD), and to provide evidence for the effect mechanism study and clinical application of acupuncture intervention for PTSD.

Methods: Sixty Sprague-Dawley (SD) rats were randomly divided into a blank control group, a model group, a grasping group, a paroxetine group and an acupuncture group, with 12 rats in each group. Except for rats in the blank control group, rats in the other groups were subjected to preparing the PTSD models using 'incarceration plus electric shock' method. After interventions, changes in rat behavior of each group were observed; changes in resting-state EEG were collected and analyzed with multichannel EEG acquisition and analysis system, and image analysis and statistical processing were performed.

Results: Compared with the blank control group, the average escape latency in the model group was significantly longer ($P<0.05$), and the times of crossing the platform and the effective areas were all significantly reduced ($P<0.01$). Compared with the grasping group, the average escape latencies in the paroxetine group and acupuncture group were significantly shortened ($P<0.05$), and the times of crossing the platform and the effective areas were all significantly increased ($P<0.05$). There were no significant differences in the average escape latency, the times of crossing the platform and the effective areas between the acupuncture group and paroxetine group (all $P>0.05$). Compared with the blank control group, the α-wave power spectrum value in the model group was significantly decreased, and the power spectrum values of β-wave, δ-wave and э-wave were significantly increased (all $P<0.01$); compared with the grasping group, α-wave power spectrum values in the paroxetine group and acupuncture group were significantly increased (both $P<0.01$), and the power spectrum values of β-wave, δ-wave and э-wave were decreased significantly (all $P<0.01$). The power spectrum values of α-wave, β-wave, δ-wave and э-wave of rats in the acupuncture group were not significantly different from those in the paroxetine group (all $P>0.05$).

Conclusion: Liver-soothing and mind-regulating acupuncture method can significantly improve the abnormal EEG activity in PTSD rats, which may be one mechanism of liver-soothing and mind-regulating acupuncture method in effectively affecting the brain function in PTSD rats.

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

【摘要】目的：观察疏肝调神针法对创伤后应激障碍(PTSD)模型大鼠静息态脑电活动的影响，为针刺干预PTSD的效应机制研究和临床应用提供依据。**方法：**将60只Sprague-Dawley (SD)大鼠随机分为空白对照组、模型组、抓取组、帕罗西汀组和针刺组，每组12只。除空白对照组外，其余各组大鼠通过“幽闭+电击”法复制PTSD模型。各组大鼠接受相应的干预后，观察其行为学的改变，并应用多通道脑电采集与分析系统检测其静息态脑电的改变，进行图像分析和统计学处理。**结果：**与空白对照组比较，模型组大鼠平均逃避潜伏期明显延长($P<0.05$)，平台穿越次数、穿越有效区域次数明显减少($P<0.01$)；与抓取组比较，帕罗西汀组和针刺组大鼠平均逃避潜伏期明显缩短(均$P<0.05$)，平台穿越次数、穿越有效区域次数明显增加(均$P<0.05$)；针刺组平均逃避潜伏期、平台穿越次数及穿越有效区域次数与帕罗西汀组比较，差异均无统计学意义(均$P>0.05$)。与空白对照组比较，模型组大鼠α波

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功率谱值明显下降, β波、δ波、϶波功率谱值明显增高(均P＜0.01); 与抓取组比较, 帕罗西汀组和针刺组大鼠α波功率谱值明显上升(均P＜0.01), β波、δ波和϶波功率谱值明显下降(均P＜0.01); 针刺组α波、β波、δ波和϶波功率谱值与帕罗西汀组比较, 差异均无统计学意义(均P＞0.05)。**结论**：疏肝调神针法可明显改善PTSD大鼠异常脑电活动, 可能是疏肝调神针法有效干预PTSD的脑功能机制之一。

【关键词】针刺疗法; 穴, 百会; 穴, 神门; 穴, 内关; 穴, 太冲; 应激障碍, 创伤后; 脑电描记术; 大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Post-traumatic stress disorder (PTSD) is a long-lasting mental disorder that can develop after a person is exposed to a sudden, threatening and/or catastrophic event[1]. It is characterized by three major symptoms: intrusive, recurrent recollections, dissociative episodes of reliving the trauma (known as 'flashbacks'), and an increase in the fight-or-flight response[2]. Epidemiological studies found that the incidence of PTSD in Wenchuan and Yushu earthquake areas in China was 85% and 60% respectively[3-4]; the average prevalence of PTSD in the United States is 8%[5]. PTSD seriously affects the physical and mental health, as well as the social function of patients, and it is difficult to be cured[6]. At present, treatment of this disease mainly relies on medication and psychological interventions. The long-term application of medication can cause drug addiction; the psychological intervention requires compliance and is time- consuming. Therefore, it has become an inevitable trend in the future to explore a relatively safe and effective therapeutic method with fewer adverse reactions for this disease.

In recent years, acupuncture has been gradually applied to the clinical treatment of PTSD[7], but there are relatively few reports on the mechanism of acupuncture in the prevention and treatment of PTSD. In this experiment, based on the effective treatment of PTSD by liver-soothing and mind-regulating acupuncture method, rat behaviors were observed and the changes in resting-state electroencephalography (EEG) in the rat models were detected, thus to reveal and investigate the regulation of neurological function by liver-soothing and mind-regulating acupuncture method in PTSD rat models, and to provide evidence for the mechanism study and clinical application of acupuncture on PTSD.

1 Materials and Methods

1.1 Laboratory animals and grouping

Sixty male adult Sprague-Dawley (SD) rats, 6-week old, weighing (180±20) g[8], were provided by the SPF Experimental Animal Center of the Research Center of Gansu University of Chinese Medicine. The sixty rats with normal memory were selected by screening with Morris water maze test. After adaptive feeding for 7 d in the rat breeding room, the rats were randomly divided into a blank control group, a model group, a grasping group, a paroxetine group and an acupuncture group, with 12 rats in each group. The disposal of animals during the experiment was in line with of the *Guiding Opinions on the Treatment of Experimental Animals* issued by the Ministry of Science and Technology of the People's Republic of China in 2006.

1.2 Main instruments and reagents

Multichannel EEG acquisition and analysis system (Blackrock Microsystem Inc., USA); WMT-100 Morris water maze video acquisition and analysis system (Chengdu Taimeng Software Technology Co., Ltd., China); self-made model preparation box for incarceration plus electric shock (output voltage: 60 V; electric current: 8 mA); paroxetine hydrochloride tablets (production batch number: 150104, specification: 20 mg/tablet, Zhejiang Jianfeng Pharmaceutical Co., Ltd., China); chloral hydrate (production batch number: 20150715, Shanghai Zhanyun Chemical Co., Ltd., China); disposable sterile acupuncture needle (specification: 0.25 mm in diameter, 25 mm in length, Beijing Keyuanda Medical Products Factory, China).

1.3 Modeling methods

Except for rats in the blank control group, rats in the other groups were subjected to preparing the PTSD models using 'incarceration plus electric shock' method[9]. The self-made model preparation box was placed above the subplate and connected to alternating current (AC) (60 V, 8 mA) power through the electrical fence. A rat was put into a model preparation box at one time, and covered with a cover plate and then pressed by a heavy object to fix the cover for creating a dark and closable environment, as well as preventing animals from escape. The power was turned on to produce an inescapable electrical stimulation to the sole of rats' paws. The power was turned off after 5 s of stimulation, and the stimulation interval was randomized for a total of 30 times. Rats were then taken out from the model preparation box, and put back into cages for breeding. The modeling time was recorded. The modeling preparation was performed once each morning and evening (30 min/time), at an interval of 4-8 h between two times of modeling preparation. Modeling preparation was continued for 7 d. Rats in each group received designated intervention from the first modeling preparation day.

1.4 Intervention methods

1.4.1 Acupuncture group

Acupoints: Baihui (GV 20), Neiguan (PC 6), Shenmen (HT 7) and Taichong (LR 3).

Methods: Acupoint location was referred to the

Experimental Acupuncture Science[10] and *Sectional Anatomical Atlas of Sprague-Dawley Rat*[11]. The acupoints were routinely sterilized after the rats were grasped and fixed. Baihui (GV 20) was punctured subcutaneously for 4-5 mm with the tip backwards after the scalp was lifted, and then the needle was retained. Neiguan (PC 6), Shenmen (HT 7) and Taichong (LR 3) on the left side were punctured obliquely for 2-3 mm on Monday, Wednesday and Friday, and Neiguan (PC 6), Shenmen (HT 7) and Taichong (LR 3) on the right side were punctured obliquely for 2-3 mm on Tuesday, Thursday and Saturday. During the needle retaining, all the 3 acupoints were successively needled for 1 min with a small-amplitude twirling manipulation. The needles were retained for 4 min, and then all the needles were removed and the needled areas were pressed with dry cotton swab. The rats were put back into the cage for feeding. The treatment was performed once a day, and 6-day was a course of treatment. A total of 12-day intervention was continuously performed.

1.4.2 Paroxetine group

Paroxetine hydrochloride solution was intragastrically administered at 5 mL/(kg·bw) after the rats were grasped and fixed. The injection was evenly performed within 1 min after the intragastric needle was inserted into the stomach. The rats were continuously grasped for 4 min and then put back into the cage for rearing after the gavage needle was withdrawn. Intragastric treatment was performed once a day, for 6 d as a course of treatment. A total of 12-day intervention was continuously performed.

1.4.3 Grasping group

Each rat in the grasping group was subjected to grasping stress for 4 min, using the same grasping and fixing methods as in the acupuncture group and paroxetine group, and then put back into the cage for rearing. The grasping was performed once a day, and a total of 12-day grasping was continuously performed.

1.5 Observation indicators and detection methods

1.5.1 General condition

The activities and mental states of rats in each group were observed.

1.5.2 Water maze test

The preliminary experiment was conducted for 1 d, followed by positioning navigation experiment for 4 d and space exploration experiment for 1 d. The platform was fixed in the first quadrant, and the third quadrant was used as the site for entering water. The navigation experiment recorded the time spent on searching the platform after the rats entered water (i.e., the escape latency). If the rats did not find the platform within 2 min, the experimenter guided them to the platform, and the escape latency was recorded as 2 min. The space exploration experiment was carried out on the next day at the end of the positioning navigation experiment. The platform was removed to explore the times of rats crossing the original platform position and the effective areas within 2 min after the rats entered water.

1.5.3 Resting-state EEG

Resting-state EEG testing was performed in a quiet room. Anesthesia was performed before data collection. Before anesthesia, rats' scalp hair was shaved, and they were fasted for 8 h and prohibited from taking water for 6 h. The grouped and numbered rats were randomly grasped and weighed, and then anesthetized by intraperitoneal injection of mixed anesthetic solution (10 g of chloral hydrate crystals were dissolved in normal saline for injection to a volume of 100 mL, stored at 4 ℃ away from light) at 5 mL/(kg·bw). The detailed anesthesia methods and assessment of rat status after anesthesia were referred to the abdominal anesthesia method by Tong S, *et al*[12]. The anesthetized rats were placed on the table top. Two recording electrodes were inserted respectively at 1 cm above the midpoint of the connection line between rat inner canthus and 5 mm away to the left and the right, respectively. The insertion depth was kept at 5 mm. A needle was inserted at the left root of ear for 5 mm and the reference electrode was inserted, with the ground wire inserted into the root of the rat tail. The EEG data collected during the experiment were processed using the BrainVision Analyzer 2.1 (USA), and the band with the frequency within 0.1-40 Hz was selected. The original EEG and the power spectrum value after the Fourier transform of the α-wave, β-wave, δ-wave and э-wave of rat's bilateral cerebral hemispheres in each group were analyzed and processed. The power spectrum was a graph in which the frequency was plotted on the abscissa and the square of the amplitude was plotted on the ordinate, and the signal energy was accorded with the frequency.

1.6 Statistical methods

Statistical processing was performed using SPSS 21.0 software. If the measurement data were in a normal distribution, they would be expressed as mean ± standard deviation ($\overline{x} \pm s$), and the comparison among multiple groups was analyzed by one-way analysis of variance; the least significant difference (LSD) *t*-test was used to compare the differences between two groups. The Dunnett *t*-test was used for the data with heterogeneity of variance. The rank sum test was used when the data were in an abnormal distribution. $P<0.05$ indicated a statistically significant difference.

2 Results

2.1 General condition

The activities and mental status of rats in each group were observed before daily intervention. For rats in the blank control group, the hair was bright and tidy; there was no increase in alertness, irritability, horripilation, gathering and scrunching; the rats were active and agile. For rats in the model group, the hair was erected and disordered in the early stage of modeling, and the alertness was increased and they were easy to be irritated. The hair color was dull in the later stage, and the rats preferred to be gathering and scrunching; the activity was decreased, which suggested that the rats showed obvious stress damage after incarceration plus electric shock, indicating that the models were established successfully. The manifests of rats in the grasping group were similar to those in a model group. Rats in the paroxetine group and acupuncture group showed transient increase in alertness and piloerection during the modeling period. After the modeling, there was no irritability or gathering and scrunching; the hair was basically normal, and the activity and agility were good.

2.2 Water maze test results

2.2.1 Comparison of positioning navigation

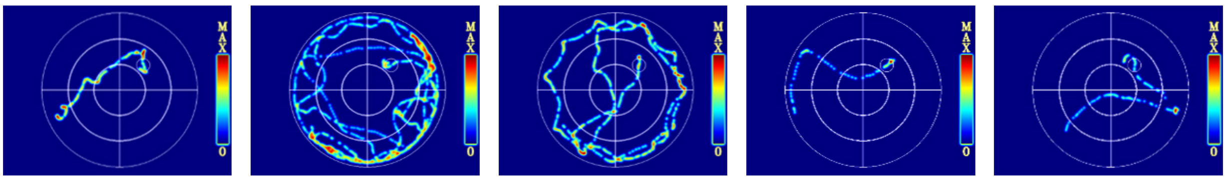
Compared with the blank control group, the average escape latency of rats in the model group was significantly prolonged (P<0.05), suggesting that stress had an effect on the learning and memory acquisition process in rats. There was no significant difference in the average escape latency between the grasping group and model group (P>0.05), suggesting that the grasping did not affect the learning and memory function of the rats. Compared with the grasping group, the average escape latencies of rats in the paroxetine group and acupuncture group were significantly shortened (P<0.05), which suggested that both paroxetine and acupuncture could improve the effects of stress on learning and memory of the rats. The average escape latency of rats in the acupuncture group was not significantly different from that in the paroxetine group (P>0.05), (Table 1).

There were mainly 4 ways for the rats to search for the hidden platforms: marginal, tendency, random and orthoscopic modes during the positioning navigation experiment. It can be seen from Figure 1 that rats in the blank control group mostly presented with orthoscopic mode, showing good learning and memory ability; rats in the model group and grasping group mainly searched by marginal and random modes, which suggested that the rats had poor learning and memory; rats in the acupuncture group and paroxetine group tended to use orthoscopic or tendency mode, showing relatively good learning and memory ability.

Table 1. Comparison of daily latency of the rats during positioning navigation experiment ($\overline{X}$ ±*s*, s)

Group	*n*	Day 1	Day 2	Day 3	Day 4
Blank control	10	117.82±3.37	48.73±13.99	26.64±12.49	10.64±4.13
Model	10	119.60±1.27	73.70±29.39[1)]	48.70±26.82[1)]	32.40±19.02[1)]
Grasping	10	119.64±1.21	80.73±22.96	47.09±19.49	27.91±13.57
Paroxetine	10	120.00±0.00	57.50±27.63[2)]	27.10±9.97[2)]	12.80±5.90[2)]
Acupuncture	10	119.60±1.26	49.40±22.76[2)]	24.90±14.26[2)]	9.10±3.93[2)]

Note: Compared with the blank control group, 1) P<0.05; compared with the grasping group, 2) P<0.05



Blank control group | Model group | Grasping group | Paroxetine group | Acupuncture group

Figure 1. Schematic diagram of rats to search the platform during positioning navigation experiment

2.2.2 Comparison of space exploration

It can be seen from Table 2, compared with the blank control group, the times of rats crossing the plateform or the effective area in the target quadrant decreased significantly in the model group (P<0.01), which suggested that stress had an effect on the learning and memory ability of rats in the model group. There were no significant differences in all indicators between the grasping group and model group (all P>0.05), which suggested that grasping did not affect the spatial learning and memory of the rats. Compared with the grasping group, the times of rats crossing the platform and the effective area in the paroxetine group and acupuncture group increased significantly (both P<0.05), suggesting that paroxetine and acupuncture improved the spatial learning and memory in the rats. There were no significant differences in all indicators between the acupuncture group and paroxetine group (all P>0.05).

2.3 EEG detection

2.3.1 Bilateral cerebral hemisphere EEG and power spectrum of rats in each group

Rats in the blank control group mainly showed high-frequency and low-amplitude wave (α-wave), (Figure 2), while waves of rats in the model group and grasping group were dominated by low-frequency δ-wave and э-wave (Figure 3 and Figure 4), while high-frequency β-wave was dominant in the paroxetine group and acupuncture group (Figure 5 and Figure 6). In Figure 2-Figure 6, 1 indicated the left cerebral hemisphere, and 2 indicated the right cerebral hemisphere.

Table 2. Comparison of the space exploration experiment ($\overline{X}$ ±s, time)

Group	*n*	Times crossing platform	Times crossing the effective areas
Blank control	10	7.55±1.37	10.36±1.50
Model	10	2.10±0.99$^{1)}$	3.10±0.99$^{1)}$
Grasping	10	1.82±0.87	2.91±1.38
Paroxetine	10	6.90±1.37$^{2)}$	9.90±1.79$^{2)}$
Acupuncture	10	7.10±1.37$^{2)}$	9.30±2.40$^{2)}$

Note: Compared with the blank control group, 1) $P<0.01$; compared with the grasping group, 2) $P<0.05$

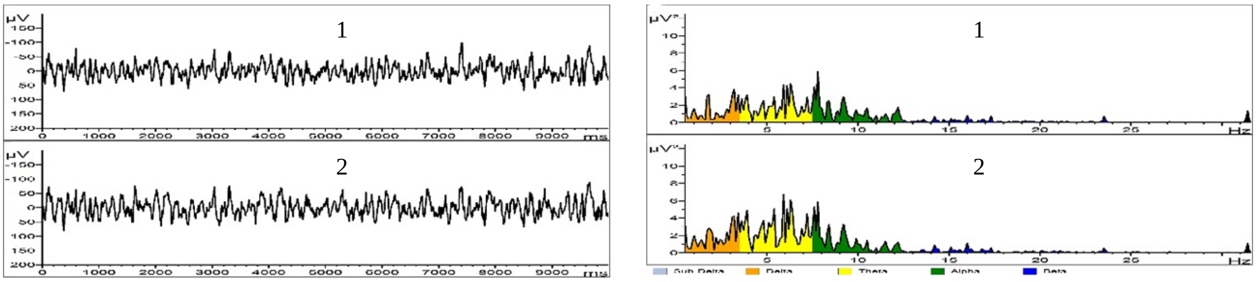


Figure 2. EEG and power of bilateral cerebral hemispheres in the blank control group

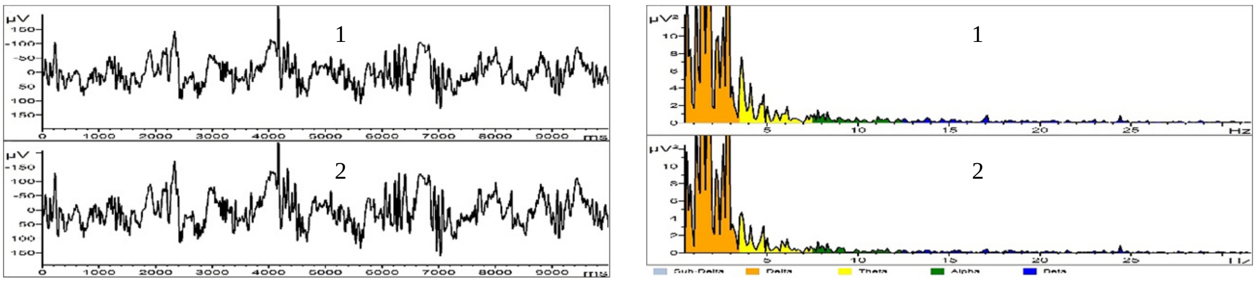


Figure 3. EEG and power of bilateral cerebral hemispheres in the model group

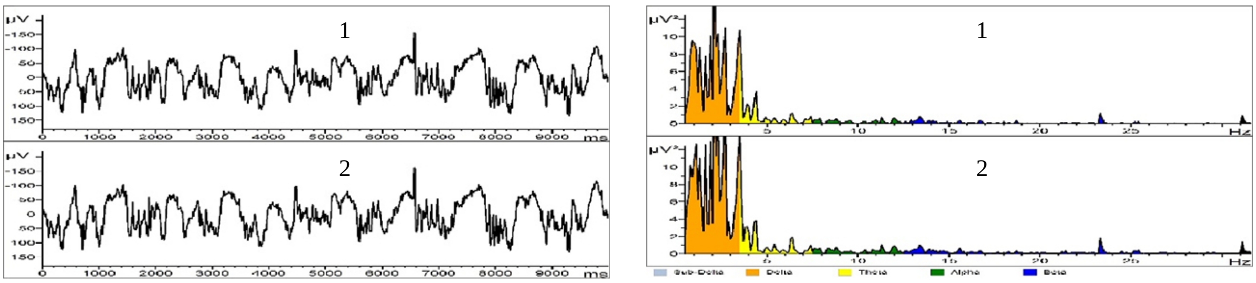


Figure 4. EEG and power of bilateral cerebral hemispheres in the grasping group

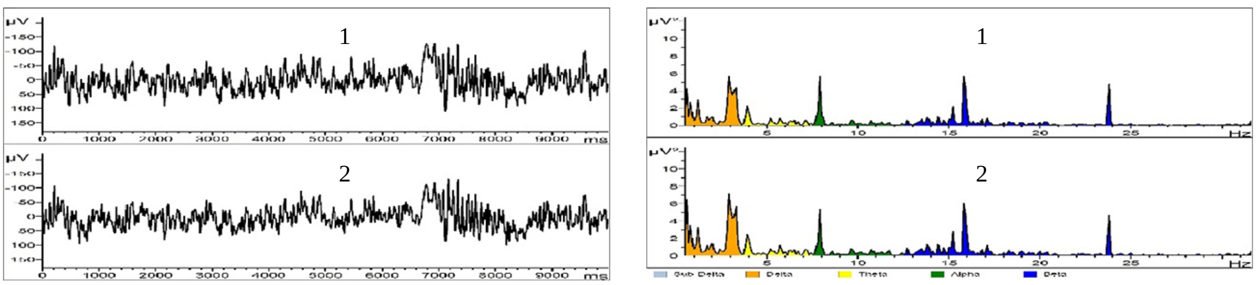


Figure 5. EEG and power of bilateral cerebral hemispheres in the paroxetine group

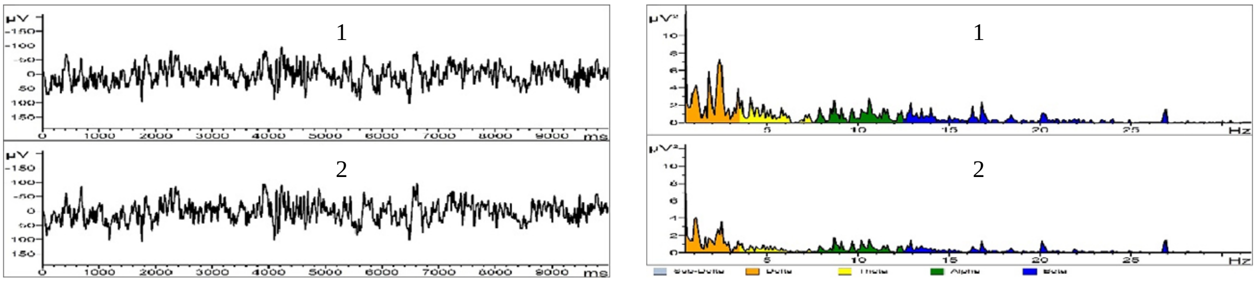


Figure 6. EEG and power of bilateral cerebral hemispheres in the acupuncture group

2.3.2 Resting EEG data of rats in each group

It can be seen from Table 3, compared with the blank control group, the α-wave power spectrum values of the bilateral cerebral hemispheres in the model group decreased significantly (*P*<0.01), suggesting that 'incarceration plus electric shock' can make α-wave distribution abnormally changed in rat's bilateral cerebral hemispheres. The α-wave power spectrum values of the bilateral cerebral hemispheres in the grasping group were insignificantly different from those in the model group (*P*>0.05), suggesting that the grasping intervention could not affect the release of α-wave in the rat brain. Compared with the grasping group, α-wave power spectrum values of the bilateral cerebral hemispheres in the paroxetine group and acupuncture group increased significantly (*P*<0.01), suggesting that paroxetine positively regulated the abnormally changed α-wave in the rat's cerebral hemisphere. There was no significant difference in α-wave power spectrum between the acupuncture group and paroxetine group (*P*>0.05). Compared with the blank control group, the power wave values of β-wave, δ-wave and э-wave in the bilateral cerebral hemispheres of the model group increased significantly (*P*<0.01), suggesting that 'incarceration plus electric shock' induced abnormal β-wave, δ-wave and э-wave in the rats, to aggravate the abnormal changes in the distribution of the normal β-wave, δ-wave and э-wave. There were no significant differences in the indicators between the grasping group and model group (*P*>0.05), suggesting that the grasping intervention could not affect the distribution of β-wave, δ-wave and э-wave in rat's brain. Compared with the grasping group, the power spectrum values of β-wave, δ-wave and э-wave in the bilateral cerebral hemispheres of the paroxetine group and acupuncture group were significantly lower (*P*<0.01), suggesting that paroxetine positively regulated the abnormally changed β-wave, δ-wave and э-wave in rat's cerebral hemispheres. There were no significant differences in each indicator between the acupuncture group and paroxetine group (*P*>0.05).

Table 3. Comparison of EEG power spectrum values of bilateral cerebral hemispheres ($\bar{x}$ ±*s*, μV^2)

Group	*n*	Site	α-wave	β-wave	δ-wave	Э-wave
Blank control	12	Left side	2.50±0.56	1.37±0.61	8.25±2.24	0.73±0.34
		Right side	2.50±0.57	1.42±0.53	8.30±2.70	0.79±0.52
Model	12	Left side	1.51±0.47$^{1)}$	2.77±0.60$^{1)}$	17.37±3.97$^{1)}$	3.80±1.51$^{1)}$
		Right side	1.47±0.64$^{1)}$	2.88±0.76$^{1)}$	17.24±4.89$^{1)}$	3.84±0.96$^{1)}$
Grasping	12	Left side	1.65±0.82	2.79±0.96	17.29±2.19	3.81±0.81
		Right side	1.58±0.72	3.07±1.15	17.37±3.24	3.83±0.62
Paroxetine	12	Left side	2.60±0.60$^{2)}$	1.42±0.59$^{2)}$	8.21±2.40$^{2)}$	0.82±0.48$^{2)}$
		Right side	2.55±0.72$^{2)}$	1.40±0.48$^{2)}$	8.25±2.42$^{2)}$	0.81±0.64$^{2)}$
Acupuncture	12	Left side	2.64±0.52$^{2)}$	1.46±0.29$^{2)}$	8.24±3.32$^{2)}$	0.84±0.51$^{2)}$
		Right side	2.60±0.51$^{2)}$	1.45±0.25$^{2)}$	8.24±2.21$^{2)}$	0.84±0.63$^{2)}$

Note: Compared with the blank control group, 1) *P*<0.01; compared with the grasping group, 2) *P*<0.01

3 Discussion

PTSD is clinically characterized by mental, psychological, and behavioral abnormalities. The disease is mainly located in the heart and brain, and the heart and brain are connected through the Governor Vessel. The regulation of the heart-mind is the basic rule for treating such diseases. PTSD belongs to the category of emotional disease in traditional Chinese medicine. The theory of traditional Chinese medicine believes that the incidence of emotional illness is mostly caused by qi stagnation, which is related to the liver failing to maintain the normal flow of qi and dysfunction of the qi movement. Therefore, emotional disorders are predominantly treated by targeting the liver[13]. This is also the embodiment of the theory that the liver qi stagnation disturbs the heart-mind. Modern research has found that 'the liver maintains the free flow of qi' is the core of regulating the stress response[14]. The Liver-soothing and mind-regulating acupuncture method is based on the treatment of liver and emphasizing the regulation of qi. Qi movement all over the body will be unblocked, thus to adjust the mind, via soothing the liver. This is also the characteristic of this study. Our previous clinical study has confirmed that liver-soothing and mind-regulating acupuncture method can effectively treat the symptoms associated with PTSD[15].

Baihui (GV 20) can adjust yin and yang, calm the mind, and refresh brain to open orifices. It is widely used in the treatment of sleep disorders, anxiety disorders, depressive disorders, schizophrenia and other diseases[16]. Neiguan (PC 6) is the Luo-Connecting point of the Pericardium Meridian, and it is also one of the Eight Confluent Points with the Eight Extraordinary Meridians. It can regulate qi and blood, smoothe the emotion, and calm the mind, thus producing a good regulating effect on mental and emotional diseases. It is clinically synergied with other acupoints to treat emotional disorders[17]. Shenmen (HT 7) is the Yuan-Primary point of the Heart Meridian, and can cure all heart-related disorders. Studies have found that acupuncture at Shenmen (HT 7) can effectively reduce the expression of arginine vasopressin (AVP) in the hypothalamic paraventricular nucleus of rats with stress and anxiety, and inhibit the activity of the hypothalamic pituitary adrenal axis (HPAA), which in turn achieves anti-stress and anxiolytic effects[18]. Taichong (LR 3) is the Yuan-Primary point and the Shu-Stream point of the Liver Meridian. It has the effect of soothing the liver, regulating the spirit, promoting qi and adjusting the emotions. The four acupoints were used together to achieve the function of soothing liver to promote qi, refreshing brain to open orifices and calming the mind.

The Morris water maze experiment can effectively evaluate the learning and memory ability of animal models. The positioning navigation experiment is mainly for detecting the spatial learning ability of animals, and the space exploration experiment is mainly for evaluating the spatial memory ability of animals[19]. In this experiment, the average escape latency, the search platform searching trajectory and the times crossing the platform and effective areas during space exploration period of the PTSD rats were observed, and the results showed that the learning and memory ability of the PTSD rats were damaged, while the acupuncture intervention improved the stress-caused damage. This confirmed the effectiveness of liver-soothing and mind-regulating acupuncture method, and also provided a behavioral basis for the reliability of resting-state EEG data analysis.

Studies have shown that abnormal brain network connectivity is closely related to the clinical manifestations of patients with neuropsychiatric diseases[20]. Resting-state EEG is an objective indicator of brain activity, as it can directly reflect the release of nerve impulses. The amplitude represents the intensity of the potential, and the high amplitude suggests that the neuron number participating in the synchronous discharge is large and the direction is consistent, and it is closer to the recording electrode [21], and vice versa. Some studies have found that α-wave is the main waveform of people in a calm state and distributed throughout the brain[22]. The higher the α-wave level, the closer the brain is to the 'zero-load' state, i.e. a lower level of cerebral cortex activation[23]. Therefore, the power spectrum of the α-wave is inversely proportional to the brain activity[24], and the low power spectrum of α-wave suggests that brain activity increases and the individual is alert. The β-wave is considered to be an excitatory expression of the cerebral cortex. An increased β-wave indicates that the person is nervous and extremely sensitive to the surrounding; the increased δ-wave suggests cognitive impairment; the increased э-wave indicates a serious cognitive impairment. Relevant data have showed that acupuncture has a significant intervention effect on the EEG result. For example, Liu YZ, *et al*[25] treated patients with depression with warm needling to reduce the symptoms of depression by adjusting the frequency and amplitude of EEG. Zhang Y, *et al*[26] found that scalp acupuncture in the abnormal discharge area guided by EEG plus body acupuncture intervention could effectively improve the abnormal power spectrum of α-wave, β-wave, δ-wave and δ-wave during seizure interval to regulate the EEG activity in patients with epilepsy.

In this study, we found that there was a certain degree of cognitive impairment in PTSD by observing the resting-state EEG of the stress model rats: the power spectrum value of α-wave decreased significantly, and the power spectrum values of β-wave, δ-wave and э-wave increased significantly. The liver-soothing and mind-regulating acupuncture method could significantly increase the α-wave power spectrum value of rat' bilateral cerebral hemispheres, and decrease the power spectrum values of β-wave, δ-wave and э-wave, which revealed the function mechanism of liver-soothing and mind-regulating acupuncture method in improving the cognitive function of PTSD rats, and further confirmed the clinical effectiveness of liver-soothing and mind-regulating acupuncture method in the 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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References

[1] Hao W. Psychiatry. 6th Edition. Beijing: People's Medical Publishing House, 2008: 140.

[2] Bracha HS. Human brain evolution and the 'Neuroevolutionary Time-depth Principle:' implications for the reclassification of fear-circuitry-related traits in DSM-V and for studying resilience to warzone-related posttraumatic stress disorder. Prog Neuropsychopharmacol Biol Psychiatry, 2006, 30(5): 827-853.

[3] Zhang Y, Ho SM. Risk factors of posttraumatic stress disorder among survivors after the 512 Wenchuan earthquake in China. PLoS One, 2011, 6(7): e22371.

[4] Wu XH, Li XL, Tao QL, Huang XH, Zhu SC. Acute stress reaction among injured persons after Yushu earthquake. Zhongguo Gonggong Weisheng, 2011, 27(7): 857-858.

[5] Cloitre M. Effective psychotherapies for posttraumatic stress disorder: a review and critique. CNS Spectr, 2009, 14(Suppl 1): 32-43.

[6] Zhang YF, Han YD, Zhao ZT, Yan XK. Exploration of acupoints selection law for post-traumatic stress disorder treated with acupuncture and moxibustion. Zhongguo Zhen Jiu, 2016, 36(11): 1229-1232.

[7] Liu WH, Wang F, Wang LL, Yang YQ, Du YH, Cheng K. Characteristics and superiorities of acupuncture medicine. Zhongguo Zhen Jiu, 2011, 31(8): 673-678.

[8] Tian YL, Yu HJ, Zhang XM, Li J, Liu JH, Bai HC, Zhu DS. Measurement of relative organ indexes and body length in SD and Wistar rats age of 5 to 7 weeks. Shiyan Dongwu Kexue, 2009, 26(6): 21-25.

[9] Zhong ZX, Wu YM. Establishment of a new rat model of posttraumatic stress disorder and its behavior test. Disan Junyi Daxue Xuebao, 2012, 34(10): 928-932.

[10] Guo Y. Experimental Acupuncture Science. Beijing: China Press of Traditional Chinese Medicine, 2012: 109.

[11] Liu Q, Gong H, Li YQ. Sectional Anatomical Atlas of Sprague-Dawley Rat. Wuhan: Huazhong University of Science & Technology Press, 2010: 25-47.

[12] Tong S, Xiong NX, Shen JY. The improvement of method of intraperitoneal injection in rat anesthetization. Shiyan Dongwu Kexue, 2014, 31(1): 52-54.

[13] Jin R, Li XY, Zheng CQ, Wang J, Zhang H. Research progress of acupuncture treatment of post-traumatic stress disorder. Zhonghua Zhongyiyao Zazhi, 2014, 29(9): 2883-2885.

[14] Zhu SC, Li XL, Huang XH, Tao QL, Wu XH, Meng YJ, Liu QX, Xu JJ, Li N. Report and analysis of 90 wounded persons' sleep disorders in acute stress in Yushu earthquake. Huaxi Yixue, 2011, 26(2): 195-197.

[15] Zhao YD, Han DY, Guo X. Warming-promotion acupuncture for post-earthquake depression: a randomized controlled study. Zhongguo Zhen Jiu, 2014, 34(8): 755-758.

[16] An CP, Cheng W, Ning SY. The current situation of acupuncture treatment of mental illness. Zhenjiu Linchuang Zazhi, 2010, 26(12): 55-56.

[17] Kong DD, Zhang YC. Application analysis of the ancient literature about Neiguan (PC 6). Shandong Zhongyi Zazhi, 2014, 33(12): 998-1000.

[18] Park HJ, Park HJ, Chae Y, Kim JW, Lee H, Chung JH. Effect of acupuncture on hypothalamic-pituitary-adrenal system in maternal separation rats. Cell Mol Neurobiol, 2011, 31(8): 1123-1127.

[19] Feng M, Lu SF, Zhang CS, Yu SG. Current status and analysis of domestic rat Morris water maze experiment. Liaoning Zhongyi Zazhi, 2011, 38(11): 2170-2172.

[20] Wang ZY, Xue Q, Xiong XC, Li PY, Tian CY, Fu CH, Wang YP, Yao DZ, Xu P. Brain function network analysis and recognition for psychogenic non-epileptic seizures based on resting state electroencephalogram. Shengwu Yixue Gongcheng Zazhi, 2015, 32(1): 8-12.

[21] Yang YM, Geng LB. An ERPs study on the psychological reality of lexical competence in the second language. Waiyu Jiaoxue Yu Yanjiu (Waiguo Yuwen Shuangyue Kan), 2008, 40(3): 163-169.

[22] Klimesch W. EEG Alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain Res Brain Res Rev, 1999, 29(2-3): 169-195.

[23] Gu CH, Wang YL, Wu CF, Xie XL, Cui CZ, Wang YX, Wang WZ, Hu BY, Zhou ZK. Brain correlates underlying social creative thinking: EEG alpha activity in trait vs. state creativity. Xinli Xuebao, 2015, 47(6): 765-773.

[24] Lai YX, Gao TT, Wu D, Yao DZ. Research on electroencephalogram of musical emotion perception. Dianzi Keji Daxue Xuebao, 2008, 37(2): 301-304.

[25] Liu YZ, Liu BG, Luo YN, Liu JX, Luo JH. Therapeutic observation on the effect of warming-needle moxibustion for 30 cases of depression and its effect on α wave of EEG. Zhongyi Zazhi, 2008, 49(11): 995-997.

[26] Zhang Y, He JL, Luo HS, Zeng JY, Ke LL, Shi Y, Huang LH. Effect of directional acupuncture on EEG power during epileptic seizure. Zhongguo Zhongyi Jizheng, 2017, 26(10): 1840-1843.

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