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Basic Study

Effect of acupuncture on blood oxygen concentration in brain of rats with post-traumatic stress disorder based on functional near-infrared spectroscopy

基于功能性近红外光谱技术观察针刺对创伤后应激障碍大鼠脑部血氧浓度的影响

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Abstract

Objective: To observe the effect of acupuncture on blood oxygen concentration in the brain of rats with post-traumatic stress disorder (PTSD) based on functional near-infrared spectroscopy (fNIRS), thus to reveal the mechanisms of acupuncture in intervening the brain function of PTSD rats.

Methods: Sixty Sprague-Dawley (SD) rats were randomly divided into a blank group, a model group, a grasping group, a paroxetine group and an acupuncture group, with 12 rats in each group. Except the blank group, rats in the other groups all received incarceration plus electric shock for 7 d to prepare the PTSD animal model. One hour before the stress model was established, rats in each group received the designated intervention: rats in the blank group and the model group did not receive any intervention; rats in the grasping group received grasping and fixation; rats in the paroxetine group received paroxetine hydrochloride solution by intragastric administration; and rats in the acupuncture group received acupuncture. Six-day treatment was a course, with 2 courses of treatment conducted for a total of 12 d. After the modeling, rats in each treatment group received intervention for 5 d, and the fNIRS system was used to collect and record the changes in the concentrations of oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (d-Hb) and total hemoglobin (t-Hb) of the involved rat's brain regions, and also to assess the brain function.

Results: Compared with the blank group, the concentration of HbO_2 was significantly increased, the concentration of d-Hb was significantly decreased, and the concentration of t-Hb was significantly increased in the model group and the grasping group after the intervention, and the differences were statistically significant (all $P<0.01$). Compared with the model group, the concentrations of HbO_2, d-Hb and t-Hb in the grasping group did not change significantly (all $P>0.05$). Compared with the grasping group, the concentration of HbO_2 was significantly decreased, the concentration of d-Hb was significantly increased, and the concentration of t-Hb was significantly decreased in the paroxetine group and the acupuncture group, and the differences were statistically significant (all $P<0.05$). There were no significant differences in the concentrations of HbO_2, d-Hb and t-Hb between the paroxetine group and the acupuncture group (all $P>0.05$).

Conclusion: Acupuncture can regulate the blood oxygen concentration in the brain of PTSD model rats, which may be an important mechanism of acupuncture in intervening 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; Spectroscopy, Near-infrared; Rats

【摘要】目的：基于功能近红外光谱技术(fNIRS)观察针刺对创伤后应激障碍(PTSD)模型大鼠脑部血氧浓度的影响，揭示针刺干预PTSD的脑功能机制。**方法**：将60只Sprague-Dawley (SD)大鼠随机分为空白组、模型组、抓取组、帕罗西汀组和针刺组，每组12只。除空白组外，其余各组大鼠均以电击幽闭法复制PTSD动物模型，模型复制共7 d。于应激造模前1 h，各组接受相应干预：空白组和模型组不接受任何干预，抓取组接受抓取固定，帕罗西汀组接受盐酸帕罗西汀溶液灌胃，针刺组接受针刺治疗，6 d为1个疗程，连续干预2个疗程，共计12 d。造模结束后，各治疗组连续治疗5 d后利用fNIRS系统采集并记录各组大鼠相关脑区组织氧合血红蛋白(HbO_2)、脱氧血红蛋白(d-Hb) 和总血红蛋白(t-Hb)浓度的变化情况，并进行脑功能评估。**结果**：干预结束后，与空白组比较，模型组和抓取组大鼠HbO_2浓度显著升高，d-Hb浓度显著降低，t-Hb浓度显著升高，差异均具有统计学意义(均$P<0.01$)；与模型组比较，抓取组大鼠HbO_2、d-Hb和t-Hb浓度均无明显变化(均$P>0.05$)；与抓取组比较，帕罗西汀组和针刺组大鼠HbO_2浓度

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显著降低，d-Hb浓度显著升高，t-Hb浓度显著降低，差异均具有统计学意义(均$P<0.05$)；帕罗西汀组与针刺组的HbO_2、d-Hb和t-Hb浓度均无统计学差异(均$P>0.05$)。**结论**：针刺对PTSD模型大鼠脑区血氧浓度有良性的调节作用，这可能是针刺干预PTSD的重要脑功能机制。

【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；光谱，近红外；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Post-traumatic stress disorder (PTSD) refers to sudden, threatening or catastrophic trauma of the body, which leads to delayed and persistent psychosomatic disorders. Clinical manifestations include reliving the trauma again, enhanced alertness and responsiveness, persistent anxiety and avoidance behavior[1]. With the frequent occurrence of natural disasters, wars, diseases and major traffic accidents at home and abroad, the incidence of PTSD is gradually increasing[2]. Long-term PTSD may lead to many symptoms, such as insomnia, depression, nightmares, and anxiety, which seriously threaten people's physical and mental health and quality of life[3]. In recent years, acupuncture has achieved remarkable results in the treatment of patients with PTSD after Wenchuan, Dingxi earthquakes and Zhouqu mudslide, and has been recognized by the medical community[4-5]. But so far, the mechanism of acupuncture in intervening PTSD brain function has been relatively less studied[6-7]. Based on the neurovascular coupling mechanism, this study used functional near-infrared spectroscopy (fNIRS) to detect the concentrations of oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (d-Hb) and total hemoglobin (t-Hb) in the brain tissues of PTSD rats, to reveal the mechanisms of acupuncture in intervening the brain function of PTSD rats.

1 Materials and Methods

1.1 Laboratory animals and grouping

Sixty male, 6-week old healthy Sprague-Dawley (SD) rats, weighing (180±20) g were provided by the SPF Laboratory Animal Center of Gansu University of Chinese Medicine [animal license number: SCXK (Gan) 2016-0001]. The rats were randomly divided into a blank group, a model group, a grasping group, a paroxetine group and an acupuncture group, with 12 rats in each group. The treatment 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 equipments and drugs

Self-made ‘incarceration plus electric shock’ model preparation box and power supply for electric shock (including circuit); brain solid positioner (Shenzhen Yuxiang Biomedical Electronics Co., Ltd., China); NIRStar 13.0 fNIRS Acquisition and Analysis System (NIRX, USA); paroxetine hydrochloride tablets (production batch number: 150104, specification: 20 mg/tablet, Zhejiang Jianfeng Pharmaceutical Co., Ltd., China); disposable sterile acupuncture needle (specification: 0.25 mm in diameter and 25 mm in length, Suzhou Medical Products Co., Ltd., China).

1.3 Animal model replication

After the animals were grouped, the PTSD rat model was replicated in the model group, the grasping group, the paroxetine group, and the acupuncture group with ‘incarceration plus electric shock’. The PTSD rat model was replicated referring to the literature[8]: cleaned the model preparation box, sprayed 75% ethanol solution, removed the odor, placed the subplate and grasped the rats into the model preparation box. Only one rat was placed in each box at a time, covered with heavy objects to fix the subplate and create a dark and closable environment, so that the rats could not escape from the electrical stimulation after the circuit was connected. The power was turned on to produce an inescapable electrical stimulation to the sole of the rat paws. Animals were kept in the model preparation box for 30 min. Then the electrical fence of each model preparation box was connected to the AC power (60 V, 8 mA). The power was turned on to produce an inescapable electrical stimulation on the soles of the rats, and the power was turned off after 4-8 s of electrical stimulation. Stimulation intervals were random and the stimulation was repeated 30 times. Rats were then taken out from the model preparation box, and put back into cages. The modeling time was recorded. The modeling preparation was performed once each morning and evening (30 min/time) for continuous 7 d. The interval between two modeling preparations was 4-8 h.

1.4 Intervention method

1.4.1 Acupuncture group

Methods: Acupoint localization was referred to *Experimental Acupuncture Science*[9] and *Sectional Anatomical Atlas of Sprague-Dawley Rat*[10]. 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 feeding after the gavage needle was withdrawn. Intragastric administration was performed once a day, 6-day was 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 feeding. The grasping was performed once a day, and a total of 12-day grasping was continuously performed.

1.5 Observation indicators

The main detection indicators of fNIRS were HbO_2, d-Hb and t-Hb concentrations. Animal studies have shown that HbO_2 change in fNIRS detection is the most sensitive marker of regional cerebral blood flow changes. Therefore, most fNIRS studies currently use HbO_2 as an analytical indicator[11]. In this study, HbO_2 was used as the main indicator, and d-Hb and t-Hb were used as auxiliary indicators to enhance the credibility of HbO_2.

1.6 Data collection

1.6.1 Pre-data collection processing

Including anesthesia, head processing, and stereotactic positioning of the brain with the solid brain positioner (after the head hair was removed, the rat was placed on the solid brain positioner smoothly, the height of the nose clip was adjusted to keep the anterior fontanelle and the lambdoid suture at the same level, and the scale of the bilateral ear rods was adjusted so that the rat was in the middle position; rat head was tightly fixed after adjustment), the light source, the detector placement and detection. Before the experiment, the light source (8) and the probe orders of the detectors (12) were set as shown in Figure 1, and covered the rat heads as closer as possible to the skin; and the acquisition software was then turned on to detect whether the light source and the detector were running normally.

S-D/1-1

Figure 1. Data model for light source and detector alignment

Note: The light source (S) is before the detector (D)

1.6.2 Data collection

Each rat was randomly grasped for 3 min without any intervention to collect data. NIRStar 13-0 software with a total of 96 channels (1 channel was composed by 1 light source and 1 detector) were used for data acquisition. After the collection was completed, the time and rat number were recorded for the following data analysis.

1.7 Statistical methods

All data were statistically analyzed using SPSS version 19.0 software. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) when the data were normally distributed and featured by homogeneity of variance, and one-way ANOVA was used for between-group comparing; the Kruskal-Wallis *H* test was used for when the data were not normally distributed and had heterogeneity of variance. The difference was statistically significant at $P<0.05$.

2 Results

2.1 Comparing concentration changes of HbO_2, d-Hb and t-Hb in the activation channels

The wave signal of fNIRS was characterized by smooth peaks and sharp valleys. The concentration changes of HbO_2, d-Hb and t-Hb in CH4 channels of each group are shown in Figure 2-Figure 6. The waveform in the blank group was dense, regular and consistent, with small waveform amplitude value and difference between the maximum value and the minimum value (Figure 2); compared with the blank group, the model group and the grasping group showed obvious fluctuations with multiple sharp peaks, wider waveform amplitude, obvious difference between the maximum value and the minimum value, and obvious variation range (Figure 3 and Figure 4); compared with the grasping group, the paroxetine group and the acupuncture group showed relatively gentle fluctuations and decreased sharp peaks with only one large amplitude peak and obvious mild variation range (Figure 5 and Figure 6).

2.2 Comparing the mean concentration changes of HbO_2, d-Hb and t-Hb in the activation channels

There were 10 activation channels after screening, CH1, CH2, CH3, CH9, CH13, CH30, CH31, CH33, CH54 and CH67. The mean concentration changes of HbO_2, d-Hb and t-Hb in 10 abnormal activation channels of each group were compared. The results showed that compared with the blank group, the concentration of HbO_2 was significantly increased, the concentration of d-Hb was significantly decreased, and the concentration of t-Hb was significantly increased in the model group, and the differences were statistically significant (all $P<0.01$); there were no significant differences in the concentrations of HbO_2, d-Hb and t-Hb between the grasping group and the model group (all $P>0.05$); compared with the grasping group, the concentration of HbO_2 was significantly decreased, the concentration of d-Hb was significantly increased, and the concentration of t-Hb was significantly decreased in the paroxetine group and the acupuncture group, and the differences between the groups were statistically significant (all $P<0.05$); there were no significant differences in HbO_2, d-Hb and t-Hb concentrations between the paroxetine group and the acupuncture group (all $P>0.05$), (Figure 7).

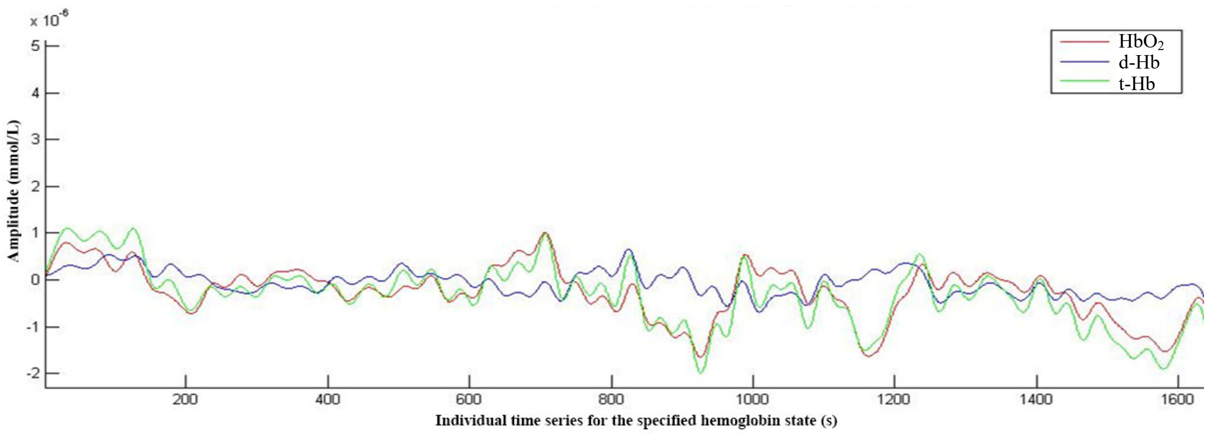

Figure 2. Changes of HbO_2, d-Hb and t-Hb of the blank group

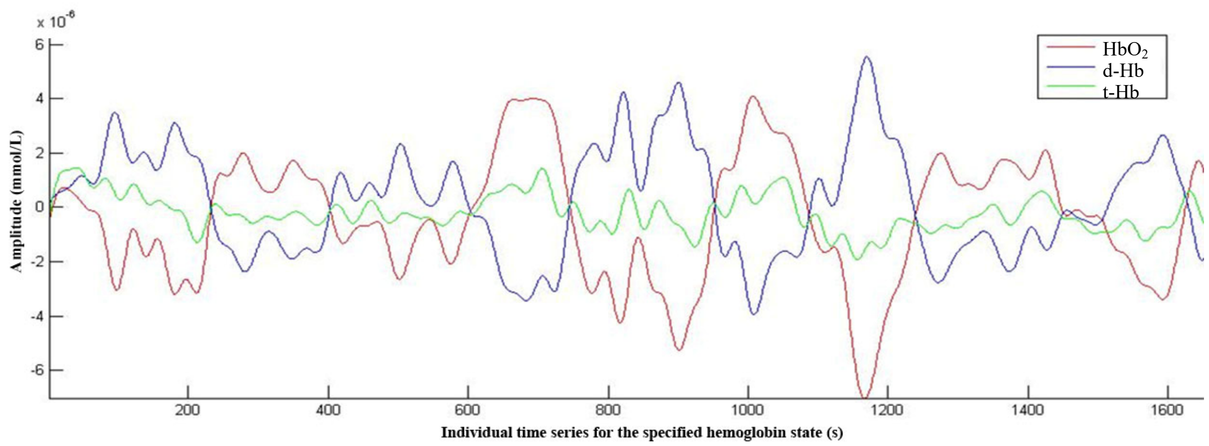

Figure 3. Changes of HbO_2, d-Hb and t-Hb of the model group

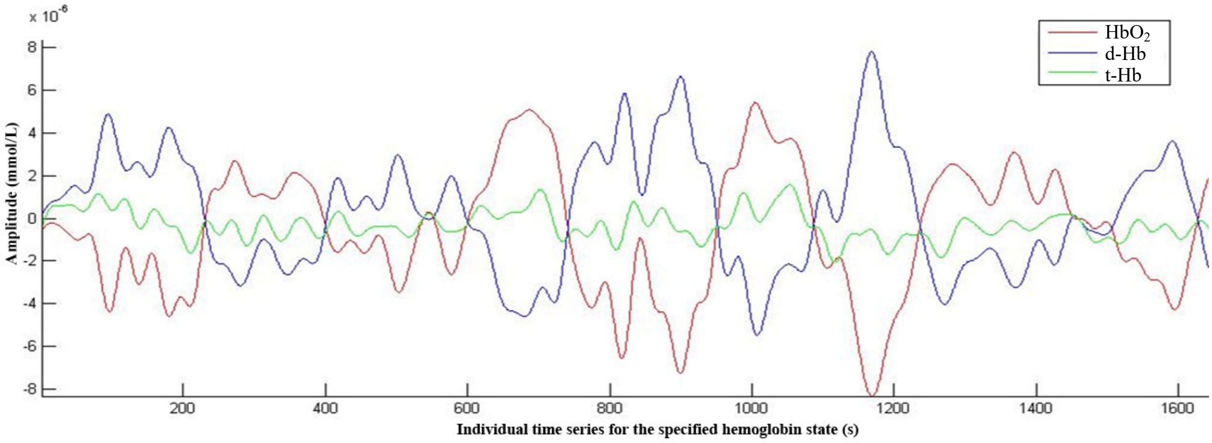

Figure 4. Changes of HbO_2, d-Hb and t-Hb of the grasping group

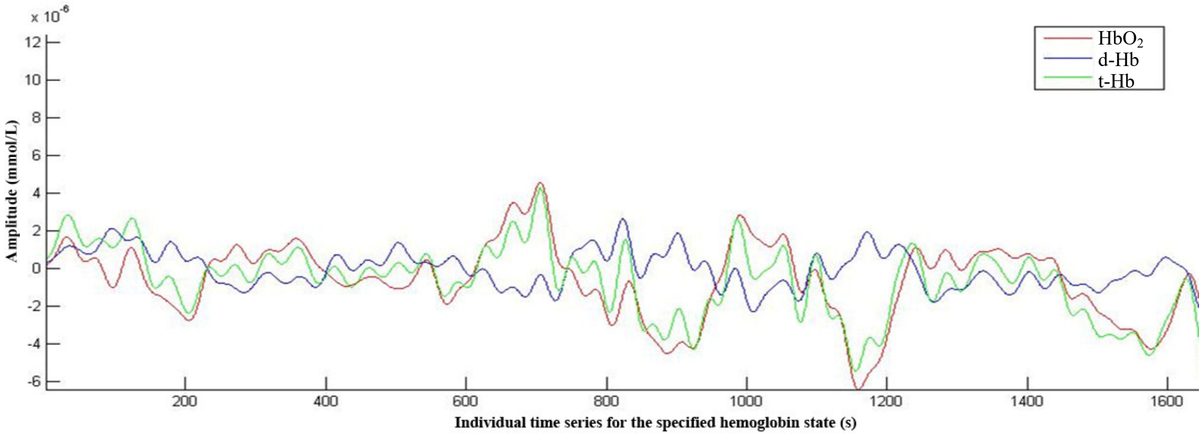

Figure 5. Changes of HbO_2, d-Hb and t-Hb of the paroxetine group

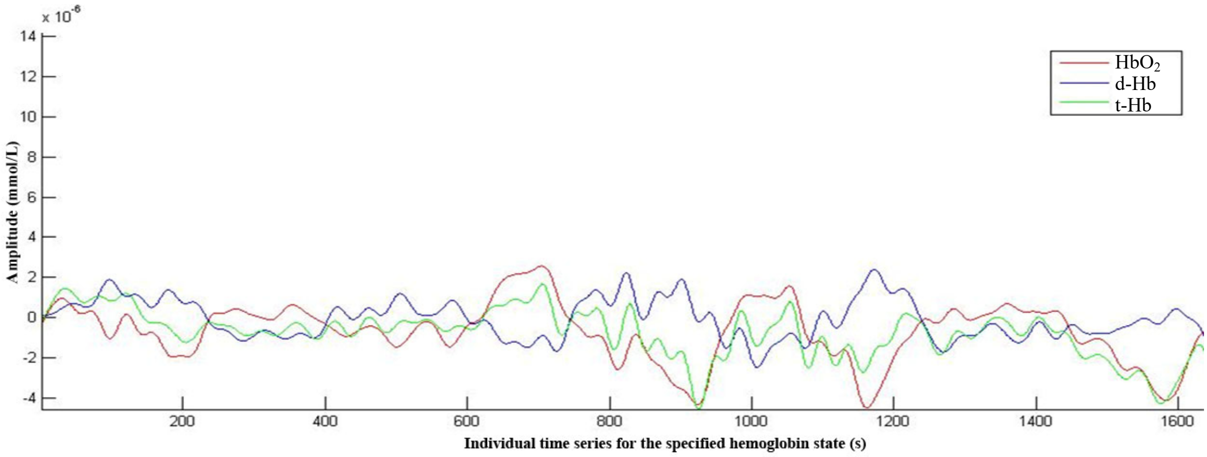

Figure 6. Changes of HbO_2, d-Hb and t-Hb of the acupuncture group

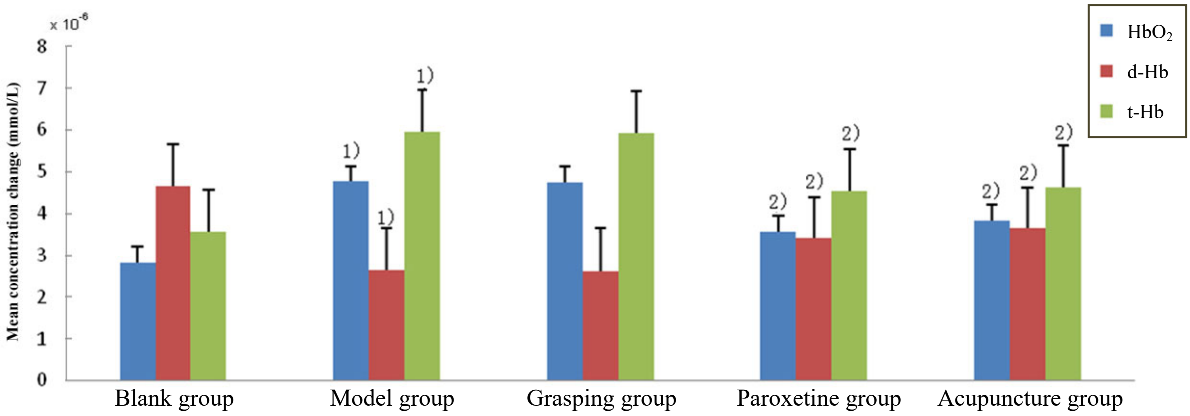

Figure 7. Histogram of mean concentration changes of HbO_2, d-Hb and t-Hb in the activation channels of each group

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

3 Discussion

The fNIRS is an emerging, safe, non-invasive indirect brain imaging method to analyze and evaluate neuronal activity, cellular energy metabolism, and hemodynamic related functions in related brain regions, to further reflect the activities of the brain structure, function, pathology and others, by detection of the hemodynamic parameters (HbO_2, d-Hb and t-Hb contents) in the cerebral cortex, based on neurovascular coupling mechanisms (that is, the activity of the brain is related to the changes in the optical properties of the brain tissues; there is a close relationship between optical parameters and cellular activities, energy metabolism and hemodynamics[12]). The fNIRS is a new neuroimaging technique for dynamically detecting nerve cell activity to monitor the brain function[13]. The nature of PTSD onset may be the result of related brain function and structural stress damage beyond a certain limit after intense traumatic stimulation[3]. The results of this study showed that compared with the blank group, the concentration of HbO_2 was significantly increased, the concentration of d-Hb was significantly decreased, and the concentration of t-Hb was significantly increased in the model group, and the differences were statistically significant (all $P<0.01$), indicating that in the brain region of the ‘incarceration plus electric shock’ model rats, the neuronal cells were abnormally discharged, the cell energy metabolism, local oxygen consumption, local blood flow and HbO_2 concentration were all increased, and that was, PTSD had brain damage; compared with the grasping group, the concentration of HbO_2 was significantly decreased, the concentration of d-Hb was significantly increased, and the concentration of t-Hb was significantly decreased in the acupuncture group, and the differences were statistically significant (all $P<0.05$), suggesting that acupuncture can affect the cerebral blood flow of rats, inhibit the abnormal neuronal activity in rat cortex to improve the energy metabolism demand of cells and recover the local oxygen consumption tends, thus playing a therapeutic role. It may be one of the mechanisms of acupuncture in intervening the brain function of PTSD rats.

As an external stimulus, acupuncture may first activate the corresponding central nervous system to regulate the nervous, endocrine and humoral networks, and then affect the target organs[14-15]. The development of neuroimaging has promoted the visualization of central effects of acupuncture. The positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and the brain functional optical imaging are representatives. The fNIRS uses animals as research objects to explore the effects of acupuncture on cerebral cortical hemoglobin, and to further analyze the mechanism of acupuncture on the brain function[16]. Related research[17] found that mid-far infrared imaging could detect the effect of acupuncture on the animal cerebral cortex activation area, to explore the corresponding relationship between acupuncture and cerebral cortical blood flow changes, providing a new method for central nervous mechanism of acupuncture effects. This may be caused by sympathetic nerves. Acupuncture changes the degree of sympathetic excitation, resulting in a series of reactions such as vascular-circulation-metabolism-temperature[18]. Studies have found that acupuncture changes blood oxygen saturation, blood flow and glucose metabolism in some functional areas of the brain[19]. Zhang H, *et al*[20] applied PET-CT imaging technique to study the effect of electroacupuncture on glucose metabolism in patients with PTSD. The results of this study showed that the brain area with metabolic abnormalities related to the pathogenesis of PTSD might be the target of electroacupuncture treatment of PTSD, especially the metabolic reduction of the brain region in the anterior cingulate cortex (ACC) and medial prefrontal cortex (MPFC), which may be one of the mechanisms of electroacupuncture in the treatment of PTSD.

The nature of PTSD onset may be the result of related brain function and structural stress damage beyond a certain limit after intense traumatic stimulation, and acupuncture can improve brain function damage after traumatic stress. Our previous research has proven that traumatic stress affects the release of neuron action potential, discharge frequency, waveform amplitude and power spectral density in rat hippocampal CA1 and CA3 regions, resulting in specific changes in the spatial and temporal patterns of neural information coding[21-22]. After comprehensively analyzing the results of this experiment, we found that acupuncture could benefit the blood oxygen concentration in the cerebral cortex of PTSD rats, to improve the abnormal activation function of cerebral cortex, which may be an important mechanism of acupuncture in intervening the brain function of PTSD rats.

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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