Effects of acupuncture on brain metabolism in patients with chronic partial sleep deprivation cognitive dysfunction
A case–control study

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Abstract

Patients with chronic partial sleep deprivation (SD) may experience cognitive dysfunction. The purpose of this study is to explore the pathways of electroacupuncture (EA) by observing the changes in brain metabolites before and after EA treatment in patients with chronic partial SD cognitive dysfunction. The research subjects included 26 chronic partial SD cognitive dysfunction patients and 27 healthy subjects. Montreal Cognitive Assessment Scale, Pittsburgh Sleep Quality Index Scale (PSQI), Stanford Sleepiness Scale, Wechsler Memory Scale, Hamilton Anxiety Scale, Hamilton Depression Scale, Stroop paradigm, psychomotor vigilance test, 2-back test task, and mood assessment test were used to assess sleep quality, cognitive function, and emotional state of subjects. Magnetic resonance spectroscopy was used to detect the basal ganglia of the brain, and the characteristics of metabolites of the 2 groups were comprehensively analyzed, and the correlation with clinical cognitive function evaluation indicators was analyzed. Compared with the control group, the Montreal Cognitive Assessment Scale and Wechsler Memory Scale scores of the observation group were reduced before treatment, while the Pittsburgh sleep quality index, Hamilton Anxiety Scale, and Hamilton Depression Scale scores were improved. The completion ability of Stroop, 2-back, and psychomotor vigilance test decreased. The GABA/Cr on the left side of the basal ganglia area increased. "Adjusting Zang-fu and Arousing Spirit" EA can improve the sleep quality and cognitive function of chronic partial sleep deprivation cognitive dysfunction patients, which may be related to regulating the levels of NAA, Cho, and GABA in the basal ganglia.

Abbreviations: CD = cognitive dysfunction, CPSDCD = chronic partial sleep deprivation cognitive dysfunction, EA = electroacupuncture, HAMA = Hamilton Anxiety Scale, HAMD = Hamilton Depression Scale, MoCA = Montreal cognitive assessment, MRS = Magnetic Resonance Spectroscopy, PSQI = Pittsburgh sleep quality index, PVT = psychomotor vigilance test, SD = sleep deprivation, SSS = Stanford Sleepiness Scale, WMS = Wechsler Memory Scale

Keywords: "Adjusting Zang-fu and Arousing Spirit" electroacupuncture, acupuncture, basal ganglia, cerebral metabolites, chronic partial sleep deprivation, cognitive dysfunction, magnetic resonance spectroscopy

1. Introduction

According to data from the World Health Organization, about one-third of the world's population experiences insomnia symptoms, with approximately 10% to 15% of people suffering from chronic insomnia. Sleep difficulties related to insomnia are usually associated with chronic sleep deprivation.[1]

Chronic sleep deprivation has a negative impact on health and is a major international public health issue in contemporary times.[2–5] Chronic partial sleep deprivation (CPSD) can lead to cognitive dysfunction (CD) in the body,[6] leading to decreased learning and memory abilities, alertness and attention, as well as an increase in negative emotions, leading to a decrease in

work efficiency and an increase in error rates.[7–12] Therefore, how to reduce the negative impact of sleep deprivation on cognitive dysfunction caused by workers in various industries, improve quality of life and work efficiency, has become a key issue that needs to be urgently addressed.

The pathogenesis of chronic partial sleep deprivation cognitive dysfunction (CPSDCD) involves the complex functions of the brain and various systems throughout the body, and the brain plays a dominant role in this process. According to research on brain functional imaging and neurobiology, the neurobiological mechanism of CPSDCD mainly involves altering the metabolism of substances in the brain, directly or indirectly damaging neurons in different regions of the brain, thereby damaging cognitive function.[13] With the continuous development of brain imaging technology, MRS has been gradually applied to detect the substance metabolism of the human central nervous system, so it can be used as a reliable technology for the early diagnosis of mild cognitive impairment, Alzheimer disease and other diseases.[14] The changes in the levels of neurotransmitters in the corresponding brain regions are related to the state of neuronal damage. By quantitatively measuring commonly used brain metabolites such as N-Acetyl-L-aspartic acid (NAA), choline (Cho), creatine (Cr), Myo-inositol (MI), as well as glutamic and glutamine (Glu + Gln/Glx), γ-aminobutyric (GABA), the degree of neuronal damage can be determined, and the neurobiological mechanism of CPSDCD can be revealed.

For CPSDCD, currently the main focus is on promoting sleep and improving cognitive symptoms, commonly treated with awakening enhancers and receptor inhibitors. Although these drugs can prolong patients' sleep time and improve cognition, long-term use can lead to corresponding side effects. Acupuncture has a significant effect in promoting sleep and improving cognition. Relevant clinical studies have reported that acupuncture has a significant improvement effect on cognitive decline caused by SD.[15,16] The previous study[17] of the research group found that "Adjusting Zang-fu and Arousing Spirit" electroacupuncture (EA) can effectively delay nerve injury and improve cognitive dysfunction related to type 2 diabetes. This study applies MRS technology to explore the potential brain effect mechanism of "Adjusting Zang-fu and Arousing Spirit" EA treatment for CPSDCD.

2. Methods

2.1. Participants

The case–control study was conducted from November 2021 to November 2022. Twenty-six CPSDCD patients were recruited from acupuncture and moxibustion department of the Third Clinical Hospital Affiliated to Changchun University of Chinese Medicine and the Affiliated to Changchun University of Chinese Medicine, including 11 men, 15 women (observation group), and 27 healthy subjects, including 11 men and 17 women (control group). All participants have signed an informed consent form, and this study has been approved by the Medical Ethics Committee of the Third Affiliated Clinical Hospital of Changchun University of Traditional Chinese Medicine on August 12, 2021 (CZDSFYLL2021-015-01).This study has been registered in the Chinese Clinical Trial Registry (ChiCTR2200061792).

2.2. Procedures

2.2.1. Inclusion criteria.

(1) Observation group: ① all are long-term fixed night shifts or overtime (aged 21–50), right-handed; ② intermittent SD lasting for more than 1 year, with <5 hours of sleep per day; ③ pittsburgh sleep quality index (PSQI) > 7 points, Montreal cognitive assessment scale (MoCA) score < 26

points; ④ regular diet, requiring that no excitable drinks such as tea and coffee, as well as cigarettes and alcohol, be consumed 1 week before the start of the experiment; Has not received acupuncture treatment within 1 month; Not taking any neuropsychiatric drugs within 6 months; ⑤ normal physical health, no history of underlying diseases, normal mental state, and no pathological or abnormal clinical findings during physical examination; ⑥ college degree or above, with fixed job responsibilities and fixed activity cities.

(2) Control group: ① all individuals (21–50 years old) who work regularly during the day and are not on night shifts, with comparable conditions such as age, gender, race, and education level compared to the observation group subjects, right-handed; ② working hours ≤ 8 hours per day, normal sleep wake rhythm, sleep time of 6.5 to 8.5 hours per night, PSQI score ≤ 7; MoCA score ≥ 26; ③ After head MRI examination, there were no serious neurological diseases or related diseases leading to cognitive impairment; ④ Normal physical health, no history of underlying diseases, normal mental state, and no pathological or abnormal clinical findings during physical examination; No history of alcohol or drug abuse; ⑤ Fixed job responsibilities, college degree or above, and fixed activity cities.

2.2.2. Exclusion criteria.
① During the night shift, the sleep time is more than 5 hours; ② individuals with a history of excessive smoking and alcohol abuse, as well as previous sleep disorders; ③ suffering from physical or psychological disorders that interfere with sleep, such as apnea syndrome and depression; ④ take prescription or over-the-counter medication within 7 days before enrollment for treatment; Those who participated in other clinical studies at the same time during the treatment period or received acupuncture and moxibustion treatment within 7 days before joining the group; ⑤ leaving the place of daily activities during the research period; ⑥ Individuals who are not suitable for MRI examination (have claustrophobia, implanted pacemakers, cardiac stents, etc). Patients with organic brain injury.

2.2.3. Culling, shedding criteria.
① Cases that do not conform to the inclusion criteria and are mistakenly included; ② cases with incomplete information or failure to treat according to regulations; ③ cases that have not completed efficacy evaluation; ④ subjects who have poor compliance and voluntarily withdraw during the course of treatment.

2.2.4. Termination criteria.
Subjects who experience serious complications or adverse events during the research process and are not suitable to continue participating in the research project.

2.2.5. Therapeutic method.
The subjects in the observation group were treated by acupuncturists with intermediate or above professional titles. Acupoints selection: Fei Shu (double), PI Shu (double), Shen Shu (double); Bai Hui, Shen Ting, Zu San li (double), Sanyinjiao (double), He Gu (double), Tai Chong (double). Operation steps:① Use Hua-Tuo brand disposable acupuncture needle, size: 0.30 × 40mm; Electronic needle therapy instrument: Hua-Huo brand SDZ-V type. ② Disinfection: the doctor performs routine hand disinfection and acupuncture site disinfection. ③ Body position: firstly, the patient was placed in prone position, acupuncture Fei Shu, Pi Shu, Shen Shu, Fei Shu, Pi Shu at a 45° to the spine oblique stabbing 15 to 20 mm; Shen-Shu straight thorn 15 to 20 mm; The retention time was 15 min. After outputting the needle, Bai Hui, Shen Ting, Zu San li, Sanyinjiao, He Gu and Tai Chong points were needled in the supine position. Bai-hui and Shen Ting were needled horizontally forward for 15 to 20 mm, He Gu and Tai Chong were needled straight for 10 to 25 mm, Zu

Figure 1. Data acquisition of magnetic resonance spectroscopy (July 23, 2022).

San li was needled straight for 20 to 30 mm, Sanyinjiao was needled for 15 to 25 mm, and the retention time was 25 min. ④ Inserting and going the needle: using needle-inserting method of both hands, according to the characteristics of the acupoints location can choose the clamping method, claw cutting method, the direction and depth of acupuncture should be strictly in accordance with the reference textbook acupuncture standard operation; After inserting the needle, lift and twist the needle with small amplitude to obtain Qi. ⑤ Electroacupuncture: after the qi was obtained by acupuncture, Pi Shu-Shen Shu, Bai Hui-Shen Ting and Zu San li-Sanyinjiao were used as the acupoints of the electroacupuncture connection, the waveform setting was selected as the dense wave, the frequency was set at 2 Hz/100 Hz, the current intensity was adjusted within the range of 0.1 to 1.0MA (depending on the patient's feeling of comfort), and the electroacupuncture was performed. ⑥ Needle out: gently press the sterilized dry cotton ball on the acupuncture site with the left hand, gently twist the needle handle with the right hand, and slowly lift the needle to the skin, leave the needle for a moment, and gently press the needle hole after the needle is out for a moment to prevent bleeding. ⑦ Course of treatment: 5 times as a course of treatment, between the course of rest 2d, the need for continuous intervention for 4 courses.

2.3. Curative effect observation

Twenty-six CPSDCD patients were recruited, due to objective reasons, some people were unable to complete the entire course of treatment or conduct timely assessment and examination of relevant indicators for group selection. 8 patients were excluded, and 18 patients, including 9 males and 9 females, were ultimately included. The observation group was evaluated with cognitive related scales, behavioral tests, and MRS examinations before and after treatment, while the control group was evaluated with cognitive related scales before treatment.

2.3.1. Outcome measures.

(1) Assessment of sleep and cognitive related scales: Including the Pittsburgh Sleep Index Scale, Montreal Cognitive Function Scale, Wechsler Memory Scale, SSS, Hamilton Anxiety Scale, and Hamilton Depression Scale (HAMD).
(2) Behavioral testing: including 2-back testing tasks, Stroop testing tasks, Psychomotor Vigilance Test, Emotional assessment testing (based on the Chinese emotional image database system).
(3) Brain Metabolite Detection: The device uses a 3.0T high field magnetic resonance scanner from the MRI Department of Jilin Provincial Hospital of Traditional Chinese Medicine (brand: Siemens; origin: Germany; equipment model: 3TMAGNETO Trio). ① The gradient echo sequence is used to obtain 3D-T2 weighted images (T2WI), with a scanning range set from the skull base to the top of the forehead. Scanning parameters: layer thickness: 1.0 mm; voxel: 1.0 mm × 1.0 mm × 1.0 mm; field of view: 256 mm × 256 mm; repetition time: 3600 ms; echo time: 415 ms; data collection time: 5:18 s. After the collection is completed, 3D-T2WI image reconstruction will be performed from sagittal, coronal, and horizontal positions. These reconstructed images will continue to be used for subsequent scanning sequences to ensure accurate positioning Perform 3D voxel spectral scanning of the bilateral basal ganglia of the subject's brain. Position: Head and foot position. Voxel: 15 × twenty × 20mm; Repetition time: 2000 ms; echo time: 68 ms; data collection time: 2.48 s. ③ The Spectracopy software of the Siemens workstation first automatically performs phase and baseline calibration, signal averaging, spectral line fitting, and metabolite identification on 1H-MRS spectral lines. Subsequently, the area under the curve and peak values of NAA, Cho, Cr, MI, Glu + Gln, PCr, and GABA in the basal ganglia are automatically read (Fig. 1). Due to the use of semi quantitative proton spectroscopy detection in this experiment, the stability of the relative values

Table 1

Comparison of general data between observation group and control group.

Group		OG	CG
Num		26	27
Age/Year	Min	25	25
	Max	50	49
	Mean ($\bar{x} \pm S$)	37.42 ± 7.58	33.78 ± 6.68
Gender/Num	M (%)	11(42.3%)	11(40.7%)
	F (%)	15(57.7%)	16(59.3%)
Education/Year ($\bar{x} \pm S$)		18.04 ± 1.99	18.93 ± 2.40
Height/m ($\bar{x} \pm S$)		166.85 ± 8.16	168.04 ± 7.95
Weight/kg ($\bar{x} \pm S$)		63.27 ± 10.41	68.11 ± 9.81

CG = control group, F = female, M = male, OG = observation group.

Table 2

Comparison of MoCA score, PSQI score, SSS score, WMS score, HAMA score and HAMD score between observation group before treatment and control group ($\bar{x} \pm$ S).

Group	OG	CG
Time	Before treatment	/
Num	26	27
MoCA	23.92 ± 1.06*	28.26 ± 1.13
PSQI	13.27 ± 1.87*	3.11 ± 1.58
SSS	9.38 ± 2.45	8.07 ± 2.51
WMS	119.88 ± 4.28†	122.74 ± 5.65
HAMA	10.69 ± 5.98*	2.59 ± 3.19
HAMD	10.46 ± 7.31*	4.15 ± 3.45

CG = control group, HAMD = Hamilton Depression Scale, OG = observation group.

*Comparison with control group, $P<.001$.

†Comparison with control group, $P<.05$.

Table 3

Comparison of MoCA score, PSQI score, SSS score, WMS score, HAMA score and HAMD score before and after treatment in the observation group ($\bar{x} \pm$ S).

Group	Before treatment	After treatment
Num	18	18
MoCA	23.94 ± 1.06	27.50 ± 1.38*
PSQI	12.89 ± 1.75	4.78 ± 2.44*
SSS	9.28 ± 2.44	8.17 ± 1.76
WMS	120.72 ± 4.47	123.50 ± 4.82‡
HAMA	9.17 ± 3.81	4.89 ± 1.60*
HAMD	8.78 ± 5.78	4.78 ± 3.15†

HAMD = Hamilton Depression Scale.

*Comparison before and after treatment within the group, $P<.001$.

†Comparison before and after treatment within the group, $P<.01$.

‡Comparison before and after treatment within the group, $P<.05$.

of the detected metabolites is higher. NAA/Cr, Cho/Cr, MI/Cr, Glu + Gln/Cr, and GABA/Cr values are used as the final statistical data.

2.3.2. Statistical analysis. SPSS 27.0 statistical analysis software was used to analyze all the data. The measurement data conforming to the normal distribution are expressed with $\bar{x} \pm S$. If it meets normal distribution, the paired samples t test is used for intragroup comparison, and the 2 independent samples t test is used for inter-group comparison. If the data does not meet normal distribution, the nonparametric test is applied. Counting data are described in frequency or percentage terms and verified with χ^2. The correlation between the ratio of differential metabolites in the basal ganglia of CPSD patients and the cognitive function assessment scale and behavioral

indicators was analyzed using Pearson correlation. $P < .05$ level indicates a statistical difference.

3. Results

3.1. Comparison of general information between 2 groups of subjects

The comparison results between the 2 groups showed that there was no significant difference in general demographic data such as age, gender, and educational years ($P > .05$) (Table 1).

3.2. MoCA score, PSQI score, SSS score, WMS score, HAMA score and HAMD score were compared between observation group before and after treatment and control group

There were significant differences in MoCA, PSQI, Wechsler Memory Scale (WMS), Hamilton Anxiety Scale (HAMA) and HAMD scores between the observation group and the control group, with statistical significance ($P < .05$), while there was no significant difference in SSS results ($P > .05$) (Table 2). There were significant differences in PSQI, WMS, HAMA and HAMD scores before and after electroacupuncture intervention ($P < .05$) (Table 3).

3.3. Comparison of Stroop paradigm, 2-back paradigm, PVT, emotion picture test and behavioral indexes between observation group before and after treatment and control group

There were significant differences ($P < .05$) between observation group and control group in terms of Stroop neutral condition response time and accuracy, Stroop conflict condition response time and accuracy, 2-back accuracy, PVT error frequency and response time, and the frequency of subjects evaluating images as positive in the image emotion test (Table 4). There were significant differences ($P < .05$) in Stroop neutral conditioned response time, accuracy, Stroop consistency conditioned response time, Stroop conflict conditioned response time, 2-back response time, PVT error frequency and response time, as well as the frequency of subjects evaluating images as positive and negative in the image emotion test before and after the intervention of electroacupuncture. Other comparisons showed no significant difference ($P > .05$) (Table 5).

3.4. Comparison of basal ganglia metabolites between observation group patients before and after treatment and control group subjects

The comparison results between the 2 groups showed that: compared with the control group, the GABA/Cr ratio on the

Table 4

Comparison of behavioral indicators of Stroop paradigm, 2-back paradigm, PVT and emotion picture test between observation group before treatment and control group ($\bar{x} \pm$ S).

Group		OG	CG
Treatment time		Before treatment	/
Number		26	27
Stroop (neutral condition)	RT/s	0.88 ± 0.04‡	0.84 ± 0.02
	Accuracy/%	0.93 ± 0.02†	0.94 ± 0.03
Stroop (consistency condition)	RT/s	0.96 ± 0.04	0.96 ± 0.04
	Accuracy/%	0.94 ± 0.03	0.95 ± 0.02
Stroop (conflict condition)	RT/s	1.01 ± 0.07*	0.93 ± 0.08
	Accuracy/%	0.89 ± 0.04*	0.93 ± 0.03
2-back	RT/s	1.19 ± 0.10	1.15 ± 0.08
	Accuracy/%	0.74 ± 0.02‡	0.75 ± 0.01
PVT	EN	2.38 ± 1.30†	1.63 ± 0.97
	RT/ms	335.15 ± 31.39†	314.85 ± 38.55
EPT (frequency)	Positive	21.50 ± 5.49†	25.59 ± 5.90
	Neutral	39.35 ± 7.97	40.78 ± 8.53
	Negative	29.15 ± 10.23	23.63 ± 11.04

CG = control group, EPT = emotion picture test, OG = observation group, RT = reaction time, EN-error number.
*Comparison with control group, $P < .001$.
†Comparison with control group, $P < .05$.
‡Comparison with control group, $P < .01$.

Table 5

Comparison of behavioral indicators of Stroop paradigm, 2-back paradigm, PVT, emotion picture test before and after treatment in observation group ($\bar{x} \pm$ S).

Group		Before treatment	After treatment
Number		18	18
Stroop (neutral condition)	RT/s	0.87 ± 0.04	0.85 ± 0.03*
	Accuracy/%	0.92 ± 0.03	0.94 ± 0.01†
Stroop (consistency condition)	RT/s	0.95 ± 0.04	0.94 ± 0.03†
	Accuracy/%	0.94 ± 0.03	0.94 ± 0.03
Stroop (Conflict condition)	RT/s	1.00 ± 0.08	0.99 ± 0.07†
	Accuracy/%	0.90 ± 0.04	0.90 ± 0.03
2-back	RT/s	1.19 ± 0.09	1.13 ± 0.05†
	Accuracy/%	0.73 ± 0.01	0.74 ± 0.02
PVT	EN	2.22 ± 1.22	1.89 ± 0.90†
	RT/ms	322.17 ± 25.44	316 ± 24.63*
EPT (frequency)	Positive	23.00 ± 5.69	27.66 ± 4.55*
	Neutral	41.83 ± 8.21	41.11 ± 7.33
	Negative	25.16 ± 9.68	21.72 ± 4.65†

EPT = emotion picture test, RT = reaction time, EN = error number.
*Comparison before and after treatment within the group, $P < .01$.
†Comparison before and after treatment within the group, $P < .05$.

left side of basal ganglia in the observation group was increased ($P < .05$) (Table 6). The results of electroacupuncture intervention showed that compared with before treatment, NAA/Cr value in the right basal ganglia and Cho/Cr value in the left basal ganglia were significantly increased after treatment ($P < .05$). After treatment, GABA/Cr value in the left basal ganglia was significantly decreased ($P < .05$). There was no significant difference in the results of other indexes ($P > .05$) (Table 7).

3.5. Correlation analysis between the difference level of metabolites in basal ganglia and the difference index of cognitive function evaluation in the observation group before and after the intervention of "regulating Zang-fu and arousing Spirit" electroacupuncture

Before intervention, there was a significant negative correlation between the GABA/Cr on the left side of the basal ganglia and the accuracy of Stroop conflict condition in the observation group ($r = -0.412$, $P = .045$) (Fig. 2).

After intervention, there was a significant negative correlation between the right NAA/Cr and HAMA ($r = -0.533$, $P = .034$) (Fig. 3), and the left Cho/Cr was significantly negatively correlated with 2-back reaction time ($r = -0.701$, $P = .002$) in the observation group (Fig. 4).

4. Discussion

The cognitive decline of CPSD patients may be related to the abnormal levels of NAA and GABA in the brain. Previous studies using 1H-MRS technology on degenerative nervous system diseases and mental diseases have found a low rate of NAA/Cr in multiple related brain regions such as the prefrontal lobe, occipital lobe, hippocampus and cerebellum. Reduced NAA/Cr is positively associated with cognitive impairment, and NAA may be the most reliable marker of mild cognitive impairment.[18–20] The decrease of NAA is mainly related to the decrease of gray matter volume and healthy neurons, and the reduced NAA level may reflect the compensatory effect of nerve cell apoptosis and neuronal energy consumption in certain brain regions. In this study, after the intervention

Table 6

Comparison of metabolite levels in basal ganglia between observation group before treatment and control group before treatment ($\bar{x} \pm$ S).

Group		OG	CG
Treatment Time		Before treatment	/
Num		26	27
NAA/Cr	R	0.99 ± 0.12	1.04 ± 0.14
	L	1.11 ± 0.33	1.09 ± 0.18
Cho/Cr	R	0.72 ± 0.17	0.74 ± 0.14
	L	0.76 ± 0.23	0.76 ± 0.19
ml/Cr	R	0.02 ± 0.05	0.02 ± 0.03
	L	0.03 ± 0.05	0.05 ± 0.09
Glu + Gln/Cr	R	0.02 ± 0.02	0.02 ± 0.02
	L	0.02 ± 0.03	0.02 ± 0.02
GABA/Cr	R	0.12 ± 0.07	0.10 ± 0.07
	L	0.18 ± 0.11*	0.11 ± 0.07

CG = control group, L = left, OG = observation group, R = right.

*Comparison with control group, $P<.05$.

Table 7

Comparison of metabolite levels in basal ganglia before and after treatment in observation group ($\bar{x} \pm$ S).

Group		Before treatment	After treatment
Number		18	18
NAA/Cr	R	0.97 ± 0.13	1.06 ± 0.13*
	L	1.13 ± 0.28	1.13 ± 0.13
Cho/Cr	R	0.70 ± 0.19	0.75 ± 0.16
	L	0.75 ± 0.16	0.86 ± 0.17*
ml/Cr	R	0.02 ± 0.06	0.02 ± 0.05
	L	0.02 ± 0.04	0.03 ± 0.05
Glu + Gln/Cr	R	0.02 ± 0.02	0.02 ± 0.01
	L	0.01 ± 0.01	0.01 ± 0.01
GABA/Cr	R	0.12 ± 0.06	0.11 ± 0.08
	L	0.19 ± 0.11	0.12 ± 0.08*

L = left, R = right.

*Comparison before and after treatment within the group, $P<.05$.

of "Adjusting Zang-fu and Arousing Spirit" electroacupuncture, the NAA/Cr level in the right basal ganglia area of patients was significantly increased, and there was a significant negative correlation with HAMA, indicating that "Adjusting Zang-fu and Arousing Spirit" electroacupuncture can improve cognitive function by activating the number and function of neurons in the basal ganglia area of the brain, especially in the regulation of negative emotions such as anxiety. Choline (Cho), as a marker of cell membrane catabolism and anabolism, is an indicator of cell membrane transport function and cell proliferation. The study found that the Cho/Cr level of the right cerebellum in middle-aged patients with depression was significantly lower than that of healthy controls, suggesting that the signal transduction system in the small brain cells of patients was damaged.[21,22] In this study, although we did not find significant differences in the Cho/Cr level between the basal ganglia area of the brain of patients in the observation group and the control group, the Cho/Cr level on the left side was significantly increased after the intervention of electroacupuncture, and was significantly negatively correlated with 2-back reaction time. Therefore, we speculated that "Adjusting Zang-fu and Arousing Spirit" electroacupuncture may improve cognitive functions such as memory ability and executive function by regulating and repairing intracellular transduction system and regulating cell proliferation. GABA is the main inhibitory neurotransmitter of the central nervous system, and is widely involved in the physiological and biochemical processes of the human body, including sleep regulation, memory and learning, emotional changes, cognitive regulation, etc.[23,24] Gaba plays an important role in aging and

neurodegenerative diseases (mild cognitive impairment, AD), and has been identified as a potential target for AD intervention.[25,26] It has been reported that changes in GABA levels in the frontal lobes of alternating shift workers may indicate a specific role of GABA in controlling circadian rhythms, and irregular work schedules often lead to disruption of normal circadian rhythms, resulting in CPSD.[27] Previous MRS Studies have shown that GABA levels in relevant brain regions will change due to different stages of the disease and the influence of age and gender.[28–30] This study found that before the intervention of "Adjusting Zang-fu and Arousing Spirit" EA, the left GABA/Cr ratio in the basal ganglia of patients in the observation group was significantly increased, and there was a significant negative correlation with the accuracy of Stroop conflict conditions. Poor sleep quality and decreased overall cognitive function in CPSD patients trigger some compensatory mechanisms in the brain, resulting in a significant increase in GABA/Cr level in the basal ganglia. It is suggested that GABA/Cr level is sensitive to cognitive function including executive function. Previous studies of our research group found that the GABA/Cr level in the basal ganglia of the brain of patients with type 2 diabetes mellitus and cognitive dysfunction showed a significant downward trend,[31] and after the intervention of "Adjusting Zang-fu and Arousing Spirit" EA, the GABA/Cr level increased significantly, which was completely contrary to the results of our research on patients with CPSD cognitive dysfunction. The reason is that although the 2 groups of subjects have symptoms of cognitive dysfunction, and the basal ganglia region of the brain is used as the brain region for MRS Detection, the comorbidity and age interval of the 2 groups are different. Meanwhile, the education level and disease course of the 2 groups of subjects were significantly different, which led to the differentiation of the research results. From the perspective of acupuncture effect, our research results reflect that "Adjusting Zang-fu and Arousing Spirit" EA has a benign bidirectional regulation effect: it increases the decreasing GABA/Cr level and decreases the increasing GABA/Cr level, and improves the sleep quality and cognitive ability of CPSD patients with cognitive dysfunction through abnormal regulation of GABA/Cr level.

According to the clinical manifestations of CPSD, such as reduced sleep time, reduced memory, decreased attention, and increased negative emotions, it can be summarized into the categories of "insomnia," "forgetfulness," "weak," "depression," "dull disease" and other early stages of traditional Chinese medicine. The loss of Yin and Yang, the excessive Yin and Yin failure, and the dysfunction of Zang-fu organs cause insomnia, which leads to the decline of normal function of the human body in the long run. The normal physiological activities of Zang -fu organs and brain spirit are affected, the deficiency of 5 Zang organs and insufficient Qi and blood are unable to nourish brains, or internal phlegm, stasis, Qi, fire and other evil forces block the brain, so that brains is insufficient, the brain spirit is useless, and cognitive dysfunction occurs, which belongs to traditional Chinese medicine. The disease is mainly caused by the deficiency of Qi - blood and Yin-Yang, and the dysfunction of Zang-fu organs is the basis of the pathogenesis. The standard is that qi, fire, phlegm and blood stasis are blocked in the brain, and are closely related to the Du and bladder channels on the meridians, and the disease is located in the brain and 5 viscera. Based on the etiology and pathogenesis, the therapeutic principle of "Adjusting Zang-fu and Arousing Spirit" was established, and the EA method of " Adjusting Zang-fu and Dredging meridians" was applied to treat the cognitive decline of CPSD, that is, " Adjusting Zang-fu and Dredging meridians" was the main point, supplemented by "Adjusting Zang-fu and Arousing Spirit." Fei Shu, Pi Shu, Shen Shu, Bai Hui and Shen Ting were selected as the main acupoints, and Zu San li, Sanyinjiao, He Gu and Tai Chong were combined. The whole recipe has 9 acupoints, corresponding to the outside and inside, matching the upper and lower levels, supplementing and purging appropriate, compatible with the subtle, a total effect of regulating Zang-fu organs, harmonizing Qi -blood, dredging meridians, arousing
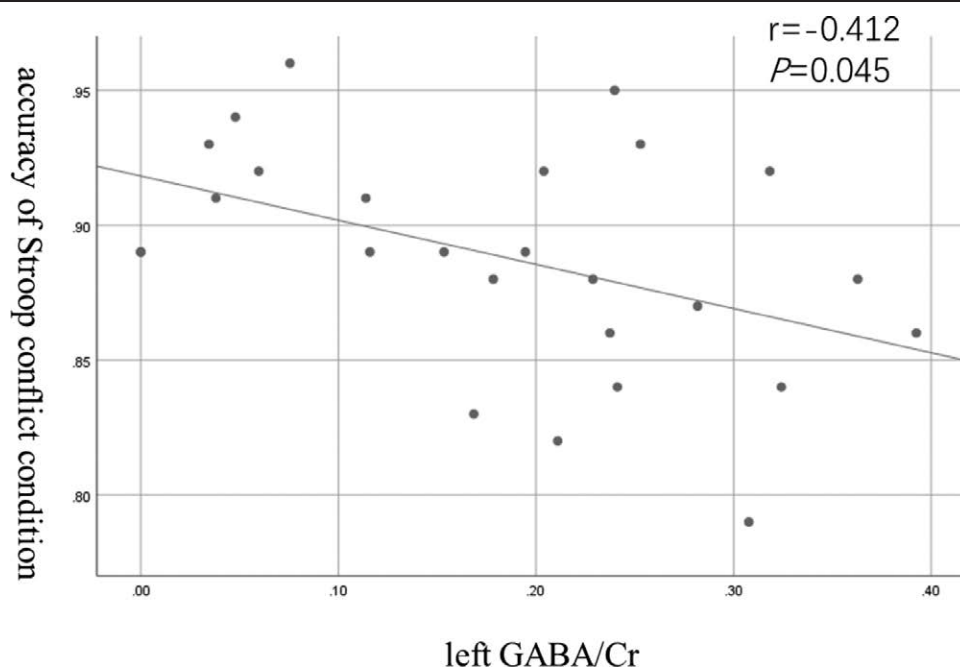
Figure 2. Correlation analyses. A negative correlation between left GABA/Cr and accuracy of Stroop conflict conditions.
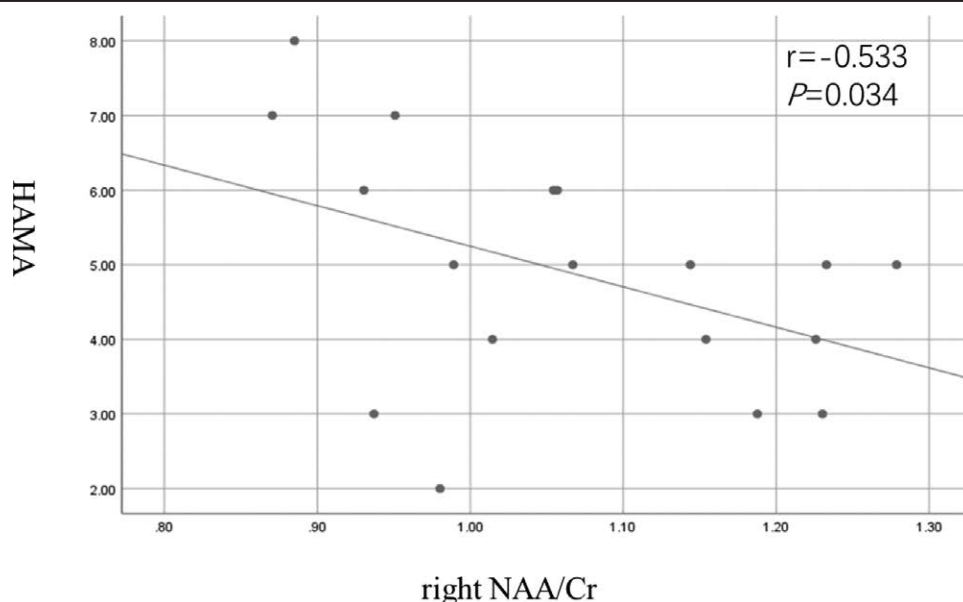

Figure 3. Correlation analyses. A negative correlation between right NAA/Cr and HAMA. HAMA = Hamilton Anxiety Scale.

spirit, reflecting the " treating diseases must be based on the root cause "of traditional Chinese medicine treatment principle.

There are some limitations that must be considered. It has been reported that the levels of some metabolites in the brain can change with the changes of women's menstrual cycle, and our study did not fully consider gender factors; At the same time, we did not real-time monitor whether the subjects' brains were completely relaxed and had no thinking activity during the examination, so the stability of the results could not be guaranteed.

5. Conclusions

Proton MRS is an advanced tool to quantify the metabolic changes in CPSD. This study showed that MRS of basal ganglia is a sensitive region for the detection of cognitive decline in CPSD. The alteration of NAA/Cr, Cho/Cr and GABA/Cr ratios in the basal ganglia may indicate the potential pathway of "Adjusting Zang-fu and Arousing Spirit" electroacupuncture for improving cognitive function in CPSD patients. It is hoped that the results of this study will lead to more extensive and comprehensive studies of CPSD patients treated with "Adjusting Zang-fu and Arousing Spirit" electroacupuncture.

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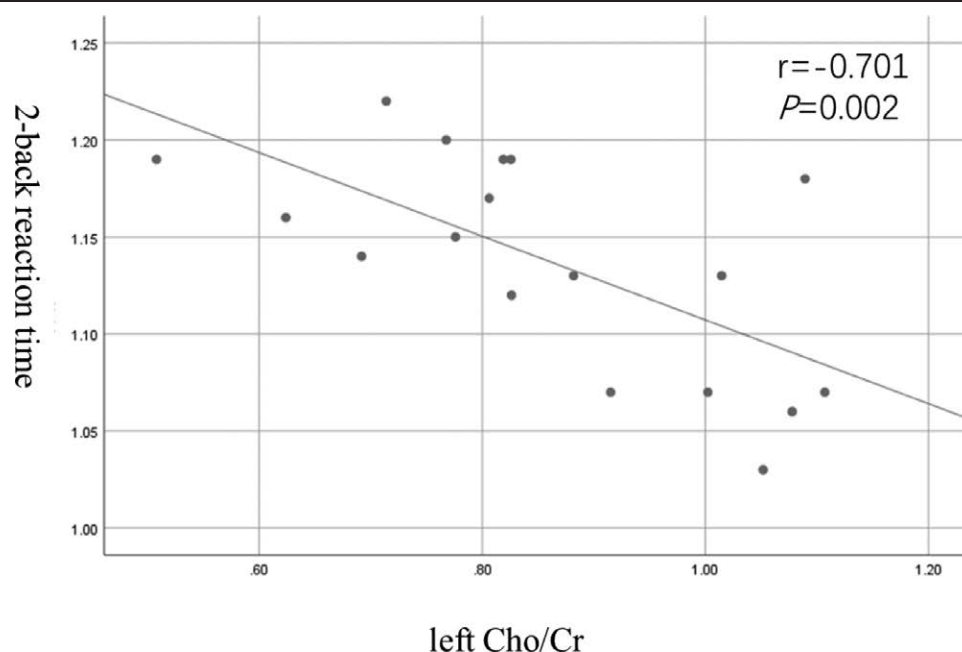**Writing – review & editing:** Hongfeng Wang.

Figure 4. Correlation analyses. A negative correlation between left Cho/Cr and 2-back reaction time.

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