Neuropsychiatric Disease and Treatment

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Modulating Gut Microbiota: The Mechanism of Electroacupuncture at the "Siguan" Acupoints in Alleviating Post-Stroke Depression

Zhuoyan Li[1,2,*], Lianqiu Li[1,*], Hui Xu[1], Zhen Kang[1], Xuehui Shi[1], Peng Zhang[1], Haimin Ye[1]

[1]The Second Affiliated Hospital of Hunan University of Chinese Medicine, Changsha, 41005, People's Republic of China; [2]The Second Clinical College of Traditional Chinese Medicine, Hunan University of Chinese Medicine, Changsha, 410208, People's Republic of China

*These authors contributed equally to this work

Correspondence: Haimin Ye; Peng Zhang, The Second Affiliated Hospital of Hunan University of Chinese Medicine, Changsha, 41005, People's Republic of China, Email 56842028@qq.com; 35401725@qq.com

Purpose: To explore the related mechanisms of electroacupuncture at the "Siguan" acupoints in treating post-stroke depression (PSD).

Methods: Fifty male SD rats were randomly divided into the blank group, stroke group, PSD group, Siguan group and Bifidobacterium group, with 10 rats in each group. The Siguan group was given electroacupuncture at the bilateral "Hegu" (LI4) and "Taichong" (LR3), using the disperse-dense wave with a frequency of 2Hz/10Hz for 30 minutes each time. The changes in depressive behaviors of rats were observed according to the sucrose consumption and the scores of the open field test of rats in each group. The gene data of the intestinal flora of rats in each group were extracted to analyze the differences in the diversity, composition structure and function of the intestinal flora among different groups.

Results: The scores of the depression behavior indexes of the PSD rats in the Siguan group were higher than those in the PSD group. Meanwhile, there were disorders in the structure and function of the intestinal flora of the PSD rats. Compared with the PSD group, both the Siguan group and the Bifidobacterium group had increased contents of beneficial bacteria (Firmicutes and Clostridia) and decreased contents of pathogenic bacteria (Bacteroidetes and Bacteroidia). Compared with the PSD group, the intestinal flora structures of the Siguan group and the Bifidobacterium group were closer to those of normal rats. The abundance of the "Metabolism of Cofactors and Vitamins" functional pathway of the intestinal flora in the Siguan group was lower than that in the PSD group.

Conclusion: Electroacupuncture at the "Siguan" acupoints can improve the depression-like behaviors of PSD rats by up-regulating intestinal probiotics, down-regulating pathogenic bacteria, restoring the physiological structure of the intestinal flora and regulating the function of the intestinal flora.

Keywords: post-stroke depression, acupuncture, electroacupuncture, siguan acupoints, gut microbiota

Introduction

At present, stroke is the number one "killer" that impairs the health of Chinese residents. The "China Stroke Surveillance Report 2021" pointed out that the incidence of stroke in China is constantly increasing, and the disease burden of stroke is also rising continuously.[1] Post-stroke depression (PSD) is the most common affective-psychiatric complication after stroke.[2] It is reported that the incidence of PSD among stroke patients is as high as 39%–52%.[3] PSD will not only impair patients' cognitive functions such as learning ability, memory and attention but also seriously reduce the quality of life of stroke patients. It is one of the important reasons leading to the complication of treatment, the prolongation of the treatment cycle, the increase in the disability rate and the increase in the mortality rate of stroke patients. At present, the pathogenic mechanism of PSD is still unclear. Studies suggest that it involves multiple aspects such as physiology, psychology, society, and genetics.[4] Clinically, antidepressant drugs are mainly used for treatment. However,

Received: 10 September 2024
Accepted: 30 January 2025
Published: 14 February 2025

antidepressant drugs have deficiencies such as a long treatment course, obvious side effects, withdrawal syndrome, and an increased risk of stroke recurrence.[5,6] A large number of studies have shown that electroacupuncture therapy has few adverse reactions and good patient compliance. While relieving the depressive mood of PSD patients, it can also promote the recovery of patients' neurological functions, reduce the side effects brought by antidepressant drugs and the dependence on antidepressant drugs.[7] Therefore, electroacupuncture has been increasingly used in the treatment and research of PSD patients in recent years.

The "microbe-gut-brain axis" (MGBA) is one of the theories that has attracted much attention in recent years. This theory proposes that in addition to directly affecting intestinal diseases, intestinal flora imbalance can also regulate the inflammatory response of the central nervous system by mediating the MGBA, and then influence diseases of the central nervous system. Among them, the mechanism of action between the disorder of intestinal flora and psychiatric disorders (such as anxiety disorders, various types of depression, etc) is currently one of the research hotspots.[8,9] Many scholars' studies have confirmed that intestinal flora and its metabolites can regulate the occurrence, development and prognosis of PSD by regulating pathways such as neurotransmitters and immune-inflammatory responses, but the specific intestinal flora and molecular mechanisms have not yet been clarified.[10,11] Our research group has confirmed in previous experimental studies[12–15] that electroacupuncture at the "Siguan" acupoints has a benign regulatory effect on the levels of brain monoamine neurotransmitters (5-hydroxytryptamine, norepinephrine, and dopamine) in the prefrontal cortex, hippocampus and hypothalamus of PSD rats, the expression of brain-derived neurotrophic factor (BDNF) and tropomyosin-related kinase B (TrkB) in the hippocampus, and the synthesis of the intestinal flora metabolite short-chain fatty acids (SCFAs). It can promote the recovery of neurological functions in PSD rats and improve the depression-like behavior of PSD rats, laying a certain preliminary foundation for this study. In this study, by applying electroacupuncture at the "Siguan" acupoints to intervene in PSD rats, we explored the effects of electroacupuncture at the "Siguan" acupoints on the depression-like behavior and intestinal flora of PSD rats, hoping to reveal the potential mechanism by which electroacupuncture at the "Siguan" acupoints exerts antidepressant effects and provides more scientific evidence for electroacupuncture in the treatment of PSD. The report is as follows.

Materials and Methods

Research Object

A total of 50 male SD rats, specific pathogen-free (SPF) grade, 3 months old, body mass 240–280 g, were provided by Hunan SJA Laboratory Animal Co., Ltd, production license no: SCXK (Xiang) 2019–0004. They were housed in the Animal Experiment Center of Hunan University of Traditional Chinese Medicine at a temperature of 20–25°C and a humidity of 50%-70%. All experimental procedures were in accordance with the ethical standards of the Animal Ethics Committee of Hunan University of Traditional Chinese Medicine on the use of laboratory animals, with the ethical approval number: LL2022022301.

Main Reagents And instruments

Reagents: Live Combined Bifidobacterium and Lactobacillus Tablets (National Pharmaceutical Approval No. S19980004, 0.5g/tablet, Inner Mongolia Shuangqi Pharmaceutical Co., Ltd).; Pentobarbital sodium (096956–001, Beijing Huan Yu Chemical Co., Ltd).; Agarose (biowest, Spain); E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA); NEXTFLEX® Rapid DNA-Seq Kit (Bioo Scientific, USA); NovaSeq Reagent Kits (Illumina, USA).

Instruments: disposable acupuncture needles (0.25mm × 25mm, Suzhou Acupuncture and Moxibustion Supplies Co., Ltd.); Electroacupuncture therapeutic apparatus (Huatuo SDZ - IV, Suzhou acupuncture and moxibustion Supplies Co., Ltd.); Open box [100 cm × 100 cm × 50 cm, Borui Weisi (Hong Kong) Technology Co., Ltd.]; Pipette (N13462C, Eppendorf, Germany); Mini Centrifuge (MiFly-6, Hefei Aibensen Scientific Instrument Co., Ltd., China); High-speed desktop freezer centrifuge (5424R, Eppendorf, Germany); Ultramicro spectrophotometer (NanoDrop2000, Thermo Fisher Scientific, USA); Electrophoresis apparatus (DYY-6C, Beijing 61 Instrument Factory, China); Micro fluorometer (TBS380, TurnerBioSystems, USA); Ultrasonic crusher (Covaris M220, Gene Company Limited, China); ELISA reader (ELx800, Biotek, USA); Vortex mixer (QL-901, Haimen Qilinbei Instrument Manufacturing Co., Ltd., China); Crushing

and grinding machine (TL-48R, Shanghai Wanbai Biotechnology Co., Ltd., China); Grinding machine (FastPrep-24 5G MP, American MP company); Sequencer (Illumina NovaSeq, Illumina, USA).

Animal Grouping and Model Preparation

After being acclimatized and fed for 1 week, 50 male SD rats were randomly divided into five groups (the blank, stroke, PSD, Siguan, and Bifidobacterium groups) using the random number table method, with 10 rats in each group.

For the four groups other than the blank group, the middle cerebral artery occlusion (MCAO) model was prepared using the modified classical Longa et al line embolism method. First, the SD rats were weighed after 12 h of fasting. Subsequently, the rats were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (4mL/kg) and then were positioned on the stereotaxic apparatus. Next, the skin was prepared and sterilized; a 2-cm-long longitudinal incision was made in the middle of the neck. After that, the neck muscles were separated to further expose and separate the right common carotid artery, internal carotid artery, external carotid artery and vagus nerve. The proximal end of the common carotid artery and the external carotid artery were ligated with sutures, and the internal carotid artery was clamped with an arterial clip. A "V"-shaped incision was made at a distance of 4–5 mm from the bifurcation of the common carotid artery. Then, a suture wire with a diameter of 0.26 mm was slowly inserted into the common carotid artery through the incision until it reached the proximal end of the middle cerebral artery. The insertion depth was calculated starting from the bifurcation of the common carotid artery and determined according to the line connecting the midline of the incisors to the bifurcation point of the common carotid artery (TC line) in different individuals. When TC = 39 mm, the insertion depth was appropriately adjusted based on the benchmark of 17 mm. Then, the suture wire was fixed, the excess wire was cut off, and the neck muscles and skin of the rats were sutured. To prevent infection after the operation, penicillin was administered intramuscularly for 3 days (once a day), at a dosage of 50,000 units/kg and 1.5 mL of 0.9% normal saline. At the 24th hour after the rats woke up from anesthesia after the operation, the Longa 5 scoring method and the horizontal stick experiment were used to evaluate the model. An MCAO model was considered to be successfully established when the Longa 5 score was between 2 and 3 points and the time in the horizontal stick experiment was less than 3 minutes.[16]

On the basis of the MCAO model, rats in the PSD, Siguan and Bifidobacterium groups were moved into individual cages for 1 week, and the PSD rat model was established by means of chronic unpredictable mild stress (CUMS). There were several methods of stimulation, and one of them was randomly adopted every day. The specific methods were as follows: (1) fasting and water fasting for 20 h. (2) water fasting for 17 h. (3) ice water swimming (4°C) for 5 min. (4) wet cage (100g sawdust and 200mL water) for 21 h. (5) continuous light for 17 h. (6) tilting the cage (45°) for 17 h. (7) horizontal oscillation (1 time/s) for 5 min. (8) behavioral restriction for 2 h. (9) tail pinching for 1 min. These stimulations were carried out for 18 consecutive days. The model was evaluated by sugar water consumption and open field test, and the PSD modeling was considered to be successful if the scores of sugar water consumption, as well as those of vertical and horizontal movements in open field test, were lower than those of both the blank group and the stroke group and also lower than the pre-modeling scores.[17]

Methods of Intervention

The Siguan group: the Siguan group used electroacupuncture to stimulate the bilateral "Siguan" acupoints (LI4 + LR3) for 30 min on the first day after the successful modeling. For the LI4 acupoint, it was punctured straight to a depth of about 3 mm; for the LR3 acupoint, it was punctured obliquely upward at an angle of 45° to a depth of about 3 mm. Then, they were connected to the electroacupuncture therapeutic instrument and were given sparse-dense waves at a frequency of 2Hz/10Hz. The intensity was adjusted until the electroacupuncture stimulation could cause slight tremors of the rats' limbs. Meanwhile, distilled water (0.01L/kg/d) was administered by gavage once a day for 21 consecutive days.

The Bifidobacterium group: The Bifidobacterium group was given Live Combined Bifidobacterium and Lactobacillus Tablets (0.63g/kg/d) by gavage on the first day after successful modeling, with a solubility of 63 g/L. The tablets were equivalent to the dosage for human use and were converted to the dosage for SD rats,[18] and the group was kept in the same restraint as that of the Siguan group for 21 days.

The blank, stroke, and PSD groups were routinely reared in the same fixed restraint and were given distilled water by gavage for 21 days.

Observation Indicators and Testing Methods

Depressive Behavior

Sugar water consumption

Sugar water consumption in rats suggests the degree of pleasure deficit in rats, and pleasure deficit is an important manifestation of PSD. The daily drinking water of rats was replaced with 10 g/L sucrose water for the first 48 h. After the rats were fasted for 20 h, 10 g/L sucrose water was given to the rats, and the weight of the drinking bottle was recorded after 24 h to obtain the drinking volume of the rats for 24 h, which was then the consumption of sugar water. Measurements were made before stroke modeling, after PSD modeling, and 21 days after intervention, respectively.

Open field test

The open field test, also known as the open box experiment, records the spontaneous behaviors of rats in an unfamiliar environment to evaluate their exploratory behaviors and motor abilities. The decline in motor and exploratory abilities is also an important manifestation of PSD. The rats were placed in the center of the open box, 1 rat per box, and their voluntary movements were recorded within 5 min. The number of times the rats walked through the squares represented the horizontal movement score, and the occurrence of 3 paws or more crossing into the neighboring squares was scored as 1 point; the number of times the rats' two forelimbs were off the ground represented the vertical movement score, and the occurrence of two forelimbs being off the ground for more than 1 cm was scored as 1 point. Assessments were performed before stroke modeling, after PSD modeling, and 21 days after intervention, respectively.

Macrogenome Sequencing to Determine Gut Microbiota Diversity

After the last intervention, the rats were fasted, their feces were taken out, frozen in liquid nitrogen, stored in an ultra-low temperature freezer (−80°C), and transported to Wuhan Anlong Heavy Science and Technology Co., Ltd. for metagenomic sequencing. Three samples were randomly selected from the eligible samples in the blank group, four samples were randomly selected from the stroke and PSD groups, and five samples were randomly selected from the Siguan and Bifidobacterium groups. Sample DNA extraction was performed using the E.Z.N.A.® Soil DNA Kit, and after completion of genomic DNA extraction, DNA concentration was measured using TBS-380, DNA purity was measured using a NanoDrop 200 Ultra-Micro Spectrophotometer, and DNA integrity was measured using 1% agarose gel electrophoresis.

DNA was fragmented by ultrasonic crusher to screen about 400bp fragments for PE library construction. The library was constructed using the NEXTFLEX® Rapid DNA-Seq Kit library construction kit. Macrogenomic sequencing was performed using an Illumina NovaSeq sequencer; Raw data were down-loaded and mass sheared using fastp, host contamination was removed by software BWA, optimized sequences were spliced and assembled using MEGAHIT software, and contigs ≥300 bp were screened for ORF prediction using MetaGene software. Genes with nucleic acid length greater than or equal to 100 bp were selected and translated into amino acid sequences. Gene sequences were clustered (parameters: 90% identity, 90% coverage) using CD-HI software to construct non-redundant gene sets. Using SOAPaligner software, high-quality reads were compared with the non-redundant gene set (95% identity), and the abundance information of genes in the corresponding samples was counted. The amino acid sequences of the non-redundant gene set were compared to the NR database using Diamond (BLASTP comparison parameter set expectation e-value of 1e-5) and species annotations were obtained from the corresponding taxonomic information database of the NR library, and then the abundance of the species was calculated using the sum of the abundance of the corresponding genes of the species. Diversity analysis of the gut microbiota was performed using the R language, Origin, and functional annotation was performed by Diamond software compared to the KEGG database (version 94.2).

Statistical Analysis

Data were processed using SPSS 26.0 software, and statistical plots were generated in R language, GraphPad Prism 9.5. Measurements were expressed as mean ± standard deviation ($\bar{x} \pm s$) if they met normal distribution, otherwise they were expressed as upper and lower quartiles [M (P25, P75)]. If normality and chi-square were satisfied, one-way analysis of variance (ANOVA) was used between multiple groups, and the LSD method was used for two-by-two comparisons. If normality or chi-square was not satisfied, nonparametric Kruskal–Wallis rank sum test was used. Gut microbiota beta diversity was analyzed using PCoA analysis, nMDS analysis and PERMANOVA test. At the test level $\alpha = 0.05$, $P > 0.05$ indicated statistically significant differences.

Results

Death of Rats

A total of 50 rats were included in this experiment, of which 4 died during the stroke modeling stage and 3 failed the Longa score. The final number of rats included in the study in each group by depressive behavioral analysis was 10 in the blank group, 9 in the stroke group, 7 in the PSD group, 8 in the Siguan group, and 9 in the Bifidobacterium group; and due to the financial constraints, the final number of rats included in the study in each group by gut microbiota analysis was: 3 in the blank group, 4 in the stroke group, 4 in the PSD group, 5 in the Siguan group, and 5 in the Bifidobacterium group.

Effects of Electroacupuncture at "Siguan" Acupoints on Depression-Like Behavior of PSD Rats

To investigate the effect of electroacupuncture at the "Siguan" acupoints on depression-like behavior in PSD rats, sugar water consumption and open field tests were used to evaluate depression-like behavior in PSD rats. If sugar water consumption, open field test vertical and horizontal movement scores increased, it indicated that depression-like behavior in PSD rats was alleviated.

Before stroke modeling, there was no statistically significant difference in sugar water consumption, vertical movement scores, and horizontal movement scores among the groups of rats. After PSD modeling, there was no statistically significant difference in sugar water consumption, vertical movement score, and horizontal movement score between the blank group and stroke group rats; Compared with the blank group, the PSD, Siguan, and Bifidobacterium group showed a decrease in sugar water consumption, vertical movement scores, and horizontal movement scores ($P<0.05$), indicating successful modeling of the PSD model. After 21 days of intervention, compared with the PSD group and self PSD modeling, the sugar water consumption, vertical movement score, and horizontal movement score of rats in the Siguan and Bifidobacterium group increased significantly ($P < 0.05$); Compared with the Bifidobacterium group, the sugar water consumption, vertical and horizontal exercise scores of the Siguan group showed an increasing trend but the differences were not statistically significant. The results showed that electroacupuncture at the "Siguan" acupoints and supplementation of Bifidobacterium alleviated depression-like behavior in PSD rats. Refer to Figures 1–3.

Effects of Electroacupuncture on the Gut Microbiota of PSD Rats at the "Siguan" Acupoints

Effects of Electroacupuncture on the Diversity of Gut Microbiota in PSD Rats at the "Siguan" Acupoints

(1) alpha diversity analysis: using the Shannon index to measure the richness of gut microbiota, the larger the Shannon index, the higher the richness of gut microbiota. After 21 days of intervention, when the Shannon index of the PSD group was compared with that of the blank and stroke group, it decreased significantly ($P < 0.01$); When the Shannon index of the Siguan and the Bifidobacterium group was compared with that of the PSD group, it increased ($P < 0.05$). There was no statistically significant difference between the Siguan and the Bifidobacterium group. The results showed that PSD decreased the richness of gut microbiota in rats, while electroacupuncture at the "Siguan" acupoints and supplementation of Bifidobacterium increased the richness of gut microbiota in PSD rats, as shown in Figure 4.

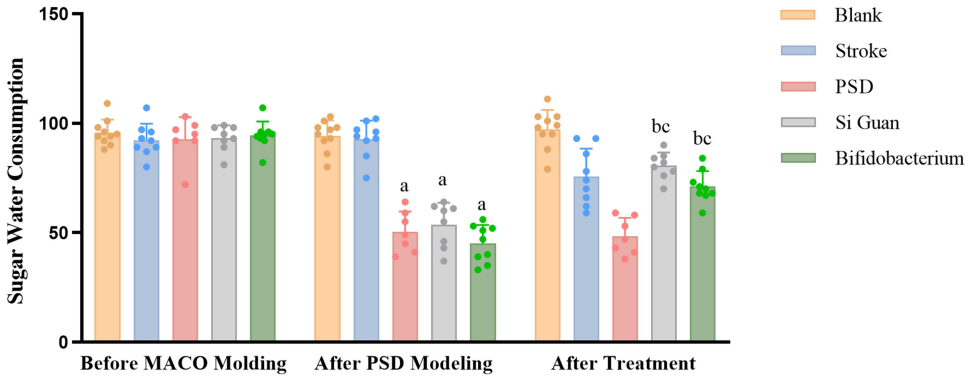

Figure 1 Comparison of sugar water consumption at different time points before and after modeling and treatment in each group of rats.
Notes: Comparison at the same time points within the group: "a" indicates a P <0.05 comparison with the blank group, and "b" indicates a *P* <0.05 comparison with the PSD group; Comparison at different time points within the group: "c" indicates a *P* <0.05 comparison with the PSD after modeling.

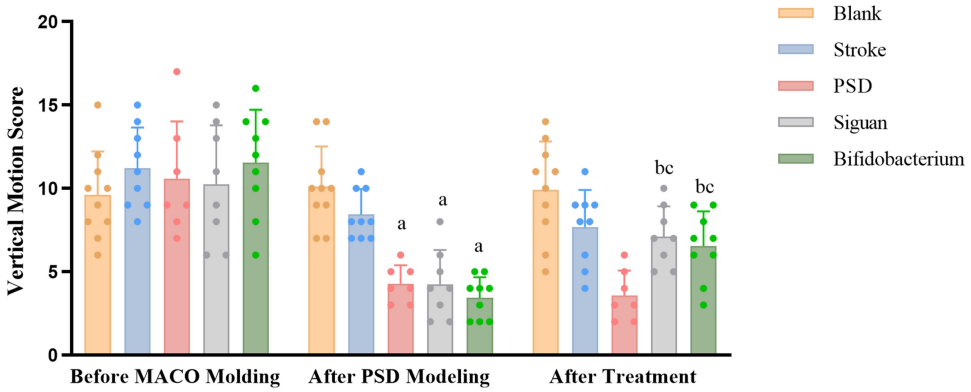

Figure 2 Comparison of vertical movement scores in open field tests before and after modeling and after treatment for rats in each group.
Notes: Comparison at the same time points within the group: "a" indicates a P <0.05 comparison with the blank group, and "b" indicates a *P* <0.05 comparison with the PSD group; Comparison at different time points within the group: "c" indicates a *P* <0.05 comparison with the PSD after modeling.

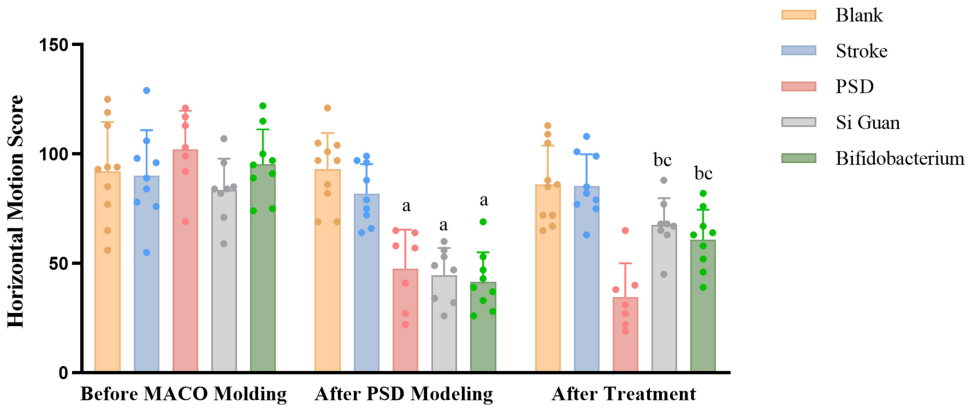

Figure 3 Comparison of horizontal exercise scores in open field tests at various time acupoints before and after modeling and treatment in each group of rats.
Notes: Comparison at the same time points within the group: "a" indicates a P <0.05 comparison with the blank group, and "b" indicates a *P* <0.05 comparison with the PSD group; Comparison at different time points within the group: "c" indicates a *P* <0.05 comparison with the PSD after modeling.

(2) Beta diversity analysis: Principal coordinate analysis (PCoA) and non-metric multidimensional scale analysis (nMDS) analysis were performed to show the differences between the gut microbiota of each group, and then Permutational multivariate analysis of variance (PERMANOVA) was performed to test whether the differences between the groups were significant.

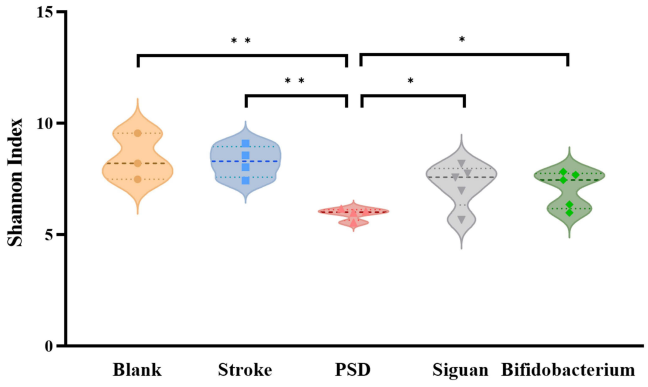

Figure 4 Comparison of Shannon index of gut microbiota of rats in each group.
Note: **indicates a difference of *P* <0.01 between the two groups; * indicates a difference of *P* <0.05 between the two groups.

The results of PCoA analysis showed that the gut microbiota of each group was discrete, and the PSD group was distributed on the rightmost side and was farther away from the blank and the stroke group, suggesting that the gut microbiota of the rats in the PSD group differed from that of the blank and the stroke group, while the Siguan and the Bifidobacterium group were farther away from the PSD group and were close to the blank group, suggesting that the diversity of the gut microbiota of the Siguan and the Bifidobacterium groups was more similar to that of the blank group and differed from that of the PSD group; The stroke group was close to the Siguan, Bifidobacterium and blank groups, suggesting that the diversity of gut microbiota in the stroke group was not significantly different from the Siguan, Bifidobacterium and blank groups. Further, PERMANOVA test was performed and the results showed that R = 0.432, $P < 0.001$, suggesting that the difference between groups was statistically significant (as shown in Figure 5).

The results of nMDS showed that the PSD group was mainly distributed along the first axis, while the structures of the Siguan and the Bifidobacterium group were similar, mainly distributed along the second axis, indicating that the PSD group had certain differences from the Siguan and the Bifidobacterium group. The PSD group changed along the first axis, while the Siguan and the Bifidobacterium group changed along the second axis. Further PERMANOVA test was performed, and the results showed that R = 0.674, $P < 0.001$, suggesting that the differences in the gut microbiota of rats

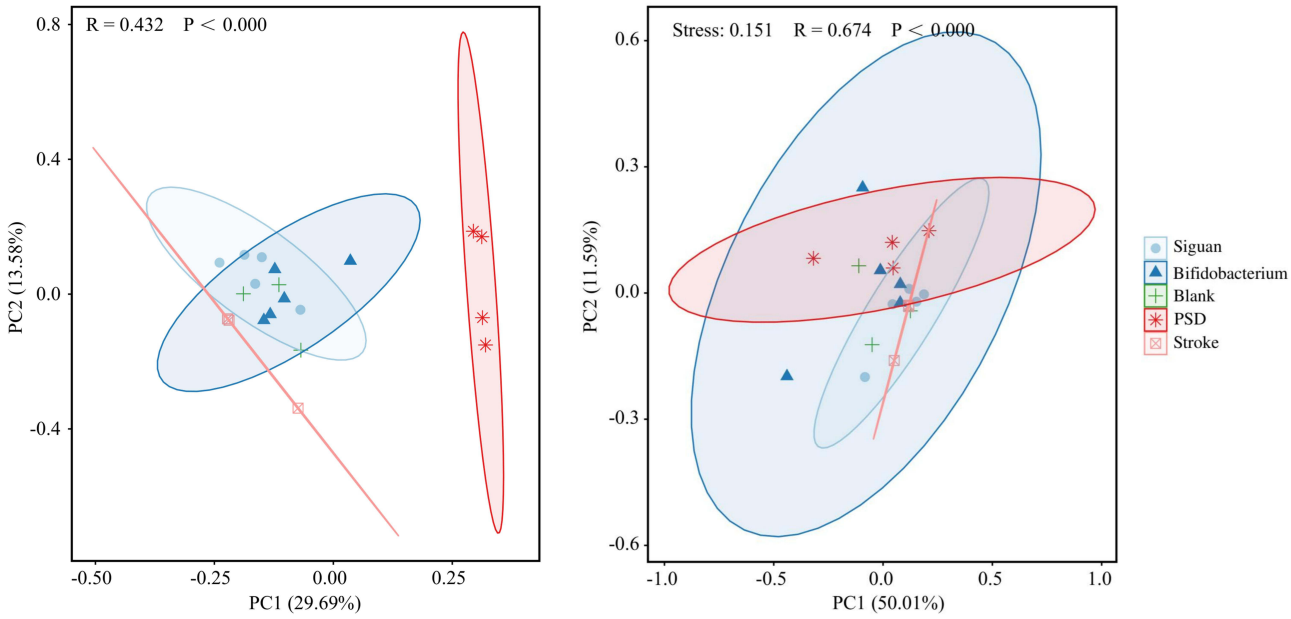

Figure 5 PCoA (left) and nMDS analysis (right) of the gut microbiota of rats in each group.

https://doi.org/10.2147/NDT.S495460

in the PSD group were statistically significant with those of rats in the Siguan and the Bifidobacterium group (as shown in Figure 5).

Effects of Electroacupuncture on the Structure of Gut Microbiota of PSD Rats at the "Siguan" Acupoints

(1) The results of phylum-level analysis showed that Firmicutes and Bacteroidetes were the two most dominant bacterial groups at the phylum level in the intestinal tract of rats in each group, and both of them accounted for more than 90% of the gut microbiota phylum level in rats, followed by Proteobacteria, Spirochaetes and Verrucomicrobia. The relative abundance of Firmicutes in each group was 76% in the blank group, 64% in the stroke group, 36% in the PSD group, 65% in the Siguan group and 57% in the Bifidobacterium group; the relative abundance of Bacteroidetes in each group was 19% in the blank group, 28% in the stroke group, 54% in the PSD group and 29% in the Siguan group, and 36% in the Bifidobacterium group, as shown in Figure 6.

Intergroup difference analysis of Firmicutes and Bacteroidetes in each group showed that compared with the blank group, the abundance of Firmicutes was decreased and the abundance of Bacteroidetes was increased in the stroke group, but the difference was not statistically significant. When compared with the blank group, the PSD group showed a decrease in Firmicutes abundance and an increase in Bacteroidetes abundance ($P < 0.05$). These results indicate that the gut microbiota of the stroke and PSD group rats undergo changes at the phylum level. When the Siguan and Bifidobacterium groups were compared with the PSD group, the abundance of Firmicutes increased and the abundance of Bacteroidetes decreased ($P < 0.05$). Additionally, there was no statistically significant difference between the Siguan and Bifidobacterium groups. All these indicate that, compared to the Siguan and Bifidobacterium groups, the gut microbiota of rats in the PSD group was altered at the phylum level. Electroacupuncture of the "Siguan" acupoints and supplementation of Bifidobacterium regulated the structure of gut microbiota at the phylum level in PSD rats, as shown in Figure 7.

(2) The results of class-level analysis showed that Clostridia and Bacteroidia were the two most dominant groups of gut microbiota at the class level in all groups of rats, and both of them accounted for more than 80% of the gut microbiota at the class level in rats, followed by Bacilli, Deltaproteobacteria and Verrucomicrobia. The relative abundance of Clostridia in each group was 70% in the blank group, 59% in the stroke group, 44% in the PSD group, 70% in the Siguan group and 62% in the Bifidobacterium group; the relative abundance of Bacteroidia in each group was 19% in the blank group, 25% in the stroke group, 38% in the PSD group, 18% in the Siguan group and 22% in the Bifidobacterium group, as shown in Figure 6.

Intergroup difference analysis of Clostridia and Bacteroidia in each group showed that the abundance of Clostridia decreased and the abundance of Bacteroidia increased in the PSD group compared with the blank and stroke groups ($P < 0.05$), suggesting that the gut microbiota of the PSD group was altered at the class level compared with that of the blank and the stroke group. However, compared with that in the PSD group, the abundance of Clostridia increased and the abundance of Bacteroidia decreased in the Siguan and Bifidobacterium groups ($P < 0.05$), suggesting that electroacupuncture of the "Siguan" acupoints and supplementation of Bifidobacterium regulated the structure of gut microbiota at the class level in PSD rats, as shown in Figure 8.

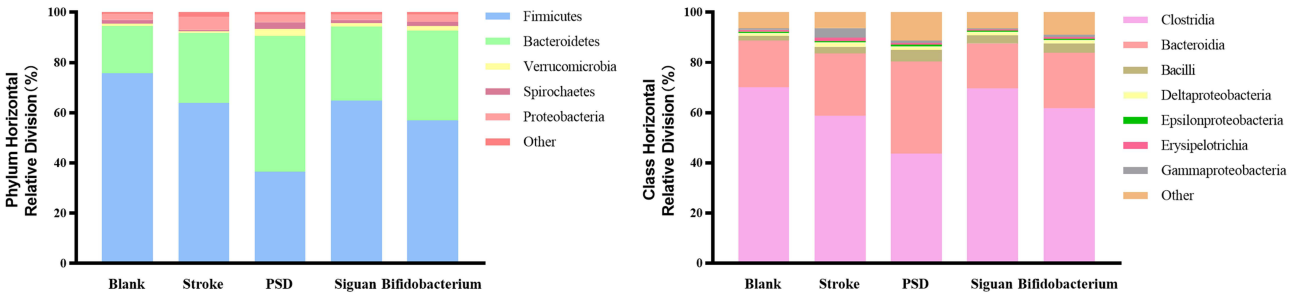

Figure 6 Comparison of relative abundance of gut microbiota in each group at the phylum and class level.

 https://doi.org/10.2147/NDT.S495460

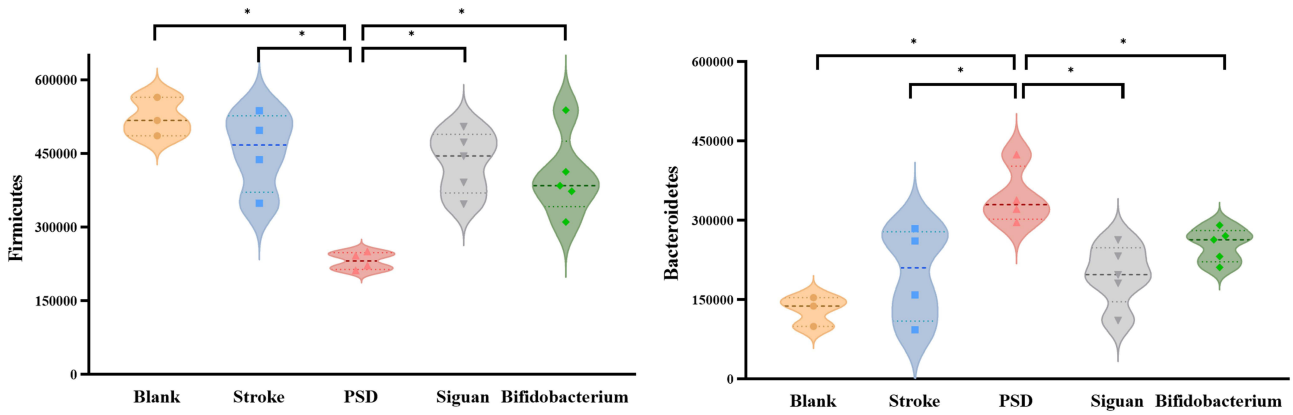

Figure 7 Comparison of Firmicutes and Bacteroidetes among groups at the phylum level.
Note: *indicates a difference of *P* <0.05 between the two groups.

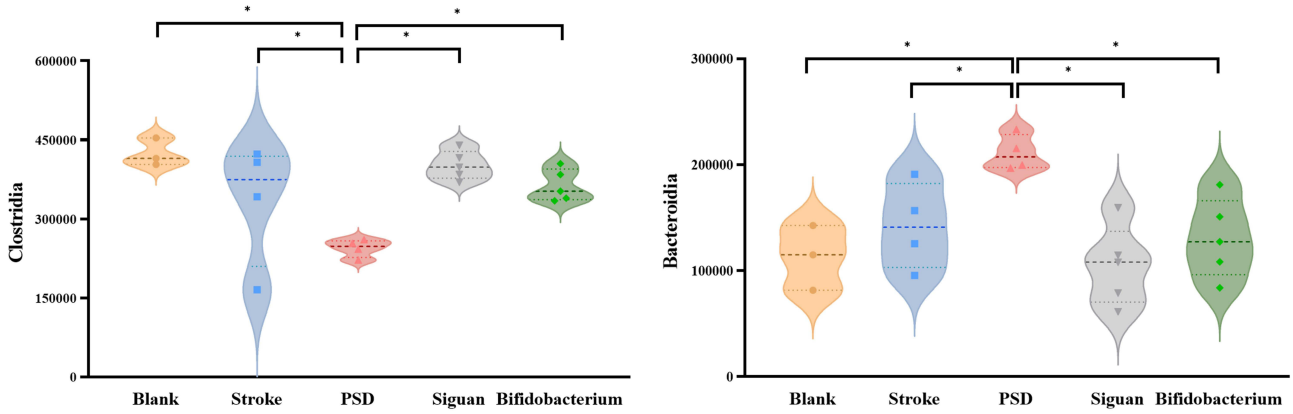

Figure 8 Comparison of Clostridia and Bacteroidia among groups at the class level.
Note: *indicates a difference of *P* <0.05 between the two groups.

Effects of Electroacupuncture on the Function of Gut Microbiota of PSD Rats at the "Siguan" Acupoints

Based on the analysis of KEGG database, the results showed that at Level B, the functions of the gut microbiota in each group of rats were mainly "Genetic Information Processing", "Signaling and Cellular Processes", "Metabolism", "Carbohydrate Metabolism", "Amino Acid Metabolism", etc. Inter-group difference analysis showed that at Level B, compared with that in the PSD group, the relative abundance of the "Metabolism of Cofactors and Vitamins" functional pathway in the Siguan group decreased (P<0.05), as shown in Figure 9.

Discussion

The clinical manifestations of PSD are mainly low mood, retardation, and loss of motor and exploratory ability, etc. There is no specific disease name corresponding to PSD in Chinese medicine, but because it is characterized by both "stroke" and "depression", it belongs to the category of "stroke" and "depression" combined in Chinese medicine. The etiology and pathogenesis of PSD in Chinese medicine are complex and varied, and the current Chinese medicine treatment system for PSD patients is mainly based on the theory of the five viscera and the five deities, such as the "Liver-Spleen Integration", which holds that regulating the spleen and stomach is one of the most important rules for treating emotional and mental illnesses.[19] The Spiritual Pivot-Nine Needles and Twelve Primordial Elements states that the five zang-organs are associated with the six fu-organs, that the six fu-organs are related to the twelve yuan-primary points, that the twelve yuan-primary points originate from the Siguan, and that the Siguan mainly governs the five zang-organs. Incidentally, it was in this text that the term "Siguan" was first introduced. According to the Interpretation of the

https://doi.org/10.2147/NDT.S495460

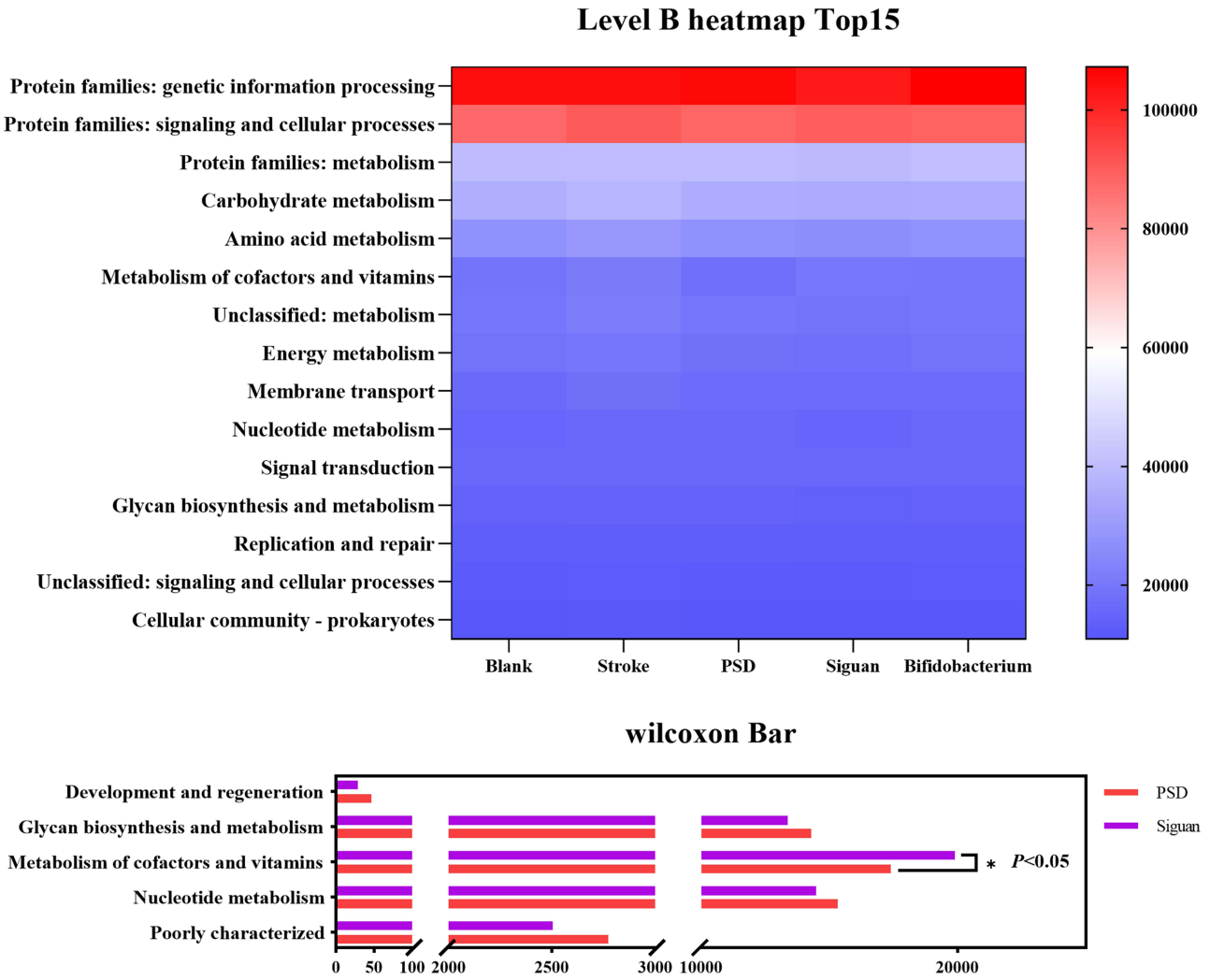

Figure 9 Functional analysis of gut microbiota in each group.

Names of Acupuncture and Moxibustion Points in Gaoshiguo, "Hegu and Taichong are each two acupoints, named Siguan, which are able to open up a lot". So far, "Siguan" has been referred to as LI4 and LR3 on both sides. The "Siguan" acupoints have the effect of harmonizing qi and blood, dredging the liver and relieving depression, and a number of experiments have proved that the "Siguan" acupoints have gained significant therapeutic effects in the treatment of PSD.[13] The possible pathogenesis of PSD in western medicine is mainly neurotransmitter mechanism, immune mechanism, endocrine mechanism, neurotrophic mechanism. According to the theory of MGBA, it is found that the gut microbiota has an important regulatory role in the above four mechanisms.[20,21] Kang Y et al[22] compared PSD patients with those who did not develop depression after stroke and showed increased abundance of conditionally pathogenic bacteria, a decreased abundance of probiotics, and an excessive inflammatory response in PSD patients. Bifidobacterium is an important component of intestinal probiotics, which has been found to secrete neurotransmitters such as tryptophan and dopamine, improving depressive behavior.[23] Therefore, this study set the Bifidobacterium treatment group as the positive control group. Previous studies conducted by our group have confirmed that electro-acupuncture stimulation of the "Siguan" acupoints alleviates the depression-like behavior of PSD rats by promoting the release of neurotransmitters, increasing the synthesis of neurotrophic factors, and promoting the secretion of metabolites from gut microbiota, and that its therapeutic pathway for treating PSD is similar to the pathway of action of gut microbiota. Based on this, in-depth investigation of whether electroacupuncture at the "Siguan" acupoints alleviates post-

stroke depression-like behavior in PSD rats by regulating the gut microbiota will help to clarify the target of electroacupuncture in the treatment of PSD.

The results of this study showed that sugar water consumption, horizontal movement score and vertical movement score of the open field test were reduced in rats after PSD modeling, suggesting that PSD modeling was successful. After 21 days of intervention, sugar and water consumption, vertical movement score and horizontal movement score in the open field test in the Siguan and Bifidobacterium groups were significantly higher than those in the PSD group and those before self-imposed intervention factors. This confirms that electroacupuncture on the "Siguan" acupoints is able to alleviate the depressive symptoms of the PSD rats, which are similar to those of the human in terms of the lack of pleasure, the reduction of horizontal activity and the reduction of exploration, which is in accordance with the results of the previous study. At the same time, this study found that supplementation of Bifidobacterium significantly alleviated the depression-like symptoms of PSD rats, and the efficacy of directly supplementing Bifidobacterium to alleviate the depression-like behaviors of PSD rats was not weaker than the efficacy of electroacupuncture stimulation of the "Siguan" acupoints in PSD rats; however, the present study was only an animal experiment, and whether probiotic supplementation could alleviate the depression-like symptoms of PSD patients needs to be further proved. In addition, this study also found that after the final intervention, the Shannon index was lower in the PSD group than in the blank and stroke groups, and the Shannon index was higher in the Siguan and Bifidobacterium groups than in the PSD group. The results of PCoA and nMDS showed that the gut microbiota diversity in the Siguan and Bifidobacterium groups was similar to that of the blank group and differed from that of the PSD group, which indicated that PSD reduced the diversity of gut microbiota in rats, and that electroacupuncture of the "Siguan" acupoints and supplementation of Bifidobacterium regulate the diversity of gut microbiota of rats with PSD, and make it close to that of normal rats. In line with the results of most studies, the diversity of gut microbiota in PSD rats/patients was lower than normal,[17,22,24] but whether there is a correlation between the degree of depression and the level of gut microbiota diversity in PSD remains controversial. Xuebin Li et al[25] found that the diversity of gut microbiota was higher in patients with post-stroke depression than in both normal subjects and stroke patients. In contrast, Shaojun Zheng et al[26] compared the diversity of gut microbiota in mild depression patients after stroke with that of normal subjects. There was no statistical difference between the two. Currently, the relationship between PSD and the diversity of gut microbiota is mainly controversial in clinical trials. The reasons for these controversies may be related to the differences in the stroke severity, depression level and age group of the PSD patients included in the studies.

In order to further study whether electroacupuncture at the "Siguan" acupoints alleviates the depression-like behavior of PSD rats by regulating the gut microbiota, this experiment compared the structure and function of the gut microbiota in each group and found that compared with the PSD group, the abundance of Firmicutes and Clostridia in the Siguan and the Bifidobacterium group increased, and that the abundance of Bacteroidetes and Bacteroidia decreased. There was no statistically significant difference in the comparison of the Siguan and the Bifidobacterium group. Firmicutes are key to the transport of saccharides and reduce the level of inflammatory factors in mice[27,28] and are typical anti-inflammatory bacteria. Moreover, Clostridia belong to the phylum Thick-walled Bacteria, which facilitates the hydrolysis and transport of synergistic polysaccharides and is one of the major producers of butyric acid;[29] Bacteroidetes, Bacteroidia release lipopolysaccharide and promote the secretion of peripheral and central pro-inflammatory cytokines.[30] Previous studies have reported that excessive neuroinflammatory responses are highly correlated with depression. Excessive neuroinflammatory responses can participate in the occurrence and development of depression through multiple neurobiological mechanisms.[31] Moreover, our research group has previously demonstrated that butyric acid among SCFAs can alleviate the depression-like behaviors of PSD rats.[15] The above indicates that electroacupuncture at the "Siguan" acupoints and supplementation of Bifidobacterium are able to bi-directionally regulate the gut microbiota of PSD rats, that is, up-regulate the intestinal probiotic bacteria and down-regulate the pathogenic bacteria, thereby maintaining the physiological structure of the gut microbiota and alleviating the depression-like behaviors of PSD rats. Existing studies have found a correlation between vitamin D deficiency and PSD, and vitamin D3 supplementation alleviates depression-like behavior in PSD rats, eg, JU Xichi et al[32] found that injecting vitamin D3 into PSD rats can up-regulate the expression of hippocampal vitamin receptors and BDNF, and then alleviate the depressive-like behavior in PSD rats. Compared with the PSD group, the relative abundance of the functional pathway of "Metabolism of Cofactors and Vitamins" in the gut

microbiota in the Siguan group was reduced, suggesting that electroacupuncture to the "Siguan" acupoints reduces the metabolic levels of cofactors and vitamins in PSD rats, which is conducive to the maintenance of vitamin levels in the body, and to the improvement of depressive behaviors in the PSD rats.

Conclusion

In conclusion, electroacupuncture at the "Siguan" acupoints effectively alleviated depression-like behaviors of PSD rats, which may be related to up-regulating intestinal probiotic bacteria, down-regulating pathogenic bacteria, restoring the physiological structure of the gut microbiota and regulating the functions of the gut microbiota. However, the shortcomings of this study are as follows: First, it did not conduct in-depth verification on whether gut microbiota dysbiosis can induce the occurrence of PSD in stroke mice. Second, although the experimental observation indicators focused on depression behavioral indicators, gut microbiota diversity, and structural and functional changes, there has not been in-depth exploration of some potential associated indicators, such as neurotransmitter levels, brain-derived nutritional factor levels, and the dynamic interaction between gut microbiota and its metabolites. This to some extent limits our comprehensive understanding of the deep mechanisms of electroacupuncture treatment for PSD. Third, although the grouping experiment of 50 rats can preliminarily reveal the mechanism of electroacupuncture at the "Four Passes" acupoint on PSD, the sample size is relatively small compared to large-scale clinical studies, and there may be some sampling errors that affect the universality of the results. Moreover, affected by various aspects such as actual conditions, sample size and treatment observation time, it cannot be excluded that there may be differences between the experimental results and the actual curative effect of electroacupuncture at the "Siguan" acupoints. In the future, further exploration can be conducted on the correlation between electroacupuncture at the "Siguan" acupoints for treating PSD and gut microbiota and their metabolites, vitamin levels in the body, and neuroinflammation, providing sufficient experimental evidence for the clinical application of electroacupuncture at the "Siguan" acupoints for treating PSD.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Ethics Approval and Consent to Participate

Ethics Approval All procedures performed in studies were in accordance with the ethical standards of the guide for the care and use of laboratory animals. The study was approved by the Animal Ethics Committee of Hunan University of Traditional Chinese Medicine (no. LL2022022301).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Hunan Provincial Natural Science Foundation General Project (grant number 2021JJ30519, 2022JJ30450), the Clinical Medical Technology Innovation Guidance Project (grant number 2021SK51304), the Scientific Research Fund of Hunan Provincial Education Department (grant number 24A0255), and the Hunan University of Chinese Medicine campus-level project (grant number 2024CX131).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Tu WJ, Wang LD, Special Writing Group of China Stroke Surveillance R. China stroke surveillance report 2021. *Mil Med Res*. 2023;10(1):33. doi:10.1186/s40779-023-00463-x
2. Krick S, Koob JL, Latarnik S, et al. Neuroanatomy of post-stroke depression: the association between symptom clusters and lesion location. *Brain Commun*. 2023;5(5):fcad275. doi:10.1093/braincomms/fcad275
3. Frank D, Gruenbaum BF, Zlotnik A, et al. Pathophysiology and current drug treatments for post-stroke depression: a review. *Int J mol Sci*. 2022;23 (23). doi:10.3390/ijms232315114
4. Ladwig S, Werheid K, Südmeyer M, et al. Predictors of post-stroke depression: validation of established risk factors and introduction of a dynamic perspective in two longitudinal studies. *Front Psychiatry*. 2023;14:1093918. doi:10.3389/fpsyt.2023.1093918
5. Strawn JR, Mills JA, Poweleit EA, et al. Adverse effects of antidepressant medications and their management in children and adolescents. *Pharmacotherapy*. 2023;43(7):675–690. doi:10.1002/phar.2767
6. Ashraf F, Mustafa MS, Shafique MA, et al. Association between depression and stroke risk in adults: a systematic review and meta-analysis. *Front Neurol*. 2024;15:1331300. doi:10.3389/fneur.2024.1331300
7. Kong D, Li Y, Zou W. Efficacy and safety of acupuncture treatment for post-stroke depression: a protocol for systematic review and meta-analysis. *PLoS One*. 2024;19(5):e0300769. doi:10.1371/journal.pone.0300769
8. Doroszkiewicz J, Groblewska M, Mroczko B. The role of gut microbiota and gut-brain interplay in selected diseases of the central nervous system. *Int J mol Sci*. 2021;22(18):10028. doi:10.3390/ijms221810028
9. Xu H, Li L, Zhou X, et al. Research progress of microbiota-gut-brain axis and post-stroke depression. *World Chin Med*. 2023;18(21):3149–3155.
10. Jiang H, Deng S, Zhang J, et al. Acupuncture treatment for post-stroke depression: intestinal microbiota and its role. *Front Neurosci*. 2023;17:1146946. doi:10.3389/fnins.2023.1146946
11. Ye HM, Li ZY, Zhang P, et al. Exploring mechanism of electroacupuncture in modulating neuroinflammation based on intestinal flora and its metabolites. *Chin J Integr Med*. 2024;31(2):183–192. doi:10.1007/s11655-024-3766-9
12. Kang Z, Ye H, Chen T, et al. Effect of electroacupuncture at siguan acupoints on expression of BDNF and TrkB proteins in the hippocampus of post-stroke depression rats. *J Mol Neurosci*. 2021;71(10):2165–2171. doi:10.1007/s12031-021-01844-4
13. Chen T, Ye H, Kang Z, et al. Effects of electroacupuncture of "four gate" point and its corresponding acupoints on behavior and hippocampal BDNF and TrkB protein expression in psd rats. *Modern Trad Chin Med Materia Medica-World Sci Tech*. 2021;23(08):2948–2954.
14. Zhang P, Ye H, Wu Q, et al. Effects of electroacupuncture of "siguan" acupoints and corresponding single point on 5-HT and NE in the hippocampus of PSD rats. *J Traditional Chinese Med Pharm*. 2016;22(08):40–42+45. doi:10.13862/j.cnki.cn43-1446/r.2016.08.012
15. Xu H, Li L, Kang Z, et al. Effects of electroacupuncture at "Siguan" points on the expression of colonic 5-hydroxytryptamine and fecal short-chain fatty acids in rats with post-stroke depression. *Chin AcupuncT Moxibustion*. 2023;43(05):545–551. doi:10.13703/j.0255-2930.20221125-k0002
16. Longa EZ, Weinstein PR, Carlson S, et al. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. 1989;20(1):84–91. doi:10.1161/01.str.20.1.84
17. Jiang W, Gong L, Liu F, et al. Alteration of gut microbiome and correlated lipid metabolism in post-stroke depression. *Front Cell Infect Microbiol*. 2021;11:663967. doi:10.3389/fcimb.2021.663967
18. Huan XH J, Chen Z, Zhen Q, Sun R. Dose conversion among different animals and healthy volunteers in pharmacological study. *Chin J Clin Pharmacol Ther*. 2004;9(09):1069–1072.
19. Zhong X, Teng J, Kang C, et al. Construction of differentiation and treatment system on post-stroke depression under the element of 'Hun and Po' based on the theory of five spirits of traditional Chinese medicine. *China J Tradition Chinese Med Pharm*. 2023;38(10):4653–4656.
20. Zhan Q, Kong F. Mechanisms associated with post-stroke depression and pharmacologic therapy. *Front Neurol*. 2023;14:1274709. doi:10.3389/fneur.2023.1274709
21. Wu SX, Li J, Zhou DD, et al. Possible effects and mechanisms of dietary natural products and nutrients on depression and anxiety: a narrative review. *Antioxidants*. 2022;11(11):2132. doi:10.3390/antiox11112132
22. Kang Y, Yang Y, Wang J, et al. Correlation between intestinal flora and serum inflammatory factors in post-stroke depression in ischemic stroke. *J Coll Physicians Surg Pak*. 2021;31(10):1224–1227. doi:10.29271/jcpsp.2021.10.1224
23. Zhang Y, Xu N, Peng Z, et al. Fecal microbiota transplantation to improve depression by modulating microbiota-gut-brain axis: a review. *Microbiol China*. 2022;49(02):756–768. doi:10.13344/j.microbiol.china.210153
24. Xiong RG, Li J, Cheng J, et al. The role of gut microbiota in anxiety, depression, and other mental disorders as well as the protective effects of dietary components. *Nutrients*. 2023;15(14):3258. doi:10.3390/nu15143258
25. Li X, Han G, Zhao J, et al. Intestinal flora induces depression by mediating the dysregulation of cerebral cortex gene expression and regulating the metabolism of stroke patients. *Front Mol Biosci*. 2022;9:865788. doi:10.3389/fmolb.2022.865788
26. Zheng S, Zhu Y, Wu W, et al. A correlation study of intestinal microflora and first-episode depression in Chinese patients and healthy volunteers. *Brain Behav*. 2021;11(8):e02036. doi:10.1002/brb3.2036
27. Natividad JM, Pinto-Sanchez MI, Galipeau HJ, et al. Ecobiotherapy rich in Firmicutes decreases susceptibility to colitis in a humanized gnotobiotic mouse model. *Inflamm Bowel Dis*. 2015;21(8):1883–1893. doi:10.1097/mib.0000000000000422
28. Bahar-Tokman H, Demirci M, Keskin FE, et al. Firmicutes/bacteroidetes ratio in the gut microbiota and IL-1β, IL-6, IL-8, TLR2, TLR4, TLR5 gene expressions in type 2 diabetes. *Clin Lab*. 2022;68(9). doi:10.7754/Clin.Lab.2022.211244
29. Dalile B, Van Oudenhove L, Vervliet B, et al. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol*. 2019;16(8):461–478. doi:10.1038/s41575-019-0157-3
30. Zhang Y, Fan Q, Hou Y, et al. Bacteroides species differentially modulate depression-like behavior via gut-brain metabolic signaling. *Brain Behav Immun*. 2022;102:11–22. doi:10.1016/j.bbi.2022.02.007
31. Wu A, Zhang J. Neuroinflammation, memory, and depression: new approaches to hippocampal neurogenesis. *J Neuroinflammation*. 2023;20 (1):283. doi:10.1186/s12974-023-02964-x
32. Song X. Correlation between serum 25-hydroxyvitamin D3 and 5-HT levels and post-stroke depression. *Smart Healthcare*. 2021;7(03):10–12. doi:10.19335/j.cnki.2096-1219.2021.3.004