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Neural mechanism and effect of acupuncture for central nervous system diseases, 2nd edition

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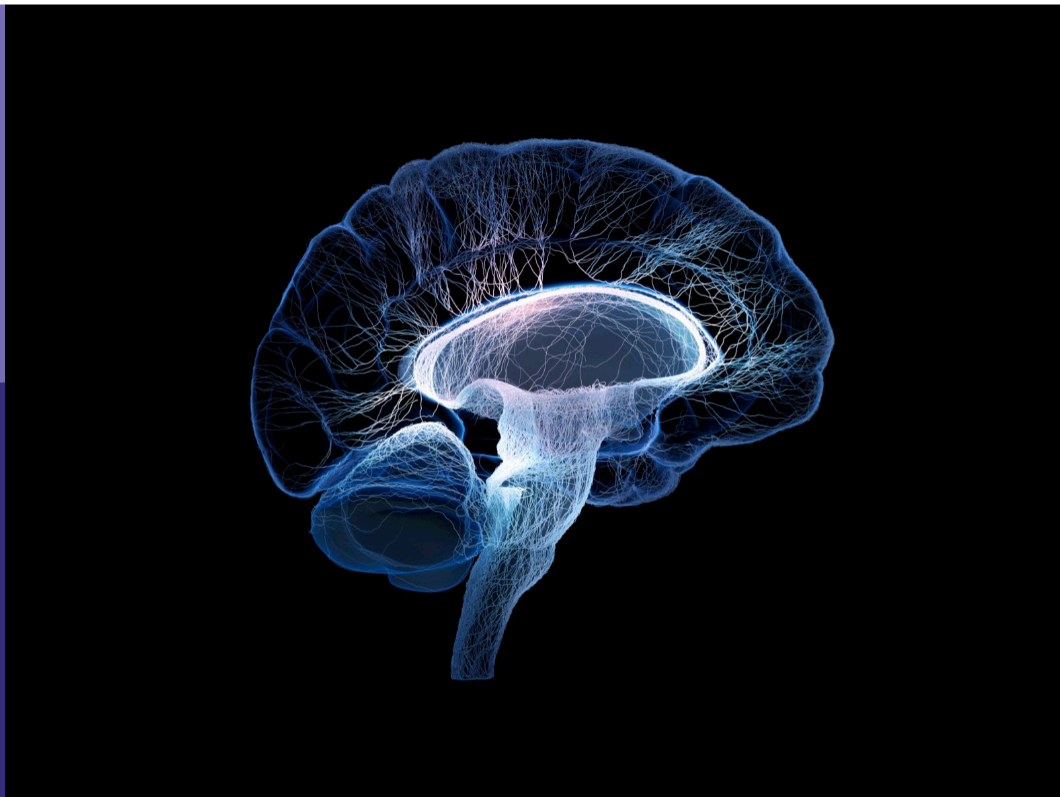

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Neural mechanism and effect of acupuncture for central nervous system diseases, 2nd edition

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Editorial: Neural mechanism and effect of acupuncture for central nervous system diseases

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Editorial on the Research Topic
Neural mechanism and effect of acupuncture for central nervous system diseases

Central nervous system (CNS) diseases encompass a wide range of disorders that affect the brain and spinal cord (Rothwell et al., 2021). These diseases involve complex neural mechanisms, which refer to the intricate workings and interactions of the neurons and other cells in the CNS (Fontes et al., 2016). The CNS controls and coordinates most of the body's functions, including movement, sensation, cognition, and regulation of vital organs. Thus, any disruption in its functioning can have profound consequences. CNS diseases not only pose a significant burden on the affected individuals but also have a considerable impact on their quality of life. Researchers and healthcare professionals strive to better understand the underlying neural mechanisms involved in these diseases, enabling the exploration of novel therapeutic approaches and targeted interventions that can help manage symptoms and enhance patient outcomes.

As for the treatment modality, acupuncture has been used as a therapeutic practice in China and various other Asian countries for over 2,000 years (Bonica, 1974). Over the years, numerous studies have been conducted to explore the benefits of acupuncture in protecting and managing nerve tissue from injuries, particularly for patients suffering from CNS diseases (Chen et al., 2014; Scheffold et al., 2015; Xiao et al., 2018). The results of these studies have consistently shown that acupuncture exhibits very few adverse effects, making it a safe option for patients with CNS diseases. However, despite the increasing attention and research dedicated to acupuncture's neural mechanism and effects, there is still much that remains unexplored. In order to shed further light on the neural mechanism and effect of acupuncture in the treatment of CNS diseases, we launched a Research Topic about "*Neural mechanism and effect of acupuncture for central nervous system diseases*" on October 24, 2022, inviting researchers to contribute their studies, which help understand the intricate neural mechanisms and therapeutic effects of acupuncture in managing CNS diseases. It is also hoped that this Research Topic expands our knowledge in this field and pave the way for more effective and targeted acupuncture treatments.

The Research Topic "*Neural mechanism and effect of acupuncture for central nervous system diseases*," published in Frontiers in Neuroscience, featured 22 articles involving 164 authors from seven countries, presenting significant contributions to our understanding of acupuncture's neural mechanism and effect for CNS diseases. These articles can be summarized into two types: neural mechanism of acupuncture and effect of acupuncture (Table 1).

TABLE 1 Summary of included literature features of acupuncture for CNS.

Study	Literature features
Neural mechanism of acupuncture on CNS	
Zhang et al.	Acupuncture alleviates neuroinflammation by inhibiting the activation of the NLRP3 inflammasome pathway
Liu J.-p. et al.	EA and MA have beneficial effects in reducing hypertension and improving cognitive function
Chen et al.	Acupuncture modulates FC between the LC and IFG, insular gyrus, and SMG
Lu et al.	Acupuncture is found to promote motor recovery after stroke by enhancing overall brain connectivity and optimizing functional interactions
Jiang, Deng et al.	Acupuncture is an effective therapeutic approach with a profound impact on the intestinal microbiota and mental health outcomes
Wang J.-X. et al.	New insights into the use of acupuncture for managing poststroke spasticity
Li Y.-Y. et al.	Acupuncture manipulations activated the corresponding brain regions involving in the anti-hypertensive effect
Yue et al.	Comprehensive investigation to gain insights into the current status, hotspots and trends in MRI of WM in AD
Yang et al.	Current research status of acupuncture for MCI
Jin et al.	Impact of scalp acupuncture on CBF and its role in improving symptoms related to brain disorders
Lee and kim	Acupuncture has potential to regulate neurological processes associated with sleep
Fu and Wang	Acupuncture may promote neuroprotection and enhance recovery after stroke
Effect of acupuncture on CNS	
Lei et al.	Acupuncture with a specific dose is associated with better therapeutic effects
Li Y.-J. et al.	Acupuncture manipulations can effectively lower blood pressure
Jiang, Zhang et al.	A case report shows that acupuncture may benefit neurological sequelae of electric shock
Fernández-Hernando et al.	Non-invasive techniques showed some positive effects on the treatment of migraines
Pu et al.	Acupuncture and traditional Chinese medical herbs may benefit for TD in children
Li L. et al.	Acupuncture has a positive influence on the short-term recovery of consciousness and long-term outcomes in acute phrase of ICH
Liu Y. et al.	Fire needle acupuncture has positive effects and significant benefits in managing autoimmune disorder
Wang Y. et al.	Cheek acupuncture has an immediate analgesic effect on severe neuralgia associated with peripheral nerve compression or permanent damage/dysfunction of CNS
Shi et al.	Acupuncture benefits episodic migraine for at least three months after the completion of the treatment

Neural mechanism of acupuncture on CNS

Acupuncture alleviates neuroinflammation by inhibiting activation of NLRP3 inflammasome

The research conducted by Zhang et al. has found that the activation of NLRP3 inflammasome plays a significant role in the development of CNS. Specifically, the activation of NLRP3 inflammasome exacerbates neuroinflammation and contributes to the progression of CNS diseases. However, the study also suggests that inhibiting the activation of NLRP3 inflammasome could potentially be a key therapeutic target for the treatment of CNS diseases. Furthermore, the research findings indicate that acupuncture may have a beneficial effect on CNS diseases by alleviating neuroinflammation. The mechanism behind this is attributed to the inhibition of the NLRP3 inflammasome pathway by acupuncture. By suppressing the activation of NLRP3 inflammasome, acupuncture interventions demonstrate the potential to improve the progression of CNS diseases by reducing neuroinflammation.

Acupuncture for hypertension reduction and cognitive function improvement

According to the study conducted by Liu J.-p. et al. electroacupuncture (EA) and manual acupuncture (MA) have been found to have beneficial effects in reducing hypertension and improving cognitive function. These acupuncture techniques were shown to enhance the functional activities in the specific brain regions associated with these conditions. Moreover, the study found that EA treatment resulted in greater activation of additional brain regions and improved functional connectivity compared to MA treatment.

Potential mechanism of acupuncture for insomnia

According to Chen et al.'s research, they reported that acupuncture has the ability to regulate the functional connectivity between the locus coeruleus and various regions within the brain such as the inferior frontal gyrus, insular gyrus, and supramarginal gyrus. This finding suggests that acupuncture may have a potential mechanism for the treatment of insomnia. This modulation of functional connectivity (FC) implies that acupuncture could influence the neural pathways and circuits involved in sleep regulation, leading to improvements in sleep quality and duration. By targeting specific brain regions associated with sleep and wakefulness, acupuncture may help restore the balance and proper functioning of the sleep-wake cycle, thereby offering a potential therapeutic approach for individuals suffering from insomnia.

Neuroimaging mechanisms of acupuncture for motor recovery after stroke

Lu et al. conducted a study where they utilized machine learning to predict the classification of minimal clinically important differences (MCID) for motor improvement after stroke. They aimed to explore the mechanisms underlying brain functional reorganization and the effects of acupuncture on motor recovery. The researchers found that machine learning algorithms were able to identify specific regions of interest (ROIs) in the brain that could accurately predict the extent of motor improvement in stroke patients. Furthermore, they observed that the FC between these ROIs and other brain regions was significantly decreased in stroke patients. The study also revealed that acupuncture treatment could effectively modulate the bilateral cerebral hemispheres and restore abnormal FC in stroke patients. This modulation occurred via different targets within the brain. As a result, acupuncture was found to promote motor recovery after stroke by enhancing overall brain connectivity and optimizing functional interactions.

Acupuncture for alterations in gut microbiota

In their review article, Jiang, Deng et al. extensively analyzed the studies that investigated the alterations in the gut microbiota following acupuncture therapy. They meticulously examined and addressed the current challenges and potential advancements in the fields of acupuncture, microbiome, and poststroke depression. Through their comprehensive analysis, Jiang, Deng et al. intended to create a foundation for future investigations, facilitating the advancement of acupuncture as an effective therapeutic approach with a profound impact on the intestinal microbiota and mental health outcomes.

New insights of acupuncture for poststroke spasticity

Based on current evidence from both clinical studies and laboratory research, Wang J.-X. et al. explored new insights into the use of acupuncture for managing poststroke spasticity (PSS), with a particular focus on the antispastic needling technique. They suggest that this technique may have potential effects on both CNS modulations and peripheral adjustments, which could contribute to the management of PSS. The authors also highlight the need for further research in several areas. First, they emphasize the importance of determining the optimal timing and duration of acupuncture intervention for PSS management. They believe that a better understanding of the ideal treatment course can lead to improved outcomes for stroke patients. Additionally, the authors call for more research into the specific application of acupuncture techniques for PSS. They suggest that different needling methods and manipulation techniques may produce varying effects on spasticity, and exploring these possibilities could contribute to the optimization of the antispastic acupuncture regimen. Furthermore, the authors propose investigating different acupoint selection strategies for PSS treatment. They believe that identifying the most effective acupoints for targeting spasticity can enhance the overall efficacy of acupuncture therapy in stroke patients. The authors also emphasize the importance of identifying predictive and aggravating factors of PSS. They argue that a better understanding of these factors can help tailor acupuncture interventions to individual patients and improve treatment outcomes. Lastly, the authors highlight the need for more rigorous study designs and valid objective assessments for spasticity in future research on acupuncture for PSS. They suggest that these improvements can enhance the reliability and validity of study findings and contribute to the overall advancement of acupuncture as a treatment modality for PSS.

Acupuncture manipulations on blood pressure and brain function

In their study, Li Y.-Y. et al. conducted experiments on spontaneously hypertensive rats to observe the effects of acupuncture manipulations on blood pressure and brain function. They aimed to elucidate the central mechanism behind the anti-hypertensive effect of these manipulations. The researchers found that acupuncture manipulations were able to achieve a hypotensive effect in the spontaneously hypertensive rats. Specifically, they noted that the twirling reducing manipulation had a significantly better hypotensive effect compared to the twirling uniform reinforcing-reducing and twirling reinforcing manipulations. To understand the central mechanism behind these effects, the researchers investigated the activation of brain regions associated with blood pressure regulation and the functional connections between them. They found that the twirling reinforcing and reducing manipulation activated these brain regions, suggesting its involvement in the anti-hypertensive effect. Interestingly, the study also revealed that brain regions involved in motor control, cognition, and hearing were activated during the acupuncture manipulations. This observation led the researchers to hypothesize that the activation of these brain regions may have additional benefits in preventing or mitigating the onset and progression of hypertensive brain damage.

Current status, hotspots and trends on magnetic resonance imaging of white matter in Alzheimer's disease

Yue et al. conducted a comprehensive investigation to gain insights into the current state of research, key areas of focus, and emerging trends in MRI of WM in patients with AD. Their study extensively reviewed existing publications on the topic, shedding light on the current understanding of WM abnormalities in AD and highlighting the significant areas of research. The findings of this study not only shed light on the current state of research but also identified the key areas of focus and hotspots within the field of MRI of WM in AD. Furthermore, the study's analysis also revealed the frontier trends in the field of MRI of WM in AD. These emerging trends suggest exciting new possibilities and avenues for future research, offering potential breakthroughs and advancements in the diagnosis, treatment, and understanding of AD. By identifying these frontier areas, this study opens up new possibilities for researchers to explore novel approaches, invent cutting-edge techniques, and develop innovative interventions that could ultimately lead to more effective management and treatment of AD.

Bibliometric analysis of acupuncture for mild cognitive impairment

The study conducted by Yang et al. utilized bibliometric methods to analyze the current research status of acupuncture in the treatment of MCI in great detail. By exploring the current research hotspots and predicting future trends, the study found that the popularity of acupuncture for MCI is steadily increasing with each passing year. The effectiveness of acupuncture for MCI is further enhanced when combined with cognitive training, as it has been found to significantly improve cognitive function. This study also investigated the role of inflammation in MCI and explored how acupuncture can effectively address this issue.

Impact of scalp acupuncture on cerebral blood flow

In their study, Jin et al. delved into an innovative approach to understand how scalp acupuncture effectively treats brain diseases. They focused on investigating the impact of scalp acupuncture on CBF and its role in improving symptoms related to brain disorders. Through extensive research and analysis, they discovered that the stimulation of specific scalp acupuncture points, targeted areas, or nerves innervating the scalp resulted in a remarkable increase in CBF.

Mechanism of acupuncture for sleep disorders

In their study, Lee and Kim conducted an investigation to explore the underlying mechanism of acupuncture's effectiveness in treating sleep disorders using a rodent model. The researchers found that sleep disorders were closely associated to various brain regions and neurotransmitters. They found that acupuncture had the potential to regulate neurological processes associated with sleep, such as the catecholamine and BDNF signaling pathways.

Acupuncture-induced endogenous neuroprotective mechanisms of poststroke

The study by Fu and Wang provides a comprehensive understanding of the potential acupuncture-induced endogenous neuroprotective mechanisms by critically examining a wide range of experimental evidence on the preventive and therapeutic effects that acupuncture exerts on cerebral ischemic stroke. By analyzing various studies and experimental findings, this review sheds light on the specific mechanisms through which acupuncture may promote neuroprotection and enhance recovery after stroke.

Effect of acupuncture on CNS

Association between acupuncture sessions and its effects on motor function of Parkinson's disease

In their study, Lei et al. aimed to investigate the relationship between acupuncture sessions and the impact on motor function in individuals with PD. The researchers discovered that for patients with motor symptoms of PD, the effectiveness of acupuncture treatment may be influenced by the dosage administered. The study revealed that acupuncture with a specific dose was associated with better therapeutic effects. However, it is important to note that excessive acupuncture stimulation could lead to the development of tolerance within the body.

Acupuncture manipulations impact blood pressure

The study conducted by Li Y.-J. et al. showed that acupuncture manipulations can effectively lower blood pressure. Among the different acupuncture techniques tested, it was found that twirling reducing manipulation had a stronger hypotensive effect on spontaneously hypertensive rats compared to twirling uniform reinforcing reducing and twirling reinforcing manipulations. Further investigations revealed that the anti-hypertensive effect of twirling reinforcing and reducing manipulation is likely associated with the activation of specific brain regions that play a role in regulating blood pressure. These brain regions are functionally connected and contribute to the overall hypotensive response. Interestingly, the activation of brain regions involved in motor control, cognition, and hearing was also observed during the acupuncture treatment. This suggests that in addition to regulating blood pressure, acupuncture may exert benefits on other aspects of brain function.

Acupuncture for neurological sequelae of electric shock

Based on the information provided by Jiang, Zhang et al. a case study was conducted on a 29-year-old patient who had been receiving MA (most likely referring to medical assistance) for

17 months. The study focused on the patient's thalamencephalic and mesencephalic injury, which were found to be secondary to electrical trauma. The results of the study revealed that the patient's self-care ability and overall quality of life had experienced significant improvements. It can be inferred that the implementation of MA played a crucial role in enhancing the patient's ability to take care of themselves and led to a considerable enhancement in their overall wellbeing.

Non-invasive techniques for migraines

In their study, Fernández-Hernando et al. examined the effectiveness of non-invasive neuromodulation techniques, specifically auricular transcutaneous vagus nerve stimulation and electro-ear acupuncture, in individuals suffering from migraine headaches. The researchers found that these non-invasive techniques showed some positive effects on the treatment of migraines, as reported in the current literature. However, it is important to note that the available data is still insufficient to draw definitive and strong conclusions regarding the effectiveness of these neuromodulation methods for migraine management.

Acupuncture and Chinese medical herbs for tic disorders in children

Pu et al. conducted a thorough investigation that aimed to assess the quality and effectiveness of acupuncture as a treatment for TD in children. They analyzed and synthesized the data from several randomized controlled trials that have been published on this topic, with a keen focus on producing reliable evidence-based medical evidence. The study revealed that both acupuncture and traditional Chinese medical herbs have shown promising results in improving tic disorders in children. In fact, the researchers strongly suggested that these interventions may be the most effective therapies available for the management of TD in this specific population. Furthermore, when comparing the outcomes of acupuncture and traditional Chinese medical herbs with the commonly used Western medicine in clinical practice, the results clearly indicate that acupuncture, especially when combined with *tuina* therapy (a form of Chinese therapeutic massage), displayed superior effects in alleviating tic disorders in children.

Acupuncture on prognosis and levels of brain-derived neurotrophic factor in acute phase of intracerebral hemorrhage

Li L. et al. conducted a research study aiming to explore the impact of acupuncture on the prognosis and levels of BDNF in patients experiencing ICH during the acute phase. The results of this study indicated that acupuncture, when administered in the acute phase of ICH, has a noteworthy positive influence on the short-term recovery of consciousness as well as long-term outcomes in patients with ICH. Furthermore, the production of BDNF may have a correlation with this beneficial effect observed through acupuncture treatment.

Fire needle acupuncture for autoimmune encephalitis

In their study, Liu Y. et al. applied a technique called fire needle acupuncture to treat patients with autoimmune encephalitis, a condition that affects the central nervous system. The results of the study showed promising outcomes, indicating that the use of fire needle acupuncture led to positive effects and significant benefits in managing this autoimmune disorder. However, it is important to note that more convincing and robust data are still needed to further support the effectiveness of fire needle acupuncture as a treatment method for autoimmune encephalitis.

Cheek acupuncture for severe neuralgia associated with peripheral nerve compression or permanent damage/dysfunction of the CNS

Wang Y. et al. reported two cases of patients who had symptoms of severe neuralgia associated with peripheral nerve compression or permanent damage/dysfunction of the CNS, and they introduced a cheek acupuncture. It can alleviate severe pain in patients with either peripheral nerve compression or permanent damage/dysfunction to the CNS, when analgesic medicines prove to be ineffective. The researchers observed an immediate and accurate analgesic effect in both cases after applying cheek acupuncture.

Acupuncture for episodic migraine

Shi et al. sought to examine the long-lasting impact of acupuncture as a treatment for episodic migraine. The researchers found compelling evidence suggesting that the beneficial effects of acupuncture can persist for at least 3 months after the completion of the treatment. This means that individuals who received acupuncture experienced a significant reduction in the frequency and intensity of their migraine attacks for an extended period.

In summary, this Research Topic focuses on the exploration of noteworthy findings in the field of acupuncture from researchers around the globe. These findings specifically revolve around the utilization of acupuncture as a potential treatment for diseases associated with the CNS. This research comprehensively analyzed the profound impact of acupuncture on these CNS-related diseases. The researchers gained a thorough understanding of the therapeutic mechanisms through which acupuncture works, and subsequently unravel the intricate neural mechanisms that contribute to its overall effectiveness. Ultimately, the findings from this research may lay the groundwork for a theoretical framework, which can greatly contribute to enhancing the outcomes of acupuncture treatments for CNS diseases in clinical settings. This theoretical foundation may help equip researchers and healthcare professionals with valuable insights and knowledge that can further optimize the application of acupuncture in treating various CNS diseases, thus significantly improving the overall quality of patient care and treatment outcomes.

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References

Bonica, J. J. (1974). Acupuncture anesthesia in the People's Republic of China: implications for American medicine. *JAMA*. 229, 1317–1325. doi: 10.1001/jama.1974.03230480033025

Chen, J., Wang, J., Huang, Y., Lai, X., Tang, C., Yang, J., et al. (2014). Modulatory effect of acupuncture at Waiguan (TE5) on the functional connectivity of the central nervous system of patients with ischemic stroke in the left basal ganglia. *PLoS ONE* 9:e96777. doi: 10.1371/journal.pone.0096777

Fontes, R., Ribeiro, J., Gupta, D. S., Machado, D., Lopes-Júnior, F., Magalhães, F., et al. (2016). Time perception mechanisms at central nervous system. *Neurol. Int.* 8:5939. doi: 10.4081/ni.2016.5939

Rothwell, J., Antal, A., Burke, D., Carlsen, A., Georgiev, D., Jahanshahi, M., et al. (2021). Central nervous system physiology. *Clin. Neurophysiol.* 132, 3043–3083. doi: 10.1016/j.clinph.2021.09.013

Scheffold, B. E., Hsieh, C. L., and Litscher, G. (2015). Neuroimaging and neuromonitoring effects of electro and manual acupuncture on the central nervous system: a literature review and analysis. *Evid. Based Complement. Alternat. Med.* 2015:641742. doi: 10.1155/2015/641742

Xiao, L. Y., Wang, X. R., Yang, Y., Yang, J. -W., Cao, Y., Ma, S. -M., et al. (2018). Applications of acupuncture therapy in modulating plasticity of central nervous system. *Neuromodulation* 21, 762–776. doi: 10.1111/ner.12724

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Efficacy of acupuncture for the treatment of Parkinson's disease-related constipation (PDC): A randomized controlled trial

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Objective: To evaluate the efficacy of acupuncture in treating Parkinson's disease-related constipation (PDC).

Materials and methods: This was a randomized, controlled trial in which patients, outcome assessors, and statisticians were all blinded. Seventy-eight eligible patients were randomly assigned to either the manual acupuncture (MA) or sham acupuncture (SA) groups and received 12 sessions of treatment over a 4-week period. Following treatment, patients were monitored until the eighth week. The primary outcome was the change in weekly complete spontaneous bowel movements (CSBMs) from baseline after treatment and follow-up. The Constipation Symptom and Efficacy Assessment Scale (CSEAS), the Patient-Assessment of Constipation Quality of Life questionnaire (PAC-QOL), and the Unified Parkinson's Disease Rating Scale (UPDRS) were used as secondary outcomes.

Results: In the intention-to-treat analysis, 78 patients with PDC were included, with 71 completing the 4-week intervention and 4-week follow-up. When compared to the SA group, weekly CSBMs were significantly increased after treatment with the MA group ($P < 0.001$). Weekly CSBMs in the MA group were 3.36 [standard deviation (SD) 1.44] at baseline and increased to 4.62 (SD, 1.84) after treatment (week 4). The SA group's weekly CSBMs were 3.10 (SD, 1.45) at baseline and 3.03 (SD, 1.25) after treatment, with no significant change from baseline. The effect on weekly CSBMs improvement in the MA group lasted through the follow-up period ($P < 0.001$).

Conclusion: Acupuncture was found to be effective and safe in treating PDC in this study, and the treatment effect lasted up to 4 weeks.

Clinical trial registration: http://www.chictr.org.cn/index.aspx, identifier ChiCTR2200059979

KEYWORDS

Parkinson's disease, constipation, acupuncture, sham acupuncture, non-motor symptom, randomized controlled trial

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease with a multifaceted etiology (Bloem et al., 2021). In addition to classical motor features, PD is also characterized by extremely complex non-motor symptoms (NMS) (Armstrong and Okun, 2020). The pathophysiological process of PD includes α- synuclein aggregation in the nigra and striatum, neuroinflammation, and mitochondrial dysfunction. The complexity of these intertwined pathophysiological processes and the resulting heterogeneity of clinical symptoms will require a targeted method (Vijiaratnam et al., 2021). Constipation, an integral part of NMS, has received greater attention in the last 15 years (Mukherjee et al., 2016). Reduced defecation frequency, excretion of hard stools, prolonged defecation, and sensations of incomplete evacuation and bloating are the most common symptoms of Parkinson's disease-related constipation (PDC) (Sung et al., 2014). Based on the Rome III criteria, the estimated prevalence of PDC ranges from 46.83 to 59.6% (Chen et al., 2015), with higher rates reported in Asia (Gan et al., 2018). Current evidence suggests that abnormally folded α-syn aggregates from Lewy bodies and neurites in the enteric nervous system play a role in the pathogenesis of PD (Cersosimo et al., 2013; Mukherjee et al., 2016). Some PD patients experience constipation years before motor symptoms appear (Dogra et al., 2022). With colon motility altered, constipation affects patients at any stage of PD and greatly reduces the efficacy of levodopa, making PD management difficult (Lesser, 2002; Cersosimo and Benarroch, 2008). As PD progresses, the increasing prevalence of constipation negatively impacts the quality of life of patients, who are more susceptible to sleep disturbances and depression (Yu et al., 2018). There is no doubt that constipation is developing into a significant problem in parallel with motor symptoms (Schapira et al., 2017).

Currently, increasing dietary fiber and fluid intake is commonly used to treat PDC (Stern and Davis, 2016). When lifestyle changes are unhelpful, probiotics and prebiotic fiber, macrogol, and lubiprostone may be used, though these have limited evidence for treating PDC (Rossi et al., 2015). Constipation in PD patients is recurrent and refractory, which requires continuous and long-term treatment (Barboza et al., 2015). Despite the fact that different prokinetics and laxatives are commonly used, they might not be the ideal choice for the long-term treatment of PDC because side effects, including cramping and bloating, could occur after continuous use (Mozaffari et al., 2020). In addition, as the condition develops, patients with PD must move to higher doses of anti-Parkinson's drugs to relieve motor symptoms (Chou et al., 2018). With the sheer number of oral medications available, they are more likely to choose non-pharmacological treatments for constipation (Seppi et al., 2019).

According to the American Parkinson's Association (APA), the management of Parkinson's disease is a multi-disciplinary effort that involves not only pharmacological but also non-pharmacological interventions (Cutson et al., 1995; Goldman and Guerra, 2020). Acupuncture is a common non-pharmacological treatment for improving gastrointestinal and neurological symptoms (Kwon et al., 2021; Fan et al., 2022a; Yao et al., 2022). Studies have shown that acupuncture can improve gastrointestinal and neurological symptoms while also relieving emotional symptoms (Wang et al., 2022a; Ying-Jia Li et al., 2022). However, it has also been questioned if this is a result of the placebo effects of acupuncture. Therefore, appropriate blinded and high-quality clinical studies are required to determine whether acupuncture has specific effects in treating PDC. High-quality clinical evidence on the efficacy of acupuncture for PDC is lacking (Zeng and Zhao, 2016). Two previous meta-analyses have discussed the effect of acupuncture on PDC; however, the conclusions of the two studies were inconsistent, mainly due to the significant heterogeneity and small sample size of the existing studies (Cheng, 2017; Li et al., 2022). As a result, we created a randomized, single-blind clinical trial with sham acupuncture (SA) as a control to evaluate the efficacy of manual acupuncture (MA) for PDC.

2. Study design and materials and methods

2.1. Recruitment and ethical review

Between May 2022 and November 2022, the patients with PDC were recruited in the outpatient department of Parkinson's disease at the First Affiliated Hospital of Guangzhou University of Chinese Medicine. All patients signed the informed consent form after being fully informed of the study's purpose and process.

This clinical trial has been approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (Ethics number: K-2022-005) and has been registered in China Clinical Trial Center (ChiCTR2200059979).

2.2. Study design and patients

The total trial period of this randomized, single-blind clinical trial was 9 weeks, consisting of a baseline phase (−1 to 0 week), an intervention phase (1 to 4 weeks), and a follow-up phase (4 to 8 weeks). We used the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) and the Consolidated Standards of Reporting Trials (CONSORT) statements as study guidelines (MacPherson et al., 2010).

The enrolled patients met the following inclusion and exclusion criteria after being evaluated by an experienced physician. Inclusion criteria included a diagnosis of PD (according to the Movement Disorder Society's revised clinical diagnostic criteria for Parkinson's disease in 2015) (Postuma et al., 2015) and functional constipation (according to the Rome IV diagnostic criteria) (Palsson et al., 2016), age between 35 and 80 years, Hoehn-Yahr grade ≤ 3, no medications taken within 2 weeks that may affect gastrointestinal function (such as prucalopride and probiotics), no participation in other clinical trials within 1 month, voluntary engagement in this study, ability to sign the informed consent, and cooperation in the completion of the bowel diary and scale filling. Exclusion criteria included organic lesions of the digestive system (such as intestinal adhesions, obstructions, tumors, or malformations in the gastrointestinal tract), a history of abdominal or anorectal surgery that may affect intestinal transit, systemic diseases that may affect the dynamics of the digestive tract (such as diabetes and hyperthyroidism), serious life-threatening diseases (such as severe cardiovascular diseases and malignant tumors), skin lesions that were inappropriate for needling, the viscose allergy that prevented acupuncture device attachment, and pregnant or lactating women.

2.3. Randomization and blinding

We used simple randomization for grouping. An independent researcher generated the random number tables by SPSS 26.0 and assigned the patients to the MA and SA groups in a 1:1 ratio according to the random numbers. The uniformly shaped cards with treatment conditions were placed inside the opaque envelopes, which were opened before treatment.

Patients, outcome assessors, and statisticians were all blinded in this study. To prevent communication between two groups, the therapy sessions were scheduled at different times of the day. To assure blindness, patients were asked to wear an eye mask during the treatment. All physicians performed the treatments in accordance with standardized procedures to maintain consistency of study. As for unblinding, patients were informed of their grouping after treatment and received the type of acupuncture after a follow-up.

For control, we used a special acupuncture device that was designed by our team members (patent number ZL202121352221.7) (Wang et al., 2022b). As shown in **Supplementary Figure 2** (**Supplementary material 1**), this acupuncture device consisted of a base and a cannula, classified into two types. In the MA group, the base had a longitudinal opening at the bottom, through which ordinary acupuncture needles could be pierced into the skin. In the SA group, the base had no opening at the bottom, so special flat-head needles could not pierce the skin. The special flat-head needle was designed to be shorter in length so that the exposed needle body length is the same in both groups, ensuring uniformity of appearance throughout operation. Besides, the cannula's angle was made to be flexible to accommodate different needling locations.

2.4. Interventions

Four licensed physicians with more than 3 years of clinical experience performed the treatments. To maintain consistency of operation, all physicians received initial training on the treatment's standardized procedures. Throughout the study, patients in both the MA and SA groups were required to maintain effective therapeutic doses of anti-Parkinson's drugs at levels similar to their baseline. Patients with any dosage adjustment, whether an increase, decrease, or adjustment, were dropped. When patients did not have complete spontaneous bowel movements (CSBMs) for more than three consecutive days or experienced intolerable symptoms such as bloating due to constipation, they were allowed to use emergency medications (including lactulose and glycerin enemas) under medical supervision with detailed documentation. The first bowel movement after each use of emergency medication could not be recorded in the results.

2.4.1. Acupuncture methods

All patients received 12 sessions of treatment, three times a week (Tuesday, Thursday, and Saturday), for a period of 4 weeks. Each session lasted 30 min. All acupoints were selected based on traditional Chinese medicine theory and previous articles on PD and constipation (Liu et al., 2016; Li et al., 2019; Nazarova et al., 2022). The acupoints used by the MA and SA groups included Sishenzhen (four acupoints, consisting of GV21, GV19, and next to GV20 1.5 cun bilateral), GV24 (Shenting), GV29 (Yintang), ST25 (Tianshu), CV4 (Guanyuan), and ST37 (Shangjuxu). All acupoints were taken bilaterally and positioned in accordance with the National Standard of the People's Republic of China (GB/T12346-2006). The location, insertion depth, and direction of the acupoints used in this study were all listed in the **Supplementary Table 3** in **Supplementary material 1**.

The patient was required to put on an eye mask while receiving treatment. The MA group's patients used acupuncture devices with a longitudinal opening at the bottom. In the SA group, sham acupuncture devices without an aperture were employed. Physicians precisely located the acupoints and sterilized the skin around them. Then, in the MA group, physicians pierced disposable sterile acupuncture needles into the skin (0.30 mm × 25 mm, 0.30 mm × 40 mm, Suzhou Tianxie Medical Supplies Co., Ltd., Suzhou, China), and in the SA group, physicians used sterile flat-head acupuncture needles without piercing the skin (0.30 mm × 10 mm, Guangzhou Suixin Medical Supplies Co., Ltd., Guangzhou, China). In both groups, the needles were left in for 30 min before being taken out.

2.5. Outcomes

Age, sex, duration of disease, and equivalent daily dose of levodopa were assessed at baseline. From the beginning of the baseline phase to the end of the follow-up phase, patients were requested to complete an electronic bowel diary, which was supervised by an independent outcome assessor.

2.5.1. Primary outcome

The primary outcome in this study was the number of weekly CSBMs. Weekly CSBMs were collected at baseline (week 0), post-treatment (week 4), and follow-up (week 8), and differences from baseline levels were compared at week 4 and week 8.

2.5.2. Secondary outcomes

Secondary outcomes included the Constipation Symptom and Efficacy Assessment Scale (CSEAS) (including the six dimensions of difficulty, Bristol, time, incompleteness, frequency, and bloating), the Patient-Assessment of Constipation Quality of Life questionnaire (PAC-QOL), and the Unified Parkinson's Disease Rating Scale (UPDRS). These were assessed at baseline, post-treatment, and follow-up.

2.5.3. Safety assessments and evaluation of blinding

Acupuncture-related adverse effects were described in detail. When adverse effects occurred, based on the patient's wishes, the physician assessed whether the patient was still appropriate for continued research involvement.

To assess the effect of blinding, at the end of the study, we asked patients to guess their assignment status and whether they believed the acupuncture needle pierced the skin.

2.6. Data monitoring and analysis

Based on the results of the pilot study we conducted, the change in weekly CSBMs from baseline was 0.964 [standard deviation (SD), 1.726] times in the MA group and −0.321 (SD, 1.310) times in the SA group. According to the calculation of PASS version 15.0, 31 patients would be needed in each group, with a two-sided significance level of 5% and a power of 90%. Given the estimated 20% loss-to-follow-up rate, we planned to enroll 78 patients in the study (39 patients in each group).

An independent statistician used SPSS 26.0 to conduct all analyses. We used the intention-to-treat (ITT) principle, regardless of whether the patients completed all assigned intervention sessions. Continuous variables were expressed as mean (SD) or median (IQR); independent *t*-tests were used for normally distributed values, and Mann-Whitney *U* tests were used for skewed data. Categorical variables were expressed as frequencies and percentages and analyzed using the $\chi 2$ or Fisher exact test, as appropriate. A repeated measures analysis of variance (ANOVA) was used to detect trends in score changes and treatment effects at different time points within groups. Multiple interpolation (MI) methods were used to estimate missing data for the primary and secondary outcomes. To assess the robustness of MI methods under the assumption of randomization, sensitivity analyses of the primary outcome were performed using per-protocol (PP) sets. Differences between groups were reported using 95% confidence intervals and bilateral *P* values, with values less than 0.05 considered significant.

3. Results

In this research, a total of 114 patients were assessed and gave their informed consent between May 2022 and November 2022. Following the exclusion of 36 patients, 78 eligible patients were enrolled and randomly assigned to two groups (39 in the MA group and 39 in the SA group).

Over the course of the treatment, seven patients dropped out [dropout rate 9.0%; MA group: $n = 3$ (7.7%); SA group: $n = 4$ (10.3%)]. The cause of withdrawal was being quarantined at home for epidemic control, a sore sensation caused by a needle, or a change in the medication regimen of an anti-Parkinson's drugs. According to the CONSORT in Figure 1, the execution and dropout details were displayed in a diagram. The features of both groups at the outset were balanced, as shown in Table 1.

3.1. Primary outcome

We used ITT data; Table 2 displayed differences in outcomes between groups, while Table 3 and Figure 2 compared outcomes within groups. Weekly CSBMs in the MA group were 3.36 [standard deviation (SD), 1.44] at baseline and increased to 4.62 (SD, 1.84) after treatment (week 4). The SA group's weekly CSBMs were 3.10

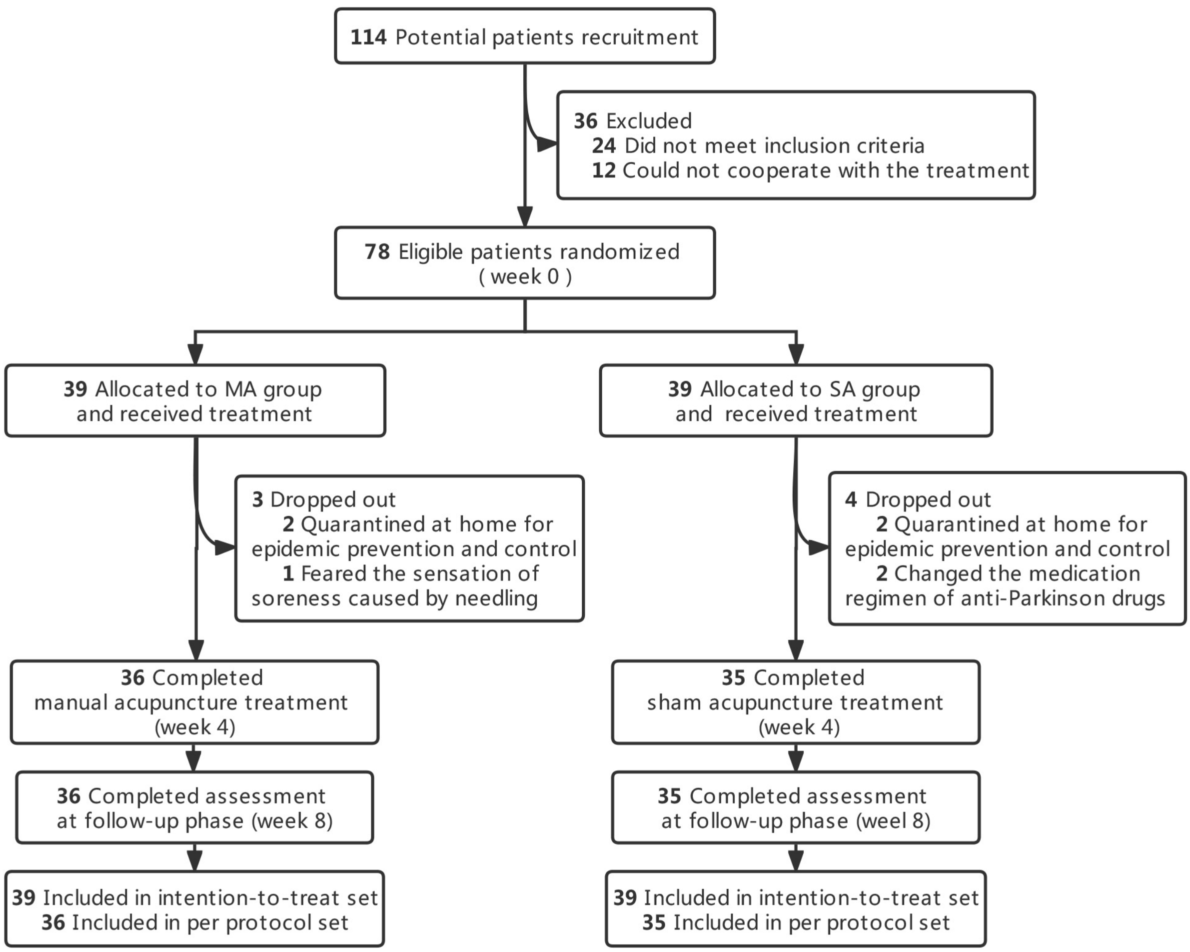

FIGURE 1
The execution and dropout details. MA, manual acupuncture; SA, sham acupuncture.

TABLE 1 Baseline characteristics of included patients.

Characteristic	Total (n = 78)	MA group (n = 39)	SA group (n = 39)
Sex, No. (%)*			
Female	43 (55.10)	22 (56.41)	21 (53.85)
Male	35 (44.90)	17 (43.59)	18 (46.15)
Age, mean (SD), y*	63.82 (8.29)	63.90 (7.34)	63.74 (9.24)
Duration of PD, mean (SD), y*	5.90 (4.14)	5.74 (3.95)	6.05 (4.37)
Duration of constipation, mean (SD), y*	9.62 (7.15)	9.84 (7.35)	9.40 (7.03)
Constipation appears earlier than motor symptoms, No. (%)*			
Yes	58 (74.36)	28 (71.79)	30 (76.92)
No	20 (25.64)	11 (28.21)	9 (23.08)
Equivalent daily dose of levodopa, mean (SD), mg*	481.01 (280.29)	479.33 (286.75)	482.69 (277.43)
Weekly CSBMs (week 0), mean (SD), times*	3.23 (1.44)	3.36 (1.44)	3.10 (1.45)
CSEAS, mean (SD)*	9.45 (3.27)	9.49 (3.39)	9.41 (3.18)
CSEAS-difficulty, mean (SD)*	2.32 (0.86)	2.33 (0.87)	2.31 (0.86)
CSEAS-bristol, mean (SD)*	1.83 (1.06)	2.03 (1.01)	1.64 (1.09)
CSEAS-time, mean (SD)*	1.49 (1.18)	1.49 (1.17)	1.49 (1.21)
CSEAS-incompleteness, mean (SD)*	1.50 (1.15)	1.46 (1.14)	1.54 (1.16)
CSEAS-frequency, mean (SD)*	1.10 (1.09)	0.95 (1.05)	1.26 (1.12)
CSEAS-bloating, mean (SD)*	1.21 (1.22)	1.23 (1.31)	1.18 (1.14)
PAC-QOL, mean (SD)*	35.87 (14.29)	35.79 (13.91)	35.95 (14.83)
UPDRS, mean (SD)*	42.67 (10.40)	42.49 (11.36)	42.85 (9.50)
UPDRS I, mean (SD)*	3.41 (2.59)	3.36 (2.28)	3.46 (2.89)

*The baseline characteristics of both groups were balanced. MA, manual acupuncture; SA, sham acupuncture; PD, Parkinson's disease; Weekly CSBMs, weekly complete spontaneous bowel movements; CSEAS, Constipation Symptom and Efficacy Assessment Scale; PAC-QOL, Patient-Assessment of Constipation Quality of Life questionnaire; UPDRS, Unified Parkinson's Disease Rating Scale.

(SD, 1.45) at baseline and 3.03 (SD, 1.25) after treatment, with no significant change from baseline. Weekly CSBMs in the MA group increased from baseline by a mean of 1.26 (95% CI, 0.75 to 1.77; $P < 0.001$) at post-treatment; at follow-up, it decreased from post-treatment but still increased by 0.92 (95% CI, 0.51 to 1.34; $P < 0.001$) from baseline. Weekly CSBMs were significantly higher in the MA group compared to the SA group both after treatment and at follow-up, with a difference between groups of 1.59 (95% CI, 0.88 to 2.30; $P < 0.001$) at week 4 and 1.31 (95% CI, 0.69 to 1.93; $P < 0.001$) at week 8. We observed similar results in the PP population, as shown in Supplementary material 2 (Supplementary Table 1).

3.2. Secondary outcomes

Constipation symptom and efficacy assessment scale (CSEAS) was used as a secondary outcome to reflect constipation symptoms. At the end of treatment and follow-up, the decrease of CSEAS scores in the MA group was significantly greater than the decrease in the SA group (CSEAS of posttreatment: between-group difference, −4.77) (95% CI, −5.97 to −3.57; $P < 0.001$); (CSEAS of follow-up: between-group difference, −4.00) (95% CI, −5.27 to −2.74; $P < 0.001$). We compared the symptoms of the six aspects of CSEAS in patients with PDC and discovered that acupuncture was more effective in improving defecation straining (Difficulty), stool properties (Bristol), and prolonged defecation (Time), as shown in Figure 2.

The PAC-QOL and the UPDRS were used to assess patients' quality of life and overall condition with PD. In terms of life improvement, the PAC-QOL score of the MA group was significantly lower than that of the SA group after treatment and at follow-up, with significant differences between groups (PAC-QOL of posttreatment: between-group difference, −7.85) (95% CI, −13.20 to −2.49; $P = 0.005$); (PAC-QOL of follow-up: between-group difference, −7.87) (95% CI, −13.15 to −2.60; $P = 0.004$). In terms of overall condition with PD, we discovered that the comparison of UPDRS scores between the MA and SA groups was not significantly different at both week 4 and week 8 (UPDRS of posttreatment: between-group difference, −3.54) (95% CI, −8.35 to 1.27; $P = 0.147$); (UPDRS of follow-up: between-group difference, −3.69) (95% CI, −8.54 to 1.16; $P = 0.134$). However, there was a significant difference in UPDRS scores in the MA group after treatment and at follow-up compared to baseline, with a change of −3.97 (95% CI, −5.83 to −2.12; $P < 0.001$) at post-treatment and −3.51 (95% CI, −5.14 to −1.89; $P < 0.001$) at follow-up.

3.3. Evaluation of safety and blinding

During the trial, six patients (7.6%) in the MA group experienced at least one adverse event, while none in the SA group did. There were no serious adverse events in either group. Supplementary Table 2 of the Supplementary material 2 depicted details of adverse events. When assessing the effectiveness of the blinded treatment at the end of the study, we discovered no discernible difference in patients' ability to correctly guess the assignment status between the MA and SA groups ($p = 0.276$). Blinding assessments is depicted in Supplementary Table 3 of Supplementary material 2.

4. Discussion

To our knowledge, this is the first randomized controlled trial to evaluate the efficacy of acupuncture for the treatment of PDC. We found that manual acupuncture was more effective than sham acupuncture at alleviating constipation symptoms and enhancing quality of life in PD patients after a 4-week course of treatment. The therapeutic effect was sustained throughout the follow-up.

Prolonged colonic transit time was described as one of the main mechanisms of PDC (Zhang et al., 2021; Safarpour et al., 2022). It has been reported that acupuncture can promote gastrointestinal motility by stimulating the distal colon through parasympathetic activation (Li et al., 2007). Weekly CSBMs were the most direct tool for evaluating colon transit in constipation. Previous research showed that manual acupuncture improves weekly CSBMs in patients with chronic constipation more effectively than sham acupuncture (Zhang et al., 2020). The PDC patients in our study had similar positive outcomes. The minimal clinically important difference (MCID) in weekly CSBMs is an increase of 1 unit, which is considered clinically

TABLE 2 Comparison of the primary and secondary outcomes in the manual acupuncture (MA) and sham acupuncture (SA) groups.

Outcome assessments	MA group (*n* = 39)	SA group (*n* = 39)	Difference (95% CI)	*P*
Weekly CSBMs, mean (SD)				
Posttreatment (week 4)	4.62 (1.84)	3.03 (1.25)	1.59 (0.88 to 2.30)	<0.001
Follow-up (week 8)	4.28 (1.47)	2.97 (1.29)	1.31 (0.69 to 1.93)	<0.001
CSEAS, mean (SD)				
Posttreatment	4.31 (2.58)	9.08 (2.76)	−4.77 (−5.97 to −3.57)	<0.001
Follow-up	5.15 (2.63)	9.15 (2.97)	−4.00 (−5.27 to −2.74)	<0.001
CSEAS-difficulty, mean (SD)				
Posttreatment	1.41 (0.88)	2.08 (1.01)	−0.67 (−1.09 to −0.24)	0.003
Follow-up	1.36 (0.84)	2.13 (0.95)	−0.77 (−1.17 to −0.36)	<0.001
CSEAS-bristol, mean (SD)				
Posttreatment	0.54 (0.68)	1.49 (1.02)	−0.95 (−1.34 to −0.56)	<0.001
Follow-up	0.85 (0.84)	1.67 (1.01)	−0.82 (−1.24 to −0.40)	<0.001
CSEAS-time, mean (SD)				
Posttreatment	0.64 (0.78)	1.54 (1.19)	−0.90 (−1.35 to −0.44)	<0.001
Follow-up	0.79 (0.89)	1.44 (1.14)	−0.64 (−1.10 to −0.18)	0.007
CSEAS-incompleteness, mean (SD)				
Posttreatment	0.69 (0.92)	1.54 (1.21)	−0.85 (−1.33 to −0.36)	0.001
Follow-up	0.85 (1.07)	1.36 (1.18)	−0.51 (−1.02 to −0.06)	0.048
CSEAS-frequency, mean (SD)				
Posttreatment	0.28 (0.56)	1.18 (0.97)	−0.90 (−1.26 to −0.54)	<0.001
Follow-up	0.59 (0.85)	1.26 (0.94)	−0.67 (−1.07 to −0.26)	0.002
CSEAS-bloating, mean (SD)				
Posttreatment	0.74 (0.88)	1.26 (1.19)	−0.51 (−0.98 to −0.42)	0.033
Follow-up	0.72 (0.86)	1.31 (1.20)	−0.59 (−1.06 to −0.12)	0.014
PAC-QOL, mean (SD)				
Posttreatment	27.46 (10.71)	35.31 (12.93)	−7.85 (−13.20 to −2.49)	0.005
Follow-up	28.00 (10.63)	35.87 (12.67)	−7.87 (−13.15 to −2.60)	0.004
UPDRS, mean (SD)				
Posttreatment	38.51 (12.17)	42.05 (8.91)	−3.54 (−8.35 to 1.27)	0.147
Follow-up	38.97 (12.36)	42.67 (8.87)	−3.69 (−8.54 to 1.16)	0.134
UPDRS I, mean (SD)				
Posttreatment	1.95 (1.83)	2.46 (1.65)	−0.51 (−1.30 to 0.27)	0.198
Follow-up	2.21 (1.56)	2.38 (1.74)	−0.18 (−0.92 to 0.57)	0.633

MA, manual acupuncture; SA, sham acupuncture; Weekly CSBMs, weekly complete spontaneous bowel movements; CSEAS, Constipation Symptom and Efficacy Assessment Scale; PAC-QOL, Patient-Assessment of Constipation Quality of Life questionnaire; UPDRS, Unified Parkinson's Disease Rating Scale.

significant for the relief of constipation (Liu et al., 2016). In this study, the change from baseline in weekly CSBMs in the MA group was 1.26 (95% CI, 0.75 to 1.77) after treatment, meeting the MCID. However, at follow-up, the increase from baseline in weekly CSBMs in the MA group was 0.92 (95% CI, 0.75 to 1.77), which did not meet the MCID. That means that during follow-up, although there was a statistically significant increase in CSMBs in the MA group, there was not a clinically significant difference. It is inconsistent with previous studies in functional constipation (Liu et al., 2016), which indicated that there were clinically significant differences during follow-up. This difference shows that due to the complex pathogenesis of PDC, it is more likely to recur than common functional constipation, which requires extended cycles of treatment to maintain a long-term therapeutic effect.

A study investigating the frequency of gastrointestinal symptoms in patients with PD showed that they were consistently plagued by defecation straining, hard stools, prolonged defecation, the sensation of incomplete evacuation, and bloating (Sung et al., 2014). In this study, we used the CSEAS with six different dimensions to measure the improvement of these symptoms in PDC. The results showed that patients in the MA group had better improvement in the above-mentioned gastrointestinal symptoms than those in the SA group during both the treatment and follow-up periods. Previous clinical trials reported similar results of acupuncture in treating

TABLE 3 Comparison of the primary and secondary outcomes from the baseline.

Variable	Mean change from baseline (95% CI) MA group (*n* = 39)	*P*	Mean change from baseline (95% CI) SA group (*n* = 39)	*P*
Weekly CSBMs				
Posttreatment (week 4)	1.26 (0.75 to 1.77)	<0.001	−0.08 (−0.36 to 0.21)	0.584
Follow-up (week 8)	0.92 (0.51 to 1.34)	<0.001	−0.13 (−0.42 to 0.16)	0.376
CSEAS				
Posttreatment	−5.18 (−6.11 to −4.25)	<0.001	−0.33 (−1.00 to 0.33)	0.318
Follow-up	−4.33 (−5.28 to −3.38)	<0.001	−0.26 (−0.88 to 0.37)	0.408
CSEAS-difficulty				
Posttreatment	−0.92 (−1.21 to −0.64)	<0.001	−0.23 (−0.47 to 0.10)	0.060
Follow-up	−0.97 (−1.32 to −0.63)	<0.001	−0.18 (−0.52 to 0.16)	0.292
CSEAS-bristol				
Posttreatment	−1.49 (−1.84 to −1.13)	<0.001	−0.15 (−0.42 to 0.11)	0.244
Follow-up	−1.18 (−1.49 to −0.87)	<0.001	0.03 (−0.18 to 0.23)	0.800
CSEAS-time				
Posttreatment	−0.85 (−1.15 to −0.54)	<0.001	0.05 (−0.13 to 0.23)	0.570
Follow-up	−0.69 (−1.05 to −0.33)	<0.001	−0.05 (−0.20 to 0.10)	0.487
CSEAS-incompleteness				
Posttreatment	−0.77 (−1.10 to −0.44)	<0.001	0.00 (−0.25 to 0.25)	1.000
Follow-up	−0.62 (−0.95 to −0.29)	0.001	−0.18 (−0.41 to 0.54)	0.128
CSEAS-frequency				
Posttreatment	−0.67 (−1.00 to −0.33)	<0.001	−0.08 (−0.34 to 0.19)	0.555
Follow-up	−0.36 (−0.69 to −0.03)	0.033	0.00 (−0.27 to 0.27)	1.000
CSEAS-bloating				
Posttreatment	−0.49 (−0.84 to −0.13)	0.009	0.08 (−0.20 to 0.35)	0.600
Follow-up	−0.51 (−0.92 to −0.11)	0.015	0.13 (−0.12 to 0.38)	0.303
PAC-QOL				
Posttreatment	−8.33 (−11.24 to −5.43)	<0.001	−0.64 (−2.31 to 1.02)	0.441
Follow-up	−7.80 (−10.94 to −4.65)	<0.001	−0.08 (−1.90 to 1.75)	0.932
UPDRS				
Posttreatment	−3.97 (−5.83 to −2.12)	<0.001	−0.80 (−2.00 to 0.41)	0.190
Follow-up	−3.51 (−5.14 to −1.89)	<0.001	−0.18 (−1.20 to 0.85)	0.725
UPDRS I				
Posttreatment	−1.41 (−2.12 to −0.70)	<0.001	−1.00 (−1.80 to −0.20)	<0.001
Follow-up	−1.15 (−1.77 to −0.54)	<0.001	−1.08 (−1.76 to −0.39)	<0.001

MA, manual acupuncture; SA, sham acupuncture; Weekly CSBMs, weekly complete spontaneous bowel movements; CSEAS, Constipation Symptom and Efficacy Assessment Scale; PAC-QOL, Patient-Assessment of Constipation Quality of Life questionnaire; UPDRS, Unified Parkinson's Disease Rating Scale.

hard bowel movements and hard stools (Xu et al., 2020), though few studies evaluated prolonged defecation as well as the sense of incomplete evacuation and bloating. The severe and frequent gastrointestinal symptoms were positively correlated with the severe motor symptoms (Sun et al., 2021). Patients with bradykinesia, tremor, and hypertonia found it difficult to stay in the bathroom for long periods of time. Additionally, patients who experienced bloating and the feeling of incomplete evacuation had a diminished appetite and had to be careful about what they ate. In this study, shorter defecation times and reduced symptoms of incomplete evacuation and bloating were seen in the MA group compared to the SA group during both treatment and follow-up. These findings offered more proof that acupuncture can effectively relieve constipation in PD.

Enhancing the quality of life in relation to health is one of PDC treatment's objectives (Sauerbier et al., 2017). In this study, the PAC-QOL, which included the dimensions of psychosocial discomfort, physical discomfort, satisfaction, and worriedness and concerns, was used to assess the quality of life in patients with PDC. We conducted the PAC-QOL in a Chinese version, which has proven reliable and valid (Zhao, 2011). The change in PAC-QOL scores revealed that MA significantly improved the quality of life of PDC patients after treatment when compared to SA. Both results during treatment and

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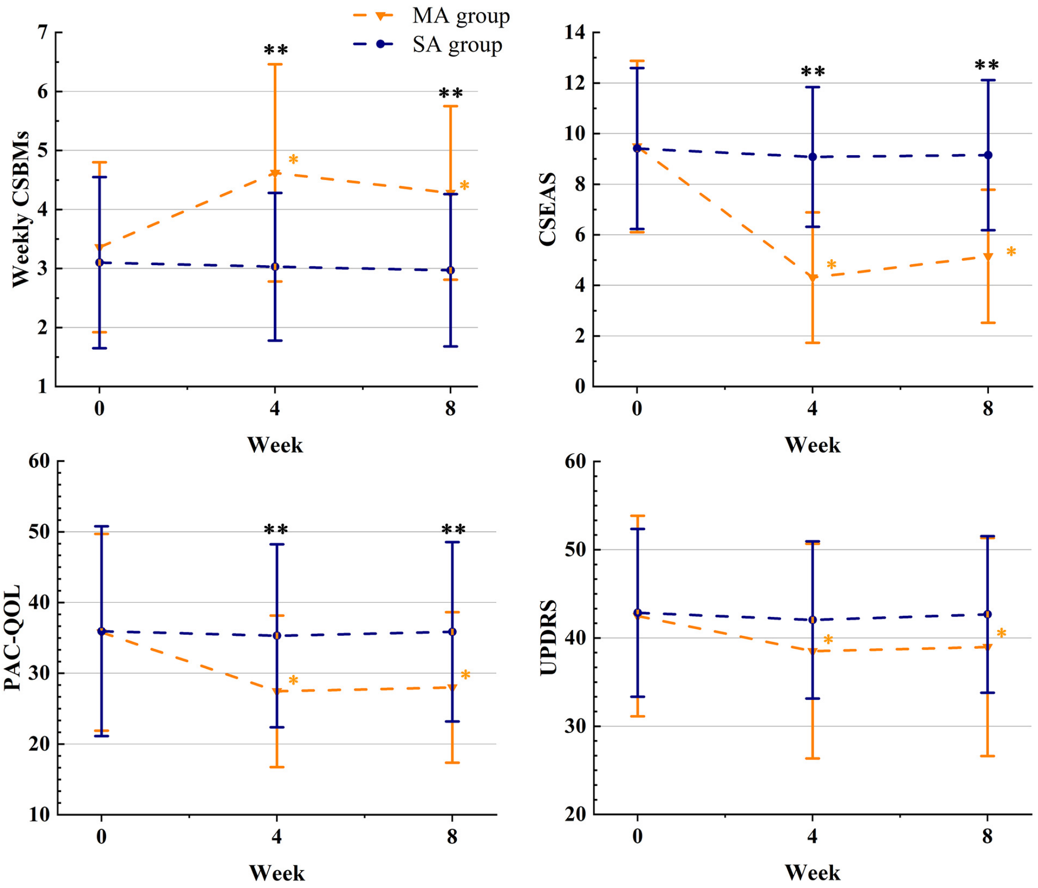

FIGURE 2
The therapeutic effects of acupuncture. *The difference with the baseline was significant within group ($P \leq 0.05$); **the difference between the groups was significant ($P \leq 0.05$); MA, manual acupuncture; SA, sham acupuncture; Weekly CSBMs, weekly complete spontaneous bowel movements; CSEAS, Constipation Symptom and Efficacy Assessment Scale; PAC-QOL, Patient-Assessment of Constipation Quality of Life questionnaire; UPDRS, Unified Parkinson's Disease Rating Scale.

follow-up in the MA group were greater than the MCID (at least a 0.5-point reduction in the total PAC-QOL score) (Liu et al., 2016), supporting the clinical effectiveness of acupuncture in improving quality of life in PDC patients.

As is well-known, PD is a progressive neurodegenerative disorder, and there is no treatment available to slow disease progression (Bloem et al., 2021). In our study, when assessing overall PD symptoms, the UPDRS scores were reduced in both the MA and SA groups, but there was no difference between the two treatment groups. Further research found that the decrease in scores was primarily concentrated in UPDRS I (the mental, behavioral, and emotional components). Previous studies have shown that placebo effects are usually observed when acupuncture is used for improving psychiatric and psychological-related symptoms in patients with functional gastrointestinal disorders as well as Parkinson's disease (Kaptchuk, 2020; Li et al., 2022; Fan et al., 2022b). Therefore, we can infer that acupuncture was helpful in improving mental and psychological symptoms in PDC patients, even though it may have been a placebo effect.

Overall, acupuncture can effectively alleviate constipation symptoms and improve the quality of life for PDC patients.

There are some strengths and limitations to this study. First, a well-designed sham acupuncture device demonstrated the reliability of the difference between manual and sham acupuncture. It maintained the sensation of the needles touching the skin without piercing it, which ensured physiologically inert blindness. Patients in both groups did not differ significantly in their ability to correctly guess their distribution at the end of the study, confirming that the blinding was psychologically credible. Secondly, we used the CSEAS, a well-designed scale, to measure the improvement of the constipation symptom. It was designed in 2015 by the Anorectal Surgery Group of the Chinese Medical Association's Surgery Branch. The CSEAS comprised six questions covering six constipation-related symptoms and was suitable for Chinese patients. Its succinct question reflected the efficiency of the treatment in a comprehensive and targeted way. In this study, we analyzed each symptom to provide more detail on the efficacy of acupuncture in improving PDC. Thirdly, no serious adverse events were found in the study, and the few adverse events that occurred in the MA group were mild and self-resolving, like those reported in previous acupuncture studies. This provided assurance of the safety of acupuncture for PDC.

In the later stages of PD, the short- and long-term responses to dopaminergic drugs are reduced, and the inability to store excess dopamine necessitates higher and more frequent doses of levodopa (Rosqvist et al., 2018). Some studies suggested that improving constipation might help regulate the absorption of dopaminergic drugs in the intestine, thereby delaying this phenomenon (Güneş and Karavana, 2022). A limitation of this study is that the study period was too short, and we were unable to observe the effect of acupuncture on levodopa dose by improving constipation. In order to better understand the association between improvement of bowel movements and levodopa dosage following acupuncture treatment, the follow-up period should be extended, and prospective cohort studies should be developed. Another limitation is the absence of a Chinese version of The Gastrointestinal Dysfunction Scale for Parkinson's disease, which might bias our evaluation of PDC patients. In the future, we will work on a Chinese version of this scale for evaluating patients with PDC. Furthermore, this study lacked objective markers that approached mechanisms. The following step will be to incorporate objective markers to advance our understanding of how acupuncture treats PDC.

5. Conclusion

Acupuncture was discovered to effectively relieve constipation symptoms and improve the quality of life of PD patients, with the treatment effect lasting up to 4 weeks.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (Ethics number: K-2022-005). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Y-JL, J-QF, and L-XZ conceived and designed the experiments. L-XZ assessed the potential patients and supervised the acupuncture scheme. M-YY, XL, W-JL, and Y-YC performed the treatment. I-IL and J-QF assessed the outcomes. Y-JL analyzed the data. W-QT generated the random allocation and assigned the participants. Y-JL, I-IL, and J-QF wrote the manuscript. Y-TW designed the acupuncture device. All authors approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1126080/full#supplementary-material

References

Armstrong, M. J., and Okun, M. S. (2020). Diagnosis and treatment of Parkinson disease: A review. *JAMA* 323, 548–560. doi: 10.1001/jama.2019.22360

Barboza, J. L., Okun, M. S., and Moshiree, B. (2015). The treatment of gastroparesis, constipation and small intestinal bacterial overgrowth syndrome in patients with Parkinson's disease. *Expert Opin. Pharmacother.* 16, 2449–2464. doi: 10.1517/14656566.2015.1086747

Bloem, B. R., Okun, M. S., and Klein, C. (2021). Parkinson's disease. *Lancet* 397, 2284–2303. doi: 10.1016/s0140-6736(21)00218-x

Cersosimo, M. G., and Benarroch, E. E. (2008). Neural control of the gastrointestinal tract: Implications for Parkinson disease. *Mov. Disord.* 23, 1065–1075. doi: 10.1002/mds.22051

Cersosimo, M. G., Raina, G. B., Pecci, C., Pellene, A., Calandra, C. R., Gutiérrez, C., et al. (2013). Gastrointestinal manifestations in Parkinson's disease: Prevalence and occurrence before motor symptoms. *J Neurol.* 260, 1332–1338. doi: 10.1007/s00415-012-6801-2

Chen, H., Zhao, E. J., Zhang, W., Lu, Y., Liu, R., Huang, X., et al. (2015). Meta-analyses on prevalence of selected Parkinson's nonmotor symptoms before and after diagnosis. *Transl. Neurodegener.* 4:1. doi: 10.1186/2047-9158-4-1

Cheng, F. K. (2017). The use of acupuncture in patients with Parkinson's disease. *Geriatr. Nurs.* 38, 302–314. doi: 10.1016/j.gerinurse.2016.11.010

Chou, K. L., Stacy, M., Simuni, T., Miyasaki, J., Oertel, W. H., Sethi, K., et al. (2018). The spectrum of "off" in Parkinson's disease: What have we learned over 40 years? *Parkinsonism Relat. Disord.* 51, 9–16. doi: 10.1016/j.parkreldis.2018.02.001

Cutson, T. M., Laub, K. C., and Schenkman, M. (1995). Pharmacological and nonpharmacological interventions in the treatment of Parkinson's disease. *Phys. Ther.* 75, 363–373. doi: 10.1093/ptj/75.5.363

Dogra, N., Mani, R. J., and Katare, D. P. (2022). The gut-brain axis: Two ways signaling in Parkinson's disease. *Cell. Mol. Neurobiol.* 42, 315–332. doi: 10.1007/s10571-021-01066-7

Fan, J. Q., Lu, W. J., Tan, W. Q., Feng, W. C., and Zhuang, L. X. (2022a). Acupuncture for Parkinson's disease: From theory to practice. *Biomed. Pharmacother.* 149:112907. doi: 10.1016/j.biopha.2022.112907

Fan, J. Q., Lu, W. J., Tan, W. Q., Liu, X., Wang, Y. T., Wang, N. B., et al. (2022b). Effectiveness of acupuncture for anxiety among patients with Parkinson disease: A randomized clinical trial. *JAMA Netw. Open* 5:e2232133. doi: 10.1001/jamanetworkopen.2022.32133

Gan, J., Wan, Y., Shi, J., Zhou, M., Lou, Z., and Liu, Z. (2018). A survey of subjective constipation in Parkinson's disease patients in shanghai and literature review. *BMC Neurol.* 18:29. doi: 10.1186/s12883-018-1034-3

Goldman, J. G., and Guerra, C. M. (2020). Treatment of nonmotor symptoms associated with Parkinson disease. *Neurol. Clin.* 38, 269–292. doi: 10.1016/j.ncl.2019.12.003

Güneş, M., and Karavana, S. Y. (2022). Non-oral drug delivery in Parkinson's disease: Current Applications and future. *Turk. J. Pharm. Sci.* 19, 343–352. doi: 10.4274/tjps.galenos.2021.95226

Kaptchuk, T. J. (2020). Placebo effects in acupuncture. *Med. Acupunct.* 32, 352–356. doi: 10.1089/acu.2020.1483

Kwon, M., Cheong, M. J., Leem, J., and Kim, T. H. (2021). Effect of acupuncture on movement function in patients with Parkinson's disease: Network meta-analysis of randomized controlled trials. *Healthcare* 9:1502. doi: 10.3390/healthcare9111502

Lesser, G. T. (2002). Frequency of bowel movements and future risk of Parkinson's disease. *Neurology* 58, 838–839. doi: 10.1212/wnl.58.5.838-a

Li, K., Wang, Z., Chen, Y., Shen, L., Li, Z., Wu, Y., et al. (2019). Efficacy of electroacupuncture for the treatment of constipation in Parkinson's disease: Study protocol for a multicentre randomised controlled trial. *BMJ Open* 9:e029841. doi: 10.1136/bmjopen-2019-029841

Li, Q., Wu, C., Wang, X., Li, Z., Hao, X., Zhao, L., et al. (2022). Effect of acupuncture for non-motor symptoms in patients with Parkinson's disease: A systematic review and meta-analysis. *Front. Aging Neurosci.* 14:995850. doi: 10.3389/fnagi.2022.995850

Li, Y. Q., Zhu, B., Rong, P. J., Ben, H., and Li, Y. H. (2007). Neural mechanism of acupuncture-modulated gastric motility. *World J. Gastroenterol.* 13, 709–716. doi: 10.3748/wjg.v13.i5.709

Liu, Z., Yan, S., Wu, J., He, L., Li, N., Dong, G., et al. (2016). Acupuncture for chronic severe functional constipation: A randomized trial. *Ann. Intern. Med.* 165, 761–769. doi: 10.7326/m15-3118

MacPherson, H., Altman, D. G., Hammerschlag, R., Youping, L., Taixiang, W., White, A., et al. (2010). Revised standards for reporting interventions in clinical trials of acupuncture (STRICTA): Extending the CONSORT statement. *PLoS Med.* 7:e1000261. doi: 10.1371/journal.pmed.1000261

Mozaffari, S., Nikfar, S., Daniali, M., and Abdollahi, M. (2020). The pharmacological management of constipation in patients with Parkinson's disease: A much-needed relief. *Expert Opin. Pharmacother.* 21, 701–707. doi: 10.1080/14656566.2020.1726319

Mukherjee, A., Biswas, A., and Das, S. K. (2016). Gut dysfunction in Parkinson's disease. *World J. Gastroenterol* 22, 5742–5752. doi: 10.3748/wjg.v22.i25.5742

Nazarova, L., Liu, H., Xie, H., Wang, L., Ding, H., An, H., et al. (2022). Targeting gut-brain axis through scalp-abdominal electroacupuncture in Parkinson's disease. *Brain Res.* 1790:147956. doi: 10.1016/j.brainres.2022.147956

Palsson, O. S., Whitehead, W. E., van Tilburg, M. A., Chang, L., Chey, W., Crowell, M. D., et al. (2016). Rome IV diagnostic questionnaires and tables for investigators and clinicians. *Gastroenterology* doi: 10.1053/j.gastro.2016.02.014 [Epub ahead of print].

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., et al. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* 30, 1591–1601. doi: 10.1002/mds.26424

Rosqvist, K., Horne, M., Hagell, P., Iwarsson, S., Nilsson, M. H., and Odin, P. (2018). Levodopa effect and motor function in late stage Parkinson's disease. *J. Parkinsons Dis.* 8, 59–70. doi: 10.3233/jpd-171181

Rossi, M., Merello, M., and Perez-Lloret, S. (2015). Management of constipation in Parkinson's disease. *Expert Opin. Pharmacother.* 16, 547–557. doi: 10.1517/14656566.2015.997211

Safarpour, D., Sharzehi, K., and Pfeiffer, R. F. (2022). Gastrointestinal dysfunction in Parkinson's disease. *Drugs* 82, 169–197. doi: 10.1007/s40265-021-01664-1

Sauerbier, A., Cova, I., Rosa-Grilo, M., Taddei, R. N., Mischley, L. K., and Chaudhuri, K. R. (2017). Treatment of nonmotor symptoms in Parkinson's disease. *Int. Rev. Neurobiol.* 132, 361–379. doi: 10.1016/bs.irn.2017.03.002

Schapira, A. H. V., Chaudhuri, K. R., and Jenner, P. (2017). Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* 18, 435–450. doi: 10.1038/nrn.2017.62

Seppi, K., Ray Chaudhuri, K., Coelho, M., Fox, S. H., Katzenschlager, R., Perez Lloret, S., et al. (2019). Update on treatments for nonmotor symptoms of Parkinson's disease-an evidence-based medicine review. *Mov. Disord.* 34, 180–198. doi: 10.1002/mds.27602

Stern, T., and Davis, A. M. (2016). Evaluation and treatment of patients with constipation. *JAMA* 315, 192–193. doi: 10.1001/jama.2015.16995

Sun, B. H., Wang, T., Li, N. Y., Wu, Q., and Qiao, J. (2021). Clinical features and relative factors of constipation in a cohort of Chinese patients with Parkinson's disease. *World J. Gastrointest. Pharmacol. Ther.* 12, 21–31. doi: 10.4292/wjgpt.v12.i1.21

Sung, H. Y., Park, J. W., and Kim, J. S. (2014). The frequency and severity of gastrointestinal symptoms in patients with early Parkinson's disease. *J. Mov. Disord.* 7, 7–12. doi: 10.14802/jmd.14002

Vijiaratnam, N., Simuni, T., Bandmann, O., Morris, H. R., and Foltynie, T. (2021). Progress towards therapies for disease modification in Parkinson's disease. *Lancet Neurol.* 20, 559–572. doi: 10.1016/s1474-4422(21)00061-2

Wang, L., Xian, J., Sun, M., Wang, X., Zang, X., Zhang, X., et al. (2022a). Acupuncture for emotional symptoms in patients with functional gastrointestinal disorders: A systematic review and meta-analysis. *PLoS One* 17:e0263166. doi: 10.1371/journal.pone.0263166

Wang, Y. T., Liu, X., Xu, Z. Q., and Zhuang, L. X. (2022b). [An auxiliary device for double-blind placebo acupuncture research]. *Zhongguo Zhen Jiu* 42, 351–354. doi: 10.13703/j.0255-2930.20210227-k0001

Xu, X. H., Zhang, M. M., Wu, X., Xu, S. B., Wang, W., Zheng, C. H., et al. (2020). Efficacy of electro-acupuncture in treatment of functional constipation: A randomized controlled trial. *Curr. Med. Sci.* 40, 363–371. doi: 10.1007/s11596-020-2188-y

Yao, J. P., Chen, L. P., Xiao, X. J., Hou, T. H., Zhou, S. Y., Xu, M. M., et al. (2022). Effectiveness and safety of acupuncture for treating functional constipation: An overview of systematic reviews. *J. Integr. Med.* 20, 13–25. doi: 10.1016/j.joim.2021.11.001

Yu, Q. J., Yu, S. Y., Zuo, L. J., Lian, T. H., Hu, Y., Wang, R. D., et al. (2018). Parkinson disease with constipation: Clinical features and relevant factors. *Sci. Rep.* 8:567. doi: 10.1038/s41598-017-16790-8

Zeng, B. Y., and Zhao, K. (2016). Effect of acupuncture on the motor and nonmotor symptoms in Parkinson's disease–a review of clinical studies. *CNS Neurosci. Ther.* 22, 333–341. doi: 10.1111/cns.12507

Zhang, M., Yang, S., Li, X. C., Zhu, H. M., Peng, D., Li, B. Y., et al. (2021). Study on the characteristics of intestinal motility of constipation in patients with Parkinson's disease. *World J. Gastroenterol.* 27, 1055–1063. doi: 10.3748/wjg.v27.i11.1055

Zhang, N., Hou, L., Yan, P., Li, X., Wang, Y., Niu, J., et al. (2020). Electro-acupuncture vs. sham electro-acupuncture for chronic severe functional constipation: A systematic review and meta-analysis. *Complement. Ther. Med.* 54:102521. doi: 10.1016/j.ctim.2020.102521

Zhao, Z. Z. (2011). *A psychometric evaluation of the Chinese version of Patient assessment of constipation quality of life questionnaire and analysis of influencing factors on quality of life*. Nanjing: School of nursing, Nanjing Medical University.

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Research progress on acupuncture treatment in central nervous system diseases based on NLRP3 inflammasome in animal models

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Central nervous system (CNS) disorders exhibit complex neurophysiological and pathological mechanisms, which seriously affect the quality of life in patients. Acupuncture, widely accepted as complementary and alternative medicine, has been proven to exert significant therapeutic effects on CNS diseases. As a part of the innate immune system, NLRP3 inflammasome contributes to the pathogenesis of CNS diseases *via* regulating neuroinflammation. To further explore the mechanisms of acupuncture regulating NLRP3 inflammasome in CNS diseases, our study focused on the effects of acupuncture on neuroinflammation and the NLRP3 inflammasome in vascular dementia, Alzheimer's disease, stroke, depression, and spinal cord injury. This study confirmed that the activation of NLRP3 inflammasome promotes the development of CNS diseases, and inhibiting the activation of NLRP3 inflammasome is a potential key target for the treatment of CNS diseases. In addition, it is concluded that acupuncture alleviates neuroinflammation by inhibiting the activation of the NLRP3 inflammasome pathway, thereby improving the progression of CNS diseases, which provides a theoretical basis for acupuncture to attenuate neuroinflammation and improve CNS diseases.

KEYWORDS

acupuncture, central nervous system diseases, NLRP3, inflammasome, mechanism of acupuncture, neuroinflammation, anti-inflammation

Introduction

Central nervous system (CNS) diseases involve complex neuropathophysiological mechanisms, which seriously affect the quality of life in these patients, such as vascular dementia (VD), Alzheimer's disease (AD), stroke, spinal cord injury (SCI), depression, Parkinson's disease (PD), etc (Wei and Qiu, 2022). CNS diseases inevitably increase the global medical financial burden due to their high mortality rates, difficulty in diagnosis, and high treatment costs. Despite the high morbidity and enormous medical costs of CNS disorders, current treatment options are limited in that an insufficient understanding of the etiopathogenesis (Yu et al., 2022).

Inflammation is a defense response in the host system against pathogen infection and various tissue damage, and appropriate inflammation can remove the damage factors in time and enhance the body's resistance to various pathogenic factors (Franceschi et al., 2018). Neuroinflammation is the key of CNS diseases. Many clinical and neuropathological studies have demonstrated that activated microglia, equivalent to macrophages in the brain and spinal cord, play a prominent role in the pathogenesis of neurodegenerative diseases as the first and most important line of immune defense in the CNS (Bellver-Landete et al., 2019). Excessive neuroinflammation leads to neuronal death and promotes the development of CNS diseases, while attenuated neuroinflammation is beneficial to neuronal survival and improves the symptoms and prognosis of CNS diseases (Dorothee, 2018). Numerous studies have shown microglia-mediated neuroinflammation is critical in CNS injury and prognosis.

Acupuncture is an effective therapeutic method, which has been widely practiced in China for more than 2,000 years. Several systematic reviews and meta-analyses have demonstrated that acupuncture can improve symptoms of CNS disorders, and its anti-inflammatory effects have long been proven (Chen et al., 2022b, He et al., 2022, Huang et al., 2022; Lin C. J. et al., 2022; Zhou Z. et al., 2022). Exhilaratingly, PROKR2 neurons are indispensable for the anti-inflammatory effect of low-intensity electroacupuncture (EA) through the vagal-adrenal axis, which was once again confirmed by the article published in the authoritative journal NATURE last year (Liu et al., 2021). In addition, EA is capable of regulating multiple cell signal transduction pathways to alleviate neuroinflammation (in animal models of stroke, AD, SCI, PD, and VD) (Chen et al., 2022b). The latest research found that EA inhibited the activation of microglia and polarized microglia to M2 phenotype, while EA reduced proinflammatory cytokines (IL-1β, TNF-α, and IL-6), increased anti-inflammatory cytokines (IL-4 and IL-10) (Xie et al., 2021). Although acupuncture, as an effective external treatment, has accumulated a lot of valuable experience in the practice of treating chronic diseases, the mechanism of acupuncture on inflammation and immune function is still unclear. Therefore, it is of great clinical significance to deeply study the mechanism of acupuncture anti-inflammation and immune regulation.

As a part of the innate immune system, nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome contributes to the pathogenesis of many diseases *via* regulating inflammation. Under physiological conditions, the NLRP3 inflammasome promotes efficient clearance of damaged cells and tissue repair through its dependent cytokines, thereby promoting tissue regeneration. At the same time, the NLRP3 inflammasome coordinates the invading pathogen-mediated immune responses and host-derived danger signals, maintaining the balance of pro-inflammatory and anti-inflammatory factors in the body. Under pathological conditions, the activation of NLRP3 inflammasome is related to the development of autoimmune diseases, which can lead to excessive inflammatory response. Recently, accumulating evidence has shown that the NLRP3 inflammasomes are activated in local tissues, the spinal cord, and brain regions in various animal models (Barczuk et al., 2022; Kou et al., 2022; Yao et al., 2022). At present, the exploration of the relationship between CNS diseases and NLRP3 inflammasome is still in its infancy, despite there being many studies on the relationship between CNS diseases and inflammatory factors. The activation of NLRP3 inflammasome mediates neuroinflammation to promote the progression of CNS diseases. Acupuncture improve of CNS diseases depends on inhibition of microglia-mediated neuroinflammation. Therefore, acupuncture targeting NLRP3 inflammasome to inhibit microglia mediated neuroinflammation is expected to become a decisive target to prevent CNS diseases. In this review, we summarized the new progress of acupuncture regulating the NLRP3 inflammasome activation-related signal pathway in the treatment of CNS diseases, which provides a theoretical basis for clarifying that acupuncture attenuates neuroinflammation and regulates immune cells.

Sources and selection criteria

We searched Web of Science, PubMed, CNKI, and Embase. The search was limited to English or non-English articles with English abstracts published since the database's inception to now. Keywords included ("acupuncture" or "electroacupuncture" or "EA") and ("NLRP3" or "NLRP3 inflammasome" or "NOD-like receptor protein 3") and ("stroke" or "vascular dementia" or "Alzheimer's disease" or "depression" or "spinal cord injury" or "bulbar palsy" or "Parkinson's disease" or "multiple sclerosis" or "traumatic brain injury" or "" or "brain tumor" or "cerebral palsy" or "headache" or "migraine" or "epilepsy" or "anxiety"). After being carefully evaluated, the information presented in the following studies was described and discussed.

Abbreviations: CNS, central nervous system; VD, vascular dementia; AD, Alzheimer's disease; stroke, SCI, spinal cord injury; PD, Parkinson's disease; MA, manual acupuncture; EA, electroacupuncture; ATP, adenosine triphosphate; TRPV, transient receptor potential vanilloid; IL-1β, interleukin-1β; IL-18, interleukin-18; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; ASC, apoptosis related spot like protein; PAMPs, pathogen associated molecular patterns; DAMPs, damage associated molecular patterns; GSDMD, gasdermin D; HMGB1, High-mobility group box 1; IFN-γ, interferon-γ; TLR, Toll-like receptor; NF-κB, nuclear transcription factor-κB; TNF-α, tumor necrosis factor-α; TXNIP, thioredoxin-interacting protein; OS, oxidative stress; ROS, reactive oxygen species; LC3, microtubule-associated proteins 1B light chain 3; POCD, postoperative cognitive dysfunction; ICH, intracerebral hemorrhage; MCAO, middle cerebral artery occlusion; LPS, lipopolysaccharide; CUMS, chronic unpredictable mild stress; PFC, prefrontal cortex; ZO-1, zonula occludens-1; CB2R, endocannabinoid receptor subtype 2; CMPK2, cytosine monophosphate kinase 2; HPA, hypothalamus-pituitary-adrenal; CGRP, calcitonin gene related peptide; Nek7, NIMA-related kinase-7.

Acupoints for CNS diseases

Acupuncture points (acupoints) are the "implicated acupoints" on the body surface during the pathological process of target organs, and the confirmed "implicated acupoints" are the parts of the body surface that play a "specific role" (Zhu, 2021). More specifically, acupoints are particular locations on the meridians, rich in nerves, blood vessels, and immune cells, which connect specific organs and regulate related body functions. Further study found that adenosine triphosphate (ATP) and transient receptor potential vanilloid (TRPV) channels were involved in acupuncture stimulation of acupoint regions (Lin J. G. et al., 2022). Based on the acupuncture theory, the selection of different acupoints has an essential impact on the efficacy of acupuncture in treating diseases, both in clinical and theoretical research. After analysis, the commonly used acupoints and the general rules of acupoint selection in CNS diseases were summarized. These acupoints include: Baihui, GV20; Dazhui, GV14; Shuigou, GV26; Yintang, GV29; Shenting, GV24; Benshen, GB13; Qubin, GB7; Shangxing, GV23; Fengfu, GV16; Zhiyang, GV9; Jizhong, GV6; Mingmen, GV4; Zusanli, ST36; Yanglingquan, GB34; Shenshu, BL23; Chize, LU5; Hegu, LI4; Sanyinjiao, SP6; Waiguan, TE5; Neiguan, PC6; Dachangshu, BL25; Taixi, KI3 (**Figure 1**).

Relationship between acupuncture and NLRP3 inflammasome in CNS diseases

Composition and activation of NLRP3 inflammasome

The NLRP3 inflammasome, a high molecular weight multiprotein complex of approximately 700 kDa, which consists of cytoplasmic NLRP3, apoptosis-associated speck-like protein (ASC) and pro-caspase-1, simplified as the receptor protein (NLRP3), adapter protein (ASC) and effector protein (caspase-1) (Swanson et al., 2019). NLRP3, as the core protein of NLRP3 inflammasome, is a pattern recognition receptor comprised of 11 leucine repeats at the C-terminal, NACHT domain in the middle, and Pyrin domain at the N-terminal. It functions in the cytoplasm to recognize exogenous microorganisms or endogenous danger signals and recruit the downstream connector protein ASC and the effector protein caspase-1. Specifically, when ASC binds to caspase-1, caspase-1 gathered on ASC splits at the junction of p20 and p10, thereby converting inactive pro-IL-1β and pro-IL-18 to mature active interleukin 1β (IL-1β) and interleukin 18 (IL-18) (Boucher et al., 2018).

Currently, the mainstream view is that the activation of NLRP3 inflammasome requires two stages: priming and activation. In the priming stage, cytokines or pathogen-associated molecular patterns (PAMPs) activate Toll-like receptor (TLR), tumor necrosis factor (TNF), or nuclear transcription factor (NF-κB) signal pathway, to promote NLRP3 and upregulate pro-IL-1β mRNA expression (Bauernfeind et al., 2009). In the activation stage, the PAMPs and DAMPs (including sodium and potassium ion flow, active oxygen generation, mitochondrial dysfunction, etc.) result in the assembly and activation of NLRP3 inflammasome. Active caspase-1 cleaves gasdermin D (GSDMD) into a pore-forming N-terminal (GSDMD-N), which mediates the secretion of IL-1β and IL-18 as well as pyroptosis. Unlike apoptosis, pyroptosis is a proinflammatory programed cell death mode mediated by the inflammasome, characterized by cell swelling, lysis, and release of cell contents (Swanson et al., 2019; **Figure 2**).

Acupuncture *via* NLRP3 inflammasome in the VD

VD is a clinical syndrome of cognitive dysfunction caused by cerebrovascular diseases, such as cerebral ischemia, cerebral hemorrhage, or brain hypoxia-ischemia (Yang et al., 2022). The main symptoms of VD are impairment of memory and executive ability. Although it has a high incidence rate, it is the only one among various types of dementia that can be prevented and treated early. The latest research has found that immune and neuroinflammation have gradually attracted the attention of researchers, excluding common risk factors for cerebrovascular diseases (Finger et al., 2022; Tian et al., 2022). Many studies have shown that acupuncture has a certain effect on VD (Chen et al., 2022b), but there are differences in efficacy due to different acupuncture methods, and there is no unified clinical standard. Scientific and reasonable selection of acupuncture methods is the key to acupuncture treatment of VD.

Thioredoxin-interacting protein (TXNIP) plays a vital role in oxidative stress (OS) and NLRP3 inflammasome activation. Manual acupuncture (MA) at ST36 and GV20 suppresses OS and inflammation by reducing TXNIP-mediated upregulation of hippocampal NLRP3 and IL-1β, thereby reducing cognitive impairment and neuronal death in VD rats (Du et al., 2018). Autophagy is a process of catabolism that relies on autophagy and lysosomes to degrade proteins, foreign bodies, and organelles to maintain the homeostasis of the internal environment. The study has found that electroacupuncture (EA) stimulation at GV20, GV14, and BL23 can improve the learning and memory ability of VD rats, reduce the ultrastructural damage of hippocampal CA1 neurons, and repair damaged neurons. It is speculated that the mechanism may be related to EA reducing the level of reactive oxygen species (ROS), the ratio of LC3-II/LC3-I, and inhibiting the expression of NLRP3 and beclin1 proteins, which potentially promotes the reduction of neuronal autophagy, inhibits the activation of the NLRP3 inflammasome, and attenuates the CNS inflammatory response (Qiu et al., 2022; **Table 1**).

Acupuncture *via* NLRP3 inflammasome in the AD

The condition of AD patients gradually deteriorates with age, manifested by various cognitive dysfunctions, including language impairment, disorientation, mood swings, loss of motivation, self-neglect, and behavioral abnormalities. Typical pathological features of AD are extracellular amyloid plaque, overexpression of tau protein and formation of nerve fiber tangle, accompanied by glial cell activation and neuroinflammation (Severini et al., 2021).

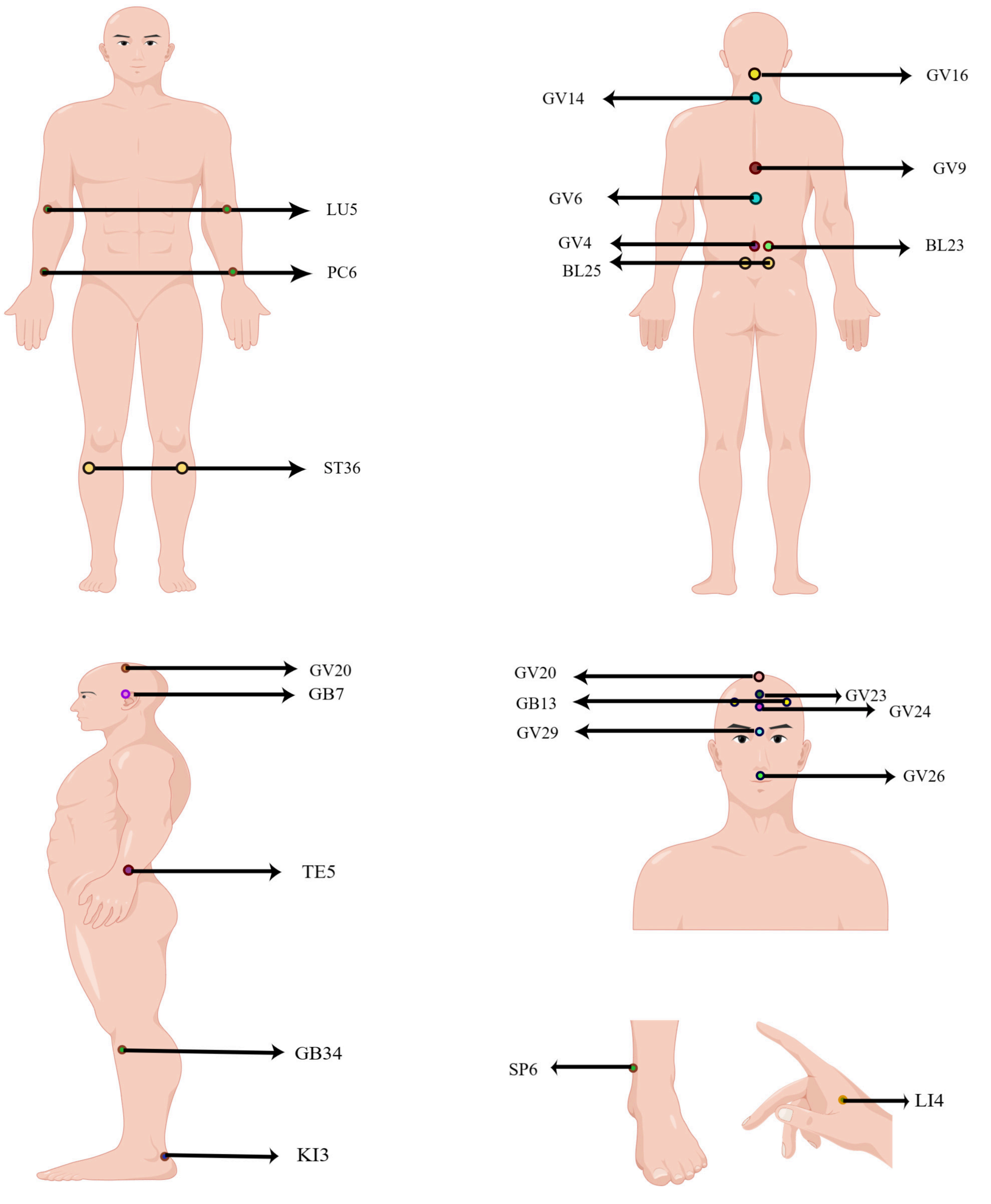

FIGURE 1
Human acupoints frequently used in CNS diseases. The locations of acupoint code are marked in the figure. GV20: Baihui; GV14: Dazhui; GV26: Shuigou; GV29: Yintang; GV24: Shenting; GB13: Benshen; GB7: Qubin; GV23: Shangxing; GV16: Fengfu; GV9: Zhiyang; GV6: Jizhong; GV4: Mingmen; ST36: Zusanli; GB34: Yanglingquan; BL23: Shenshu; LU5: Chize; LI4: Hegu; SP6: Sanyinjiao; TE5: Waiguan; PC6: Neiguan; BL25: Dachangshu; KI3: Taixi.

A growing number of studies have shown that the NLRP3 inflammasome plays a vital role in the pathogenesis of AD by stimulating the innate immune response and activating the NLRP3 inflammasome (Ising et al., 2019; Moonen et al., 2022).

EA pretreatment on ST36 and GV20 acupoints can prevent learning/memory dysfunction in AD-like rats, the mechanism of which may be related to the down-regulation of hippocampal NLRP3, caspase-1, IL-1β protein expression, and inhibition of microglial activation (He et al., 2020). Moreover, EA at GV20 markedly preserved cognitive function in postoperative cognitive dysfunction (POCD) mice, associated with the inhibition of neuroinflammation as evidenced by reduced microglial activation and decreased IL-1β and IL-6 levels in brain tissue. Mechanistically, the activation of NLRP3 inflammasome and NF-κB was inhibited

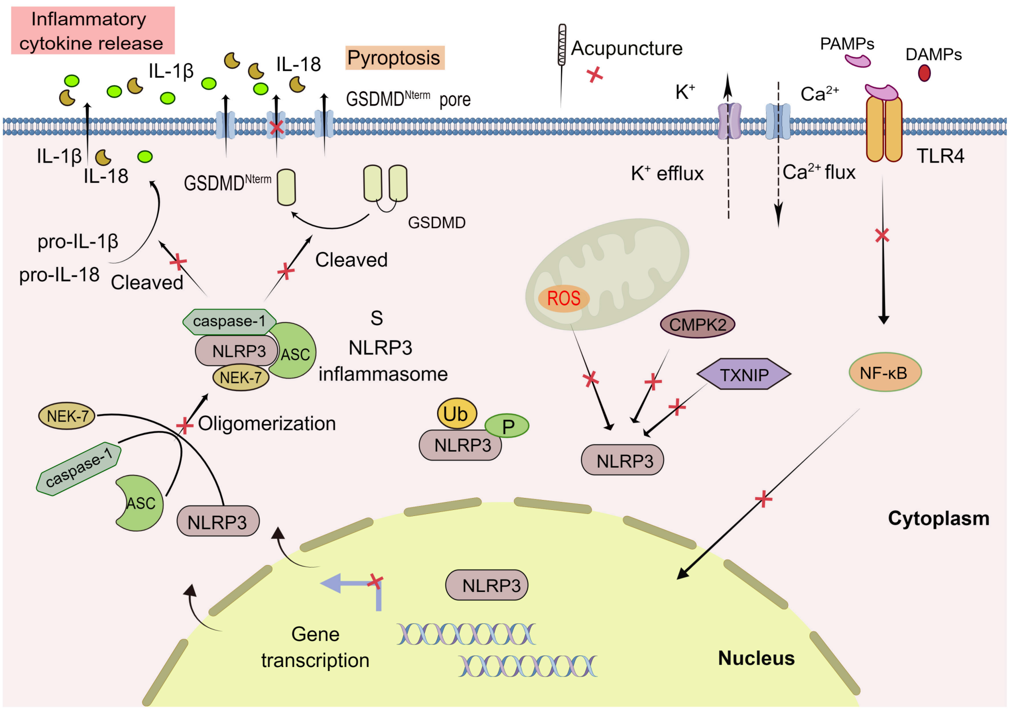

FIGURE 2
Acupuncture treatment in CNS diseases based on NLRP3 inflammasome. The activation of NLRP3 inflammasome involves the assembling of the components of NLRP3 inflammasome (NLRP3, ASC, caspase-1, and NEK7) to form a complete NLRP3 inflammasome complex. PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; GSDMD, Gasdermin D; ROS, reactive oxygen species. Formation of the inflammasome activates caspase 1, which in turn cleaves pro-IL-1β and pro-IL-18. TLR, Toll-like receptor; NEK7, NIMA-related kinase 7; NF-κB, nuclear factor-κB; TXNIP, thioredoxin-interacting protein; CMPK2, cytosine monophosphate kinase 2; Ub, ubiquitylation; P, phosphorylation; Red X, represents a potential target for acupuncture treatment of CNS.

by EA, while the agonist of NLRP3 eliminates the therapeutic effect of EA on cognitive function. EA also preserved hippocampal neurons and tight junction proteins zonula occludens-1 (ZO-1) and claudin 5 (Sun et al., 2022). Besides, studies have confirmed that stimulating ST36 and GV20 acupoints with 2 Hz and 10 Hz EA ameliorates cognitive impairment. Intriguingly, cognitive function, hippocampal morphology, and TUNEL-positive cell counts were improved by stimulation with both EA frequency. Notably, 10 Hz EA was more effective than 2 Hz EA in reducing the number of TUNEL-positive cells in the CA1 area and serum IL-1β and IL-6 levels. Its mechanism may be that EA significantly reduced NLRP3, ASC, caspase-1, GSDM-D, IL-1β, and IL-18, but 2 Hz EA failed to effectively down-regulate the expression of ASC protein (Hou et al., 2020; **Table 1**).

Acupuncture *via* NLRP3 inflammasome in the stroke

Stroke, a localized cerebral dysfunction caused by acute blood flow interruption, which includes 85% of ischemic stroke (manifested as focal infarction) and 15% of hemorrhagic stroke (manifested as cerebrovascular rupture), has been the leading cause of disability and death worldwide (Feigin and Krishnamurthi, 2016). The pathological progress of neuroinflammation in stroke requires the core involvement of the immune system. Ischemic stroke increases DAMPs levels, activating innate immune system sensors such as macrophages/microglia, neutrophils, and Toll-like receptors (TLRs) to co-amplify the inflammatory response (Lunemann et al., 2021). In addition, neuroinflammation induced by cerebral hemorrhage can release various inflammatory cytokines (such as IL-1β and IL-18), further aggravating the neuroinflammation (Anthony et al., 2022).

In recent years, mounting evidence has indicated that the inflammasomes have critical functions in inflammatory reactions and innate immunity. The NLRP3 inflammasome has been confirmed to be participated in brain injury after intracerebral hemorrhage (ICH) to mediate neuronal injury and neuroinflammation by serving as an important mediator of neuroinflammation after cerebral ischemia (Zhou et al., 2019). Inflammation, in particular, is one of the core pathological mechanisms of secondary injury of ischemic stroke (Chen et al., 2016). MA downregulates the expression of NLRP3, IL-1β, and IL-18 in the brain of ICH rats through GV20 to GB7 and inhibits the inflammatory response to promote the recovery of neurological function (Liu et al., 2020). Moreover, miR-223, a biomarker of multiple human metabolic ailments, was upregulated, while the levels of NLRP3, IL-1β, and caspase-1 decreased in the peri-infarct cortex of EA-treated rats with middle cerebral artery occlusion (MCAO). Nevertheless, the neuroprotective effect of EA was partially blocked by antagomir-223 (Sha et al., 2019).

EA ameliorates cognitive impairment by inhibiting NLRP3 inflammasome activation in stroke rats. EA at GV20 and GV24 attenuates cognitive impairment by regulating endogenous

TABLE 1 Acupuncture methods for VD, AD, and stroke *via* NLRP3 inflammasome.

Methods	Model	Tissue	Acupoints	Acupuncture strategy	Mechanisms	Animals	References
MA	VD	Hippocampal	ST36; GV20	MA were twisted 2 times per second for 30 s, once a day, 2 weeks, with a rest on the seventh day, total 12 treatments	TXNIP↓; SOD↓; ROS↓; NLRP3↓; ACS↓; caspase-1↓; IL-1β↓;	Wistar rats	Du et al. (2018)
EA	VD	Hippocampal	GV20; GV14; BL23	Dilatational wave, 10/50 Hz, 1 mA, 30 min, once a day, 4 weeks	NLRP3↓; LC3-II/LC3-I↓; beclin-1↓; ROS↓	SD rats	Qiu et al. (2022)
EA	CI	Hippocampal	ST36; GV20	Low frequency 2 Hz and high-frequency 10 Hz, 1 mA, 30 min, once a day, 14 consecutive days	NLRP3↓; caspase-1↓; ASC↓; IL-1β↓; IL-18↓; GSDMD↓; Aβ↓	SAMP8 mice; SAMR1 mice	Hou et al. (2020)
MA	HIE	Hippocampus	GB13	10 min, once a day, 14 consecutive days	NLRP3↓; ASC↓; caspase-1↓; IL-1β; IL-18↓	SD rats	Qu et al. (2021)
EA	CI	Hippocampus and colon	GV20; BL25; ST36	Continuous wave, 2 Hz, 1.0 mA, 15 min, once a day, 5 day a week, 5 weeks	NLRP3↓; TLR4↓; TNF-α↓; ASC↓; IL-1β↓; caspase-1↓; NF-κB p65↓; claudin-5; ZO-1	APP/PS1 mice	Liao et al. (2022)
EA	POCD	Hippocampus	GV20	2 Hz, 0.5 mA, 20 min, twice a day, 7 days	NLRP3↓; ASC↓; caspase-1↓; IL-1β↓; IL-6↓; NF-κB↓;	C57BL/6 mice	Sun et al. (2022)
EA	AD	Hippocampal	ST36; GV20	Continuous wave, 50 Hz, 1 mA, 20 min, once a day, 8 weeks	NLRP3↓; caspase-1↓; IL-1β↓	SD rats	He et al. (2020)
EA	AD	Hippocampus	GV20; GV26; GV29	Sparse wave, 2 Hz, 0.6 mA, 20 min, once a day, 15 days except on the 8th day	NLRP3↓; ACS↓; caspase-1↓; IL-1β↓	SAMP8 mice; SAMR1 mice	Jiang et al. (2018)
EA	AD	Hippocampus	GV20; GV24	2 Hz, 1 mA, 15 min, once a day, 5 consecutive days per week, 3 weeks	NLRP3↓; ASC↓; caspase-1↓; IL-1β; IL-18↓	PS cDKO mice	Li K. et al. (2021)
MA	Hemorrhagic stroke	Brain	GV20; GB7	Twist at the speed of 180–200 r/min, once every 5 min, with an interval of 5 min, 3 times in total, once every 12 h, for 7 days	NLRP3↓; IL-18↓; IL-1β↓	SD rats	Liu et al. (2020)
EA	Cerebral I/R model	Brain	LU5; LI4; SP6; ST36	Dilatational wave, 5/10 Hz, 2 mA, 20 min, 24 h	NLRP3↓; GSDMD↓; pro-caspase-1↓; cleaved-caspase-1 p20↓; pro-IL-1β↓; cleaved-IL-1β↓	C57BL/6 mice; Caspase-1 knockout mice	Cai et al. (2022)
EA	Stroke	Brain	GV20	Dilatational wave, 2/15 Hz, 1 mA, 30 min, once a day, five consecutive days	NLRP3↓; pro-caspase-1↓; caspase-1↓; pro-IL-1β↓; IL-1β↓; GSDMD↓; GSDMD-N↓	SD rats	Jiang et al. (2019)
EA	Stroke	Peri-infarct cortex	TE5; ST36	Continuous wave, 20 Hz, 1 mA, 30 min, once a day, seven consecutive days	NLRP3↓; miR-223↑; IL-18↓; caspase-1↓; IL-1β↓	SD rats	Sha et al. (2019)
EA	Stroke	Hippocampus	GV20; GV24	Sparse wave, 4 Hz, dense wave, 20 Hz, 0.5 mA, 30 min, once a day, 7 days	ROS↓; NLRP3↓; ASC↓; IL-18↓; caspase-1↓; LC3-II/LC3-I↓; IL-1β↓; Parkin↑; PINK1↑	SD rats	Zhong et al. (2022)

EA, electroacupuncture; MA, manual acupuncture; CI, cognitive impairment; HIE, hypoxic-ischemic encephalopathy; RIP3, receptor-interacting protein 3; POCD, postoperative cognitive dysfunction; SOD, superoxide dismutase; PS cDKO mice, PS1 and PS2 double knockout mice.

melatonin secretion through aralkylamine N-acetyltransferase gene synthesis in the pineal gland in MCAO rats and plays neuroprotective effects by upregulating mitophagy-associated proteins and suppressing ROS-induced NLRP3 inflammasome activation after ischemia-reperfusion injury (Zhong et al., 2022). Moreover, the results of Cai et al. (2022) revealed that the neuroprotective effect of EA is reflected in the inhibition of caspase-1 mediated neuronal pyroptosis and inflammatory response after cerebral ischemia/reperfusion. EA at LU5, LI4, SP6 and ST36 could decrease the score of neurological deficit, reduce the volume of cerebral infarction and improve the degree of nerve cell injury, and inhibit NLRP3, pro-caspase-1, cleaved-caspase-1 p20, pro-IL-1β, cleaved-IL-1β and GSDMD protein expression (Cai et al., 2022). This research shows that EA plays a neuroprotective role by interfering with the priming stage of NLRP3 inflammasome activation (Table 1).

Acupuncture *via* NLRP3 inflammasome in the depression

Major depression is the most common mood disorder in China, with a lifetime prevalence of 3.4% and an annual prevalence of

2.1% (Huang et al., 2019). Conversely, it has a low treatment rate and few people receive proper treatment (Lu et al., 2021). In China, depression in elderly patients with chronic diseases leads to an increase in medical costs of 3.1–85.0% (Wu et al., 2022). EA can not only reduce the HAMD score, synergistically improve the efficacy of antidepressants, but also effectively reduce the side effects (Sun et al., 2013; Zhou Z. et al., 2022). In particular, the researchers found that GV20 and GV29 are the most commonly used acupoints for the treatment of depression. Given that the close relationship between neuroinflammation and depression has been widely confirmed (Troubat et al., 2021), it is speculated that neuroinflammation may be the critical therapeutic target for future depression treatment strategies. NLRP3 inflammasome is an intracellular multiprotein complex responsible for the innate immune processes associated with infection, inflammation, and depression. Subsequently, we analyzed how the inhibition of acupuncture on the activation of NLRP3 inflammasome alleviates depression.

The chronic unpredictable mild stress (CUMS) increases NLRP3 levels in the hippocampus. EA may improve the cognitive impairment of APP/PS1 mice by up-regulating the expression of claudin-5 and ZO-1, reducing the transposition of gut-derived lipopolysaccharide (LPS) to the CNS, inhibiting the over-activation of TLR4/NF-κB/NLRP3 pathway, and alleviating the inflammatory reaction of the CNS (Liao et al., 2022). Furthermore, EA stimulation of GV20, BL23 and KI3 acupoints inhibits the NF-κB/NLRP3 inflammasome pathway and improves CUMS-induced depressive behavior (Wang et al., 2022). In addition to EA, MA can significantly improve the depressive behavior of CUMS-induced rats at GV23 and GV16, the mechanism of which involves inhibiting the expression of NLRP3, ASC, caspase-1, IL-1β, IL-18, GSDMD, HMGB1, IFN-γ, IL-6, and TNF-α in serum and hippocampus. The above reports indicate acupuncture prevents CUMS-induced depression-like behaviors by reducing NLRP3-mediated pyroptosis and inflammatory responses (Chen et al., 2022a). Moreover, the antidepressant effect of acupuncture seems to be related to the inhibition of apoptosis in the prefrontal cortex (PFC). Acupuncture at GV20 and GV29 acupoints can reduce the number of TUNEL-positive cells and lower the protein expression of NLRP3, ASC coupled with caspase-1 in PFC (Wang H. M. et al., 2020; Li X. et al., 2021).

Other studies have found that patients with inflammatory bowel disease (IBD) are more susceptible to depression, with a prevalence rate of 33.1% (Gao et al., 2021). We all know, IBD is closely related to the activation of the NLRP3 inflammasome, inferring that NLRP3 is expected to become a new therapeutic target for IBD (Song et al., 2021). It was found that EA alleviated depression-like behavior in colitis model rats through their effects on the gut microbiome by modulating the hippocampal inflammatory response and metabolic disorders, as well as the hypothalamus-pituitary-adrenal (HPA) axis. EA at ST36 and SP6 not only significantly improved behavioral tests, but mechanistically it also upregulated the expression of ZO-1 and altered the composition of the gut microbiome (statistically increasing the density of producers of short-chain fatty acids such as *Ruminococcaceae*, *Phascolarctobacterium*, and *Akkermansiaceae*). Meanwhile, EA blocked the TLR4/NF-κB signaling pathways and NLRP3 inflammasome, along with downregulated the IL-1β level (Zhou F. et al., 2022; **Table 2**).

Acupuncture *via* NLRP3 inflammasome in the SCI

The traumatic event of SCI triggers a signaling cascade leading to glial activation, neuroinflammation, and neuron death. The activation of endocannabinoid receptor subtype 2 (CB2R) can reduce neuroinflammation by promoting the clearance of NLRP3, thus improving functional recovery of SCI. The mechanism of inhibiting neuroinflammation may be that CB2R promotes the differentiation of M2 macrophages/microglia, inhibits the differentiation of M1 macrophages/microglia, increases the expression of IL-10, and reduces IL-1β and IL-6 expression. In addition, activated CB2R also increases the ubiquitination of NLRP3, and interacts with autophagy related protein p62 and microtubule-associated proteins 1B light chain 3 (LC3) (Jiang et al., 2022). EA at GV14 and GV4 has the effect of promoting functional recovery after SCI and improving neuronal apoptosis. Furthermore, p38MAPK-mediated microglia activation and inflammatory reaction and JNK/p66Shc-mediated ROS generation and OS damage were both attenuated by EA. However, except for 50, 0.2, and 100 Hz EA fails to completely reverse the activation of microglia, apoptosis, inflammation, and the cascade of p38MAPK and NF-κB (Cheng et al., 2020).

Activation of NLRP3 is a vital mechanism of NLRP3 inflammasome activation and the inflammatory response following SCI. The dependence on cytosine monophosphate kinase 2 (CMPK2) catalytic activity provides opportunities for more effective control of NLRP3 inflammasome-associated diseases (Zhong et al., 2018). By constructing an adeno-associated virus (AAV) CMPK2 model to knock down the CMPK2 gene, Chen et al. found that EA at Jiaji (T9-T11) group and AAV CMPK2 group significantly improved the Basso Beattie Bresnahan score. EA and AAV CMPK2 group significantly reduced the protein expression levels of CMPK2, NLRP3, ASC, caspase-1, IL-18, and IL-1β, while the AAV CMPK2 blank group had the opposite results. In summary, CMPK2 promotes the expression of NLRP3, and EA downregulated the expression of CMPK2 and inhibited activation of NLRP3 inflammasome, which could elevate locomotion function in rats with SCI (Chen et al., 2022c). Furthermore, EA stimulation of GV9 and GV6 improves the locomotion of SCI rats, which is speculated to be inseparable from the up-regulation of calcitonin gene related peptide (CGRP) expression and the down-regulation of NLRP3, ASC, and caspase 1 expression in the spinal anterior horn tissue (Guo et al., 2021; **Table 2**).

Discussion

Acupuncture is one of the traditional methods of treating CNS diseases in traditional Chinese medicine. It has the function of regulating qi and blood, and dredging the meridians. Its therapeutic effect on stroke, AD, VD, SCI, depression and other CNS diseases has been confirmed. According to our retrieval strategy, we searched the CNS diseases listed above through the database, and found that acupuncture regulating NLRP3 inflammasome mainly concentrated Alzheimer's disease, vascular dementia, spinal cord injury, stroke and depression, and only one article about acupuncture treatment of Parkinson's disease. For example, EA

TABLE 2 Acupuncture methods for depression and SCI *via* NLRP3 inflammasome.

Methods	Disease	Tissue	Acupoints	Acupuncture strategy	Mechanisms	Animals	References
EA	Depression	Hippocampus	GV20; GB34	Dilatational wave, 2/100 Hz, 0.3 mA, 30 min, once every other day, 4 weeks	NLRP3↓; pro-IL-1β↓; IL-1β↓; ASC↓; IL-18↓; P2 × 7R↓; caspase-1↓; cleaved-caspase-1↓	SD rats	Yue et al. (2018)
MA	Depression	Hippocampus	GV23; GV16	20 min, once every other day, 28 days	NLRP3↓; IFN-γ; IL-6↓; ASC↓; IL-1β↓; caspase-1↓; IL-18↓; HMGB1↓; GSDMD↓; TNF-α↓	SD rats	Chen et al. (2022a)
EA	Colitis- related depression	Hippocampus	ST36; SP6	5 Hz, 0.2 mA, 30 min, once a day, 14 consecutive days	NLRP3↓; IL-1β↓; TLR4↓; NF-κB↓; p-NF-κB p65↓	SD rats	Zhou F. et al. (2022)
EA	Depression	Hippocampus	GV20; BL23; KI3	Sparse wave, 2 Hz, 0.6 mA, 15 min, once a day, 3 weeks	NLRP3↓; NF-κB↓; IL-6↓; IL-1β↓; IL-18↓; TNF-α↓	C57BL/6J mice	Wang et al. (2022)
MA	Depression	Prefrontal cortex	GV20; GV29	Twist at the speed of 60 r/min, 10 min, treatment 6 days per week, 6 weeks	NLRP3↓; ASC↓; IL-1β↓; caspase-1↓; IL-18↓	SD rats	Wang H. M. et al. (2020)
EA	SCI	Spinal cord	Jiaji (T9–T11)	2/100 Hz, 1 mA, 20 min, once a day, 1, 3, and 7 days	NLRP3↓; CMPK2↓; ASC↓; IL-1β↓; caspase-1↓; IL-18↓	SD rats	Chen et al. (2022c)
EA	SCI	Spinal cord	GV9; GV6	Continuous wave, 2 Hz, 30 min, once a day, 7 and 14 days	NLRP3↓; CGRP↑; ASC↓; caspase-1↓;	SD rats	Guo et al. (2021)

MA, manual acupuncture; EA, electroacupuncture; SCI, spinal cord injury.

ameliorated dopaminergic neuron damage in PD rats through inhibiting NLRP3/Caspase-1 mediated neuronal pyrosis (Liu et al., 2022). Since there are few studies involving acupuncture to regulate NLRP3 inflammasome in the treatment of other CNS diseases, our study only discusses five diseases, including AD, VD, depression, stroke, and SCI.

Acupuncture regulate microglia neuroinflammation in CNS diseases

With the development of modern advanced science and technology and scientists' in-depth exploration of the physiological and biological mechanisms of acupuncture, so far, the mechanisms of acupuncture have involved central sensitization, neuroinflammation, neurotransmitters, immune regulation, oxidative stress, intestinal flora, etc. (Zhang et al., 2022). After integrating information in the brain, acupuncture modulates multiple neuroimmune pathways (including the vagus-adrenal medulla-dopamine, cholinergic anti-inflammatory, and sympathetic pathways, as well as the HPA axis) that ultimately act on immune cells by releasing crucial neurotransmitters and hormones (Li N. et al., 2021).

Microglia is an innate immune effector cell of the CNS, which plays the role of immune surveillance. Since microglia are the main source of inflammatory factors, neuroinflammation caused by its overactivation is the key to many CNS diseases. Further, activation of microglia and inflammation-mediated neurotoxicity are suggested to have essential roles in the pathogenesis of several neurodegenerative disorders. The activated microglia are classified into M1 and M2 phenotypes, which exert pro-inflammatory and anti-inflammatory effects, respectively. More and more studies have verified that acupuncture can inhibit neuritis by regulating the microglia phenotype, thereby improving or reversing the pathological process of CNS diseases (Wang L. et al., 2020; Li D. et al., 2021).

Mechanism of acupuncture regulating NLRP3 inflammasome in CNS diseases

In CNS diseases animal models, such as AD, VA, depression, stroke and SCI, acupuncture regulated the NLRP3 inflammasome activation with the characteristics of multiple targets, multiple links and multiple pathways. In the priming stage, acupuncture reduced inflammatory factors (TLR4, TNF-α, IL-1β etc.) to inhibit the expression of NLPR3. In the activation stage, acupuncture constricted the oligomerization of NRLP3 inflammasome by lessening OS reaction and the expression level of each component of NLRP3 inflammasome (NLRP3, ASC, pro caspase-1). EA also decreased pro-caspase-1, caspase-1, pro-IL-1β and GSDMD by regulating the NLRP3 inflammasome mediated pyroptosis, inhibited the release of inflammatory factor (IL-1β, HMGB1, IL-18, INF- γ) and eventually mitigated neuroinflammatory response.

Additionally, EA diminished the level of ROS, the ratio of LC3-II/LC3-I, and inhibited the expression of NLRP3 and beclin 1 proteins, which potentially inhibited the activation of NLRP3 inflammasome. However, how EA regulated autophagy pathway and the interaction between autophagy and NLRP3 inflammasome need further study (Qiu et al., 2022). In addition, EA played a neuroprotective role by increasing miR-223 in the periinfarct cortex of MCAO rats to inhibit the levels of NLRP3, IL-1β and caspase-1 (Sha et al., 2019). Acupuncture suppressed TXNIP-mediated NLRP3 and IL-1β in VD rats (Du et al., 2018). EA downregulated the expression of CMPK2 and restrained activation of NLRP3 inflammasome, which could restore locomotion function in rats with SCI (Chen et al., 2022c). Moreover, EA downregulated of NLRP3, ASC, and caspase-1 expression in the spinal anterior horn tissue by raising CGRP expression (Guo et al., 2021).

It was noteworthy that the therapeutic effects of EA with different frequencies were different. 10 Hz EA was more efficient than 2 Hz EA in reducing the number of TUNEL positive cells in CA1 area of SAMR1 mice. The potential reason was that 10 Hz EA significantly reduces NLRP3, ASC, caspase-1, GSDM-D, IL-1 β, and IL-18 levels, but 2 Hz EA failed to effectively reduce the expression of ASC protein (Hou et al., 2020). However, excepted for 50, 0.2, and 100 Hz EA failed to completely reverse the activation of microglia, apoptosis, inflammation, and the cascade of p38MAPK and NF-κB (Cheng et al., 2020).

Regulation of NLRP3 inflammasome

The activation of NLRP3 inflammasome is often accompanied by a variety of regulatory mechanisms. Ubiquitination of NLRP3 exerts a bidirectional regulatory role in activating the NLRP3 inflammasome, depending on the type of ubiquitin ligase and ubiquitination. Moreover, phosphorylation of NLRP3 may have a reaction to the activation of inflammasome, but the specific mechanism is unclear. The activation process of NLRP3 inflammasome is also related to the role of other proteins. For example, TXNIP can activate NLRP3 inflammasome after interacting with NLRP3 as an oxidation sensor. CMPK2, belonging to the nucleotide kinase family, can activate NLRP3 and play a crucial role in chronic inflammatory diseases. In turn, activation of the NLRP3 inflammasome relies on the catalytic activity of CMPK2, which provides a target for more effective control of NLRP3 inflammasome-associated diseases (Zhong et al., 2018).

Significantly, it has been found that all stimuli to NLRP3, whether or not they induce K^+ efflux or NLRP3 mutations, require NIMA-related kinase-7 (Nek7) to activate the NLRP3 inflammasome. Thus, as a proximal regulator of NLRP3 oligomerization and an assembly component of the NLRP3 inflammasome, Nek 7 is a crucial regulatory molecule during the activation (He et al., 2016; Swanson et al., 2019). Besides, DDX3X is also a protein necessary for stimulating NLRP3 inflammasome (Samir et al., 2019). To sum up, since it involves a myriad of factors, the activation process of NLRP3 is sophisticated and indeterminate, which depends on in-depth studies to elaborate on the exact regulatory principles.

Summary and future directions

This article summarized the role of NLRP3 inflammasome in a variety of common CNS diseases, expounded on its critical activation and regulation mechanisms, and detailed how acupuncture can improve CNS diseases *via* regulating the activation of NLRP3. With the development of immunological research, it has become increasingly clear that NLRP3 plays an indispensable role in different CNS diseases. Neuroinflammation is a cascade of immune responses mediated by innate immune residents of the CNS that can be triggered by damaging processes such as ischemia and hypoxia (Sha et al., 2021). In one respect, neuroinflammation maintains the stability of the microenvironment, but it can also cause damage to brain cells and neurons due to excessive activation of the inflammatory response (Uddin et al., 2020; Dhapola et al., 2021). Despite its importance in infections and sterile tissue damage, the exact mechanisms controlling and enabling NLRP3 activation are still being elucidated. Diverse cellular perturbations trigger NLRP3 activation, including the disruption of cellular ion homeostasis, lysosomes, and mitochondrial function or metabolism. However, how these perturbations relate to each other and how they converge on a common molecular mechanism that activates NLRP3 remain ongoing areas of research. Deeply clarifying the role of NLRP3 inflammasome in CNS diseases will lay a foundation for comprehensively understanding the occurrence and development of diseases and proposing specific targeted treatment methods with NLRP3 inflammasome inhibitors as the core.

Up to now, the research on the mechanism of acupuncture has been conducted by means of analysis and reduction. It is undeniable that the research method with reductionism as the main body has brought us a lot of new knowledge in the exploration of the mechanism of acupuncture, as well as a series of reliable evidence for the scientific nature of acupuncture therapy. However, due to the complexity of individual, as well as the bidirectional, multi-level and multi-target nature of acupuncture regulation, it is difficult for the research method with reductionism as the main body to give a complete answer to the relevant mechanism. Although some studies have provided partial evidence for EA in remedying CNS diseases, there is still a lack of high-quality randomized controlled trials to ascertain the efficacy and safety of EA in CNS diseases from an overall level. MA differs from EA with electrical stimulation signals. Many studies have shown that the frequency and intensity of acupuncture will produce different therapeutic effects. Future research directions, it is suggested, further explore the frequency, intensity and duration of MA or EA on CNS diseases.

Author contributions

H-MZ, RC, and F-XL conceived the main ideas. H-MZ, J-LZ, and DL wrote the initial manuscript. J-LZ and F-XL revised and edited the manuscript. S-HW and Y-JZ designed the framework. M-FZ and J-XL helped search the references. Z-MY helped illustrate the figures. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Anthony, S., Cabantan, D., Monsour, M., and Borlongan, C. V. (2022). Neuroinflammation, stem cells, and stroke. *Stroke* 53, 1460–1472. doi: 10.1161/STROKEAHA.121.036948

Barczuk, J., Siwecka, N., Lusa, W., Rozpedek-Kaminska, W., Kucharska, E., and Majsterek, I. (2022). Targeting NLRP3-mediated neuroinflammation in Alzheimer's disease treatment. *Int. J. Mol. Sci.* 23:8979. doi: 10.3390/ijms23168979

Bauernfeind, F. G., Horvath, G., Stutz, A., Alnemri, E. S., Macdonald, K., Speert, D., et al. (2009). Cutting edge: Nf-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183, 787–791. doi: 10.4049/jimmunol.0901363

Bellver-Landete, V., Bretheau, F., Mailhot, B., Vallieres, N., Lessard, M., Janelle, M. E., et al. (2019). Microglia are an essential component of the neuroprotective scar that forms after spinal cord injury. *Nat. Commun.* 10:518. doi: 10.1038/s41467-019-08446-0

Boucher, D., Monteleone, M., Coll, R. C., Chen, K. W., Ross, C. M., Teo, J. L., et al. (2018). Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J. Exp. Med.* 215, 827–840. doi: 10.1084/jem.20172222

Cai, L., Yao, Z. Y., Yang, L., Xu, X. H., Luo, M., Dong, M. M., et al. (2022). "Mechanism of electroacupuncture against cerebral ischemia-reperfusion injury: Reducing inflammatory response and cell pyroptosis by inhibiting NLRP3 and caspase-1. *Front. Mol. Neurosci.* 15:822088. doi: 10.3389/fnmol.2022.822088

Chen, Y. J., Nguyen, H. M., Maezawa, I., Grossinger, E. M., Garing, A. L., Kohler, R., et al. (2016). The potassium channel Kca3.1 constitutes a pharmacological target for neuroinflammation associated with ischemia/reperfusion stroke. *J. Cereb. Blood Flow Metab.* 36, 2146–2161. doi: 10.1177/0271678X15611434

Chen, Y., Wang, H., Sun, Z., Su, X., Qin, R., Li, J., et al. (2022b). Effectiveness of acupuncture for patients with vascular dementia: A systematic review and meta-analysis. *Complement Ther. Med.* 70:102857. doi: 10.1016/j.ctim.2022.102857

Chen, Y., Hao, C., Chen, W., Cheng, W., Li, P., Shen, J., et al. (2022a). Anti-depressant effects of acupuncture: The insights from NLRP3 mediated pyroptosis and inflammation. *Neurosci. Lett.* 785:136787. doi: 10.1016/j.neulet.2022.136787

Chen, Y., Wu, L., Shi, M., Zeng, D., Hu, R., Wu, X., et al. (2022c). Electroacupuncture inhibits NLRP3 activation by regulating Cmpk2 after spinal cord injury. *Front. Immunol.* 13:788556. doi: 10.3389/fimmu.2022.788556

Cheng, M., Wu, X., Wang, F., Tan, B., and Hu, J. (2020). Electro-acupuncture inhibits p66Shc-mediated oxidative stress to facilitate functional recovery after spinal cord injury. *J. Mol. Neurosci.* 70, 2031–2040. doi: 10.1007/s12031-020-01609-5

Dhapola, R., Hota, S. S., Sarma, P., Bhattacharyya, A., Medhi, B., and Reddy, D. H. (2021). Recent advances in molecular pathways and therapeutic implications targeting neuroinflammation for Alzheimer's disease. *Inflammopharmacology* 29, 1669–1681. doi: 10.1007/s10787-021-00889-6

Dorothee, G. (2018). Neuroinflammation in neurodegeneration: Role in pathophysiology, therapeutic opportunities and clinical perspectives. *J. Neural Transm.* 125, 749–750. doi: 10.1007/s00702-018-1880-6

Du, S. Q., Wang, X. R., Zhu, W., Ye, Y., Yang, J. W., Ma, S. M., et al. (2018). Acupuncture inhibits TXNIP-associated oxidative stress and inflammation to attenuate cognitive impairment in vascular dementia rats. *CNS Neurosci. Ther.* 24, 39–46. doi: 10.1111/cns.12773

Feigin, V. L., and Krishnamurthi, R. (2016). Stroke is largely preventable across the globe: Where to next? *Lancet* 388, 733–734. doi: 10.1016/S0140-6736(16)30679-1

Finger, C. E., Moreno-Gonzalez, I., Gutierrez, A., Moruno-Manchon, J. F., and Mccullough, L. D. (2022). Age-related immune alterations and cerebrovascular inflammation. *Mol. Psychiatry* 27, 803–818. doi: 10.1038/s41380-021-01361-1

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., and Santoro, A. (2018). Inflammaging: A new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14, 576–590. doi: 10.1038/s41574-018-0059-4

Gao, X., Tang, Y., Lei, N., Luo, Y., Chen, P., Liang, C., et al. (2021). Symptoms of anxiety/depression is associated with more aggressive inflammatory bowel disease. *Sci. Rep.* 11:1440. doi: 10.1038/s41598-021-81213-8

Guo, M., Xiang, J. Q., Wei, L. H., and Cui, Y. J. (2021). [Effect of electroacupuncture of "Zhiyang" (Gv9) and "Jizhong" (GV6) on expression of CGRP and NLRP3 in rats with spinal cord injury]. *Zhen Ci Yan Jiu* 46, 679–683.

He, C., Huang, Z. S., Chen, H. R., Yu, C. C., Wang, X. S., Jiang, T., et al. (2020). [Effect of pretreatment of acupuncture on learning-memory ability and related protein of NLRP3 inflammasome in hippocampus in Alzheimer's disease like rats]. *Zhongguo Zhen Jiu* 40, 1323–1327.

He, K., Hu, R., Huang, Y., Qiu, B., Chen, Q., and Ma, R. (2022). Effects of acupuncture on neuropathic pain induced by spinal cord injury: A systematic review and meta-analysis. *Evid. Based Complement Alternat. Med.* 2022:6297484. doi: 10.1155/2022/6297484

He, Y., Zeng, M. Y., Yang, D., Motro, B., and Nunez, G. (2016). Nek7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature* 530, 354–357. doi: 10.1038/nature16959

Hou, Z., Qiu, R., Wei, Q., Liu, Y., Wang, M., Mei, T., et al. (2020). Electroacupuncture Improves Cognitive Function in Senescence-Accelerated P8 (Samp8) Mice via the NLRP3/Caspase-1 Pathway. *Neural Plast* 2020, 8853720. doi: 10.1155/2020/8853720

Huang, Y., Wang, Y., Wang, H., Liu, Z., Yu, X., Yan, J., et al. (2019). Prevalence of mental disorders in China: A cross-sectional epidemiological study. *Lancet Psychiatry* 6, 211–224. doi: 10.1016/S2215-0366(18)30511-X

Huang, Z., Chen, Y., Xiao, Q., Kuang, W., Liu, K., Jiang, Y., et al. (2022). Effect of acupuncture for disorders of consciousness in patients with stroke: A systematic review and meta-analysis. *Front. Neurol.* 13:930546. doi: 10.3389/fneur.2022.930546

Ising, C., Venegas, C., Zhang, S., Scheiblich, H., Schmidt, S. V., Vieira-Saecker, A., et al. (2019). NLRP3 inflammasome activation drives tau pathology. *Nature* 575, 669–673. doi: 10.1038/s41586-019-1769-z

Jiang, F., Xia, M., Zhang, Y., Chang, J., Cao, J., Zhang, Z., et al. (2022). Cannabinoid receptor-2 attenuates neuroinflammation by promoting autophagy-mediated degradation of the NLRP3 inflammasome post spinal cord injury. *Front. Immunol.* 13:993168. doi: 10.3389/fimmu.2022.993168

Jiang, J., Ding, N., Wang, K., and Li, Z. (2018). Electroacupuncture could influence the expression of Il-1beta and NLRP3 inflammasome in hippocampus of Alzheimer's disease animal model. *Evid. Based Complement Alternat. Med.* 2018:8296824. doi: 10.1155/2018/8296824

Jiang, T., Wu, M., Zhang, Z., Yan, C., Ma, Z., He, S., et al. (2019). Electroacupuncture attenuated cerebral ischemic injury and neuroinflammation through alpha7nachR-mediated inhibition of NLRP3 inflammasome in stroke rats. *Mol. Med.* 25:22. doi: 10.1186/s10020-019-0091-4

Kou, L., Chi, X., Sun, Y., Han, C., Wan, F., Hu, J., et al. (2022). The circadian clock protein Rev-erbalpha provides neuroprotection and attenuates neuroinflammation against Parkinson's disease via the microglial NLRP3 inflammasome. *J. Neuroinflammation* 19:133. doi: 10.1186/s12974-022-02494-y

Li, D., Zhao, Y., Bai, P., Li, Y., Wan, S., Zhu, X., et al. (2021). Baihui (DU20)-penetrating-Qubin (GB7) acupuncture regulates microglia polarization through miR-34a-5p/Klf4 signaling in intracerebral hemorrhage rats. *Exp. Anim.* 70, 469–478. doi: 10.1538/expanim.21-0034

Li, K., Shi, G., Zhao, Y., Chen, Y., Gao, J., Yao, L., et al. (2021). Electroacupuncture ameliorates neuroinflammation-mediated cognitive deficits through inhibition of NLRP3 in presenilin1/2 conditional double knockout mice. *Neural Plast.* 2021:8814616. doi: 10.1155/2021/8814616

Li, N., Guo, Y., Gong, Y., Zhang, Y., Fan, W., Yao, K., et al. (2021). The anti-inflammatory actions and mechanisms of acupuncture from acupoint to target organs via neuro-immune regulation. *J. Inflamm. Res.* 14, 7191–7224. doi: 10.2147/JIR.S341581

Li, X., Wang, H., Li, C., Wu, J., Lu, J., Guo, J. Y., et al. (2021). Acupuncture inhibits NLRP3 inflammasome activation in the prefrontal cortex of a chronic stress rat model of depression. *Anat. Rec.* 304, 2470–2479. doi: 10.1002/ar.24778

Liao, D. M., Pang, F., Zhou, M., Li, Y., Yang, Y. H., Guo, X., et al. (2022). [Effect of electroacupuncture on cognitive impairment in App/Ps1 mice based on TLR4/NF-kappaB/NLRP3 pathway]. *Zhen Ci Yan Jiu* 47, 565–572.

Lin, C. J., Yeh, M. L., Wu, S. F., Chung, Y. C., and Lee, J. C. (2022). Acupuncture-related treatments improve cognitive and physical functions in Alzheimer's disease: A systematic review and meta-analysis of randomized controlled trials. *Clin. Rehabil.* 36, 609–635. doi: 10.1177/02692155221079117

Lin, J. G., Kotha, P., and Chen, Y. H. (2022). Understandings of acupuncture application and mechanisms. *Am. J. Transl. Res.* 14, 1469–1481.

Liu, H., Zhang, B., Du, J., Feng, P. P., Ruan, C., Zhang, W. B., et al. (2020). [Effect of acupuncture on NLRP3 inflammatory corpuscle in rats with intracerebral hemorrhage]. *Zhongguo Zhen Jiu* 40, 757–763.

Liu, S., Wang, Z., Su, Y., Qi, L., Yang, W., Fu, M., et al. (2021). A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis. *Nature* 598, 641–645. doi: 10.1038/s41586-021-04001-4

Liu, Y. Y., Guo, Y. B., Zhai, H. Y., Lei, D. B., Wang, H., Zhao, S. C., et al. (2022). [Effect of electroacupuncture regulating NLRP3/Caspase-1 pathway on pyroptosis of dopaminergic neurons in rats with Parkinson's disease]. *Zhen Ci Yan Jiu* 47, 983–992.

Lu, J., Xu, X., Huang, Y., Li, T., Ma, C., Xu, G., et al. (2021). Prevalence of depressive disorders and treatment in China: A cross-sectional epidemiological study. *Lancet Psychiatry* 8, 981–990. doi: 10.1016/S2215-0366(21)00251-0

Lunemann, J. D., Malhotra, S., Shinohara, M. L., Montalban, X., and Comabella, M. (2021). Targeting inflammasomes to treat neurological diseases. *Ann. Neurol.* 90, 177–188. doi: 10.1002/ana.26158

Moonen, S., Koper, M. J., Van Schoor, E., Schaeverbeke, J. M., Vandenberghe, R., von Arnim, C., et al. (2022). Pyroptosis in Alzheimer's disease: Cell type-specific activation in microglia, astrocytes and neurons. *Acta Neuropathol.* 145, 175–195. doi: 10.1007/s00401-022-02528-y

Qiu, R. R., Zhang, H., Deng, C., Chen, D. F., Xu, Y. Y., Xiong, D., et al. (2022). [Effects of electroacupuncture on Ros-NLRP3 inflammatory pathway and autophagy related proteins in hippocampus of vascular dementia rats]. *Zhen Ci Yan Jiu* 47, 298–304.

Qu, Y., Wu, J. Y., and Yuan, Q. (2021). [Effect of acupuncture on hippocampal inflammation in rats with intrauterine distress induced hypoxic-ischemic brain injury]. *Zhen Ci Yan Jiu* 46, 14–20.

Samir, P., Kesavardhana, S., Patmore, D. M., Gingras, S., Malireddi, R., Karki, R., et al. (2019). DDX3X acts as a live-or-die checkpoint in stressed cells by regulating NLRP3 inflammasome. *Nature* 573, 590–594. doi: 10.1038/s41586-019-1551-2

Severini, C., Barbato, C., Di Certo, M. G., Gabanella, F., Petrella, C., Di Stadio, A., et al. (2021). Alzheimer's disease: New concepts on the role of autoimmunity and NLRP3 inflammasome in the pathogenesis of the disease. *Curr. Neuropharmacol.* 19, 498–512. doi: 10.2174/1570159X18666200621204546

Sha, R., Zhang, B., Han, X., Peng, J., Zheng, C., Zhang, F., et al. (2019). Electroacupuncture alleviates ischemic brain injury by inhibiting the miR-223/NLRP3 pathway. *Med. Sci. Monit.* 25, 4723–4733. doi: 10.12659/MSM.917213

Sha, S., Tan, J., Miao, Y., and Zhang, Q. (2021). The role of autophagy in hypoxia-induced neuroinflammation. *DNA Cell Biol.* 40, 733–739. doi: 10.1089/dna.2020.6186

Song, Y., Zhao, Y., Ma, Y., Wang, Z., Rong, L., Wang, B., et al. (2021). Biological functions of NLRP3 inflammasome: A therapeutic target in inflammatory bowel disease. *Cytokine Growth Factor Rev.* 60, 61–75. doi: 10.1016/j.cytogfr.2021.03.003

Sun, H., Zhao, H., Ma, C., Bao, F., Zhang, J., Wang, D. H., et al. (2013). Effects of electroacupuncture on depression and the production of glial cell line-derived neurotrophic factor compared with fluoxetine: A randomized controlled pilot study. *J Altern. Complement Med.* 19, 733–739. doi: 10.1089/acm.2011.0637

Sun, L., Yong, Y., Wei, P., Wang, Y., Li, H., Zhou, Y., et al. (2022). Electroacupuncture ameliorates postoperative cognitive dysfunction and associated neuroinflammation via NLRP3 signal inhibition in aged mice. *CNS Neurosci. Ther.* 28, 390–400. doi: 10.1111/cns.13784

Swanson, K. V., Deng, M., and Ting, J. P. (2019). The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* 19, 477–489. doi: 10.1038/s41577-019-0165-0

Tian, Z., Ji, X., and Liu, J. (2022). Neuroinflammation in vascular cognitive impairment and dementia: Current evidence, advances, and prospects. *Int. J. Mol. Sci.* 23:6224. doi: 10.3390/ijms23116224

Troubat, R., Barone, P., Leman, S., Desmidt, T., Cressant, A., Atanasova, B., et al. (2021). Neuroinflammation and depression: A review. *Eur. J. Neurosci.* 53, 151–171. doi: 10.1111/ejn.14720

Uddin, M. S., Kabir, M. T., Mamun, A. A., Barreto, G. E., Rashid, M., Perveen, A., et al. (2020). Pharmacological approaches to mitigate neuroinflammation in Alzheimer's disease. *Int. Immunopharmacol.* 84:106479. doi: 10.1016/j.intimp.2020.106479

Wang, H. M., Li, C., Li, X. Y., Zhao, Y., Lu, J., Wu, J. H., et al. (2020). [Effects of acupuncture on Nod-like receptor protein 3 inflammasome signal pathway in the prefrontal cortex of rat with depression]. *Zhen Ci Yan Jiu* 45, 806–811.

Wang, L., Yang, J. W., Lin, L. T., Huang, J., Wang, X. R., Su, X. T., et al. (2020). Acupuncture attenuates inflammation in microglia of vascular dementia rats by inhibiting miR-93-mediated Tlr4/MyD88/Nf-kappaB signaling pathway. *Oxid. Med. Cell Longev.* 2020:8253904. doi: 10.1155/2020/8253904

Wang, Q., Bi, H., Huang, H., Wang, Y., Gong, L., Qi, N., et al. (2022). Electroacupuncture prevents the depression-like behavior by inhibiting the NF-kappaB/NLRP3 inflammatory pathway in hippocampus of mice subjected to chronic mild stress. *Neuropsychobiology* 81, 237–245. doi: 10.1159/000521185

Wei, W., and Qiu, Z. (2022). Diagnostics and theranostics of central nervous system diseases based on aggregation-induced emission luminogens. *Biosens. Bioelectron.* 217:114670. doi: 10.1016/j.bios.2022.114670

Wu, Y., Jin, S., Guo, J., Zhu, Y., Chen, L., and Huang, Y. (2022). The economic burden associated with depressive symptoms among middle-aged and elderly people with chronic diseases in China. *Int. J. Environ. Res. Public Health* 19:12958. doi: 10.3390/ijerph191912958

Xie, L., Liu, Y., Zhang, N., Li, C., Sandhu, A. F., Williams, G. R., et al. (2021). Electroacupuncture improves M2 microglia polarization and glia anti-inflammation of hippocampus in Alzheimer's disease. *Front. Neurosci.* 15:689629. doi: 10.3389/fnins.2021.689629

Yang, Y., Zhao, X., Zhu, Z., and Zhang, L. (2022). Vascular dementia: A microglia's perspective. *Ageing Res. Rev.* 81:101734. doi: 10.1016/j.arr.2022.101734

Yao, H., Zhang, D., Yu, H., Yuan, H., Shen, H., Lan, X., et al. (2022). Gut microbiota regulates chronic ethanol exposure-induced depressive-like behavior through hippocampal NLRP3-mediated neuroinflammation. *Mol. Psychiatry*. 28, 919–930. doi: 10.1038/s41380-022-01841-y

Yu, C. C., Du, Y. J., Li, J., Li, Y., Wang, L., Kong, L. H., et al. (2022). Neuroprotective mechanisms of puerarin in central nervous system diseases: Update. *Aging Dis.* 13, 1092–1105. doi: 10.14336/AD.2021.1205

Yue, N., Li, B., Yang, L., Han, Q. Q., Huang, H. J., Wang, Y. L., et al. (2018). Electro-acupuncture alleviates chronic unpredictable stress-induced depressive- and anxiety-like behavior and hippocampal neuroinflammation in rat model of depression. *Front. Mol. Neurosci.* 11:149. doi: 10.3389/fnmol.2018.00149

Zhang, B., Shi, H., Cao, S., Xie, L., Ren, P., Wang, J., et al. (2022). Revealing the magic of acupuncture based on biological mechanisms: A literature review. *Biosci. Trends* 16, 73–90. doi: 10.5582/bst.2022.01039

Zhong, X., Chen, B., Li, Z., Lin, R., Ruan, S., Wang, F., et al. (2022). Correction to: Electroacupuncture ameliorates cognitive impairment through the inhibition of NLRP3 inflammasome activation by regulating melatonin-mediated mitophagy in stroke rats. *Neurochem. Res.* 47, 1931–1933. doi: 10.1007/s11064-022-03590-4

Zhong, Z., Liang, S., Sanchez-Lopez, E., He, F., Shalapour, S., Lin, X. J., et al. (2018). New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature* 560, 198–203. doi: 10.1038/s41586-018-0372-z

Zhou, F., Jiang, H., Kong, N., Lin, J., Zhang, F., Mai, T., et al. (2022). Electroacupuncture attenuated anxiety and depression-like behavior via inhibition of hippocampal inflammatory response and metabolic disorders in TNBS-induced Ibd rats. *Oxid. Med. Cell Longev.* 2022:8295580. doi: 10.1155/2022/8295580

Zhou, K. Q., Green, C. R., Bennet, L., Gunn, A. J., and Davidson, J. O. (2019). The role of connexin and pannexin channels in perinatal brain injury and inflammation. *Front. Physiol.* 10:141. doi: 10.3389/fphys.2019.00141

Zhou, Z., Xu, G., Huang, L., Tian, H., Huang, F., Liu, Y., et al. (2022). Effectiveness and safety of electroacupuncture for depression: A systematic review and meta-analysis. *Evid. Based Complement Alternat. Med.* 2022:4414113. doi: 10.37766/inplasy2022.1.0068

Zhu, B. (2021). [On the acupoint and its specificity]. *Zhongguo Zhen Jiu* 41, 943–950.

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Acupuncture for the treatment of thalamencephalic and mesencephalic injury secondary to electrical trauma: A case report

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In a case of thalamencephalic and mesencephalic injury secondary to electrical trauma, a 29-year-old patient has been receiving manual acupuncture for 17 months in National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion. As a result of treatment, the patient's self-care ability and quality of life have greatly improved. In order to fully understand how acupuncture can benefit neurological sequelae resulting from electrical trauma, further research is needed. Additionally, there should be consideration given to the promotion of acupuncture therapy in the neurological sequelae of electric shock.

KEYWORDS

acupuncture, central nervous system disease, brain injury, neurological sequelae, electrical trauma, cerebral infarction

Introduction

An electric injury is caused by artificial current flowing through the body. Patients are more likely to be male, and the majority of these injuries are caused by low voltage (<1,000 V) accidents or work-related accidents. The burn ward or intensive care unit (ICU) is typically the first place a patient is admitted due to severe systemic burns after an electrical injury. However, due to the diversity of its clinical manifestations involving multiple organs and systems, it is often requires the cross-pollination of multiple disciplines for unconventional diagnosis and treatment, moreover, the prevention, follow-up, and treatment of complications following electrical trauma are vital.

When the current penetrates irregularly into the deep tissue of the body, aside from the appearance of clear boundary burning on the skin (redness, blisters, breakage, etc.), it can also damage the nervous system directly. There are several types of nerve injuries, including ischemic encephalopathy, cerebellar ataxia, hypoxic encephalopathy, intracranial hemorrhage, damage to other areas of the central nervous system, and peripheral nerve injuries. In electrical shocks, nerve tissue has a low electrical resistance compared to other tissues, explaining the high incidence (31∼81.6%) of nervous system injury (Singerman et al., 2008; Warenits et al., 2020). Furthermore, owing to the gap between developing and developed countries in health care construction and the results of epidemiological investigations of electrical injuries reported in different regions are inherently different, it is possible that the true incidence of nervous system injury after electrical shock is higher in developing countries and higher than the figures reported above.

According to current literature, neurologic syndromes after electrical injury can be classified in two classifications. Cherington' classification: Cherington (2003, 2005) proposed four groups of electrical injury based on time of onset, duration of symptoms, and severity of the clinical situation and whether a secondary event to other mishaps (I. immediate and transient, II. immediate and prolonged or permanent, III. delayed, IV. traumatic lesions secondary to falls and blast effects). Andrews' classification: Andrews and Reisner (2017) framed the electrical injury in an anatomic classification (I. Central syndromes, II. Cerebellar syndromes, III. Cranial nerve and related disorders, IV. Autonomic syndromes, V. Spinal cord disorders, VI. Paralyzes, VII. Peripheral nerve disorders, VIII. Sensory abnormalities). Understanding the damage to the nervous system after electrical trauma is crucial for developing effective treatment plans to improve quality of life for survivors. In spite of the fact that neurological sequelae are very common after electric injury, there are no guidelines or reports that give a clear treatment plan for acute ischemic stroke after electric shock, which is one of the most severe neurological complications.

Acupuncture is one of the traditional Chinese medicine treatments, which is a therapeutic operation *via* nondrug afferent stimuli. As a representative of the external treatment of traditional Chinese medicine, acupuncture has been inherited through the generations and still plays an important role in clinical treatment. The research results of acupuncture have been published many times in top international journals such as Nature, BMJ, JAMA and Annals of Internal Medicine, indicating that the effectiveness of acupuncture therapy is gradually gaining international recognition (Liu, 2022). The disease spectrum of acupuncture has been continuously expanded, especially in nervous system diseases, and its safety and effectiveness have been continuously confirmed by modern evidence-based medicine (Xu et al., 2018; Lu et al., 2022). In China, acupuncture treatment for ischemic strokes has been included in traditional Chinese medicine (TCM) rehabilitation guidelines. There are a variety of regulating effects of acupuncture on the nervous system, which are capable of promoting the healing of various injuries of the nervous system and improving neurological function problems. Hence, it is recommended as a level A evidence in the TCM rehabilitation guidelines for the treatment of ischemic stroke and as a level I evidence (Zhang et al., 2021). Considering this, we came up with an interesting question—could acupuncture be used to treat neurological sequelae caused by electrical trauma? However, only four cases of acupuncture as a treatment for electrical injury-induced neurological sequelae have been reported to date and are all reported in China (Huang, 1957; Zheng, 1986; Shao, 1997; Hao, 2005). Despite electrical current is known to cause damage to the nervous system, the pathogenesis of nerve injury after electrical trauma is unknown. The evidence that acupuncture can treat various nervous system diseases after electrical trauma has not been fully explored.

We report a case of thalamencephalic and mesencephalic injury secondary to electrical trauma. By Cherington's classification, he was categorized as a group II, and By Andrew's classification, he showed symptoms of types I and III. In the second month after electrical trauma, the National Institute of Health stroke scale (NIHSS) score was significantly reduced after 3 weeks of acupuncture treatment (Score dropped from 9 to 6, and language function improved). The patient was able to walk independently after 17 months of continuous acupuncture treatment (the NIHSS score had reduced to 3, and motor function improved). There has been a significant improvement in quality of life. We hope to share this case report with colleagues around the world *via* communication platforms.

Case description

In April 2021, a 29-year-old right-handed man suffered an electrical injury after coming into contact with a 220 v wire in the staff dormitory. When a colleague discovered the condition, the patient was unconscious and had urinary incontinence. Then, the patient was rushed to the hospital's emergency room. At that time, the patient had partial exfoliation of the epidermis of both great toes, as well as subcutaneous congestion around the orbit of the left eye and left shoulder. After analeptic and diuretic treatment, the patient was transferred to the burn department for indwelling gastric tube and urinary tube, and received a higher level of care. Additionally, neurological physicians and neurosurgeons was consulted immediately.

Initially, the patient was in a coma with bilateral pupils of equal size and roundness, both 8 mm in diameter, loss of light reflex, and positive Babinski sign. On admission, magnetic resonance imaging (MRI) of the cervical spine showed that the spinal cord measured normal in size, shape, and signal, the cervical disc herniation compressed the dural sac, the left vertebral artery's flow void signal was decreased, and the right vertebral artery was thin; magnetic resonance angiography (MRA) revealed the localized stenosis of P1 segment of bilateral posterior cerebral arteries (panel A), a poor local visualization of the C5 segment of bilateral internal carotid arteries, and the basal artery ring to be normal in size and shape, as shown in Figure 1; MRI of the head showed abnormal signals in midbrain and bilateral thalamus, considered cerebral infarction (top of the basilar syndrome), and soft tissue swelling was observed in the bilateral frontal, left parietal temporal, and left orbit areas. The patient became incapable of spontaneously expectorating 2 days after admission to the hospital, so a tracheotomy was performed. After examining the patient's neurological system, it was recommended that the patient repeat the MRI. A repeat imaging performed 10 days after the original MRI showed abnormal signals in the bilateral thalamic region, and midbrain area. MRA of the head showed localized stenosis in the clinoid segment of the bilateral internal carotid artery, slender right vertebral artery (panel B), and localized stenosis in the A2 segment of the bilateral anterior cerebral artery (panel C), as shown in Figure 1. As the patient was being hospitalized, his state of consciousness changed from coma to drowsiness, and he was able

Abbreviations: ICU, intensive care unit; TCM, traditional Chinese medicine; NISHH, National Institute of Health stroke scale; MRI, magnetic resonance imaging; MRA, magnetic resonance angiography; DWI, diffusion-weighted imaging; DTI, diffusion tensor imaging; RCT, randomized controlled trials; PTSD, post-traumatic stress disorder; LMN, lower motor neuron; TCD, transcranial doppler.

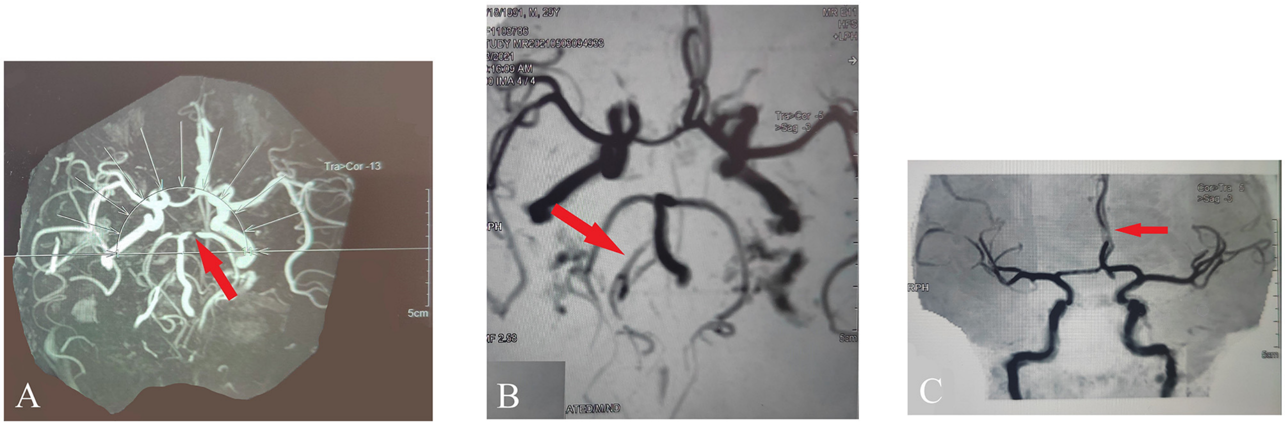

FIGURE 1
MRA revealed the artery's condition. **(A)** Localized stenosis of P1 segment of bilateral posterior cerebral arteries. **(B)** Slender right vertebral artery. **(C)** Localized stenosis in the A2 segment of the bilateral anterior cerebral artery.

to cooperate with simple commands (See Supplementary Video 1). A 41-day period after the electrical injury, the muscle strength of the patient's limbs recovered to grade 3. Approximately 70 days after the shock, the patient came to National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion for acupuncture treatment in hopes of improving his quality of life and self-care abilities.

At the time of his arrival at center's inpatient ward, the patient had a NIHSS score of 9 (2 points for the level of consciousness question and 2 points each for the upper and lower extremities movements, 3 points for language), dilated and stiff pupils. After 24 days of hospitalization, the patient was discharged with a NIHSS score of 6 (2 points each for the upper and lower extremities movements, 1 points for language, and 1 point for dysarthria) and there was no significant change in the pupil condition, eye movement showed external rotation, but no internal rotation, up rotation, or down rotation was demonstrated, and both right and left eyelid levator muscles were grade 0 in strength. In Figure 2, abnormal signals are still visible in the thalamus and midbrain after the MRI scan taken on December 28, 2021. As part of his rehabilitation treatment, the patient received regular acupuncture sessions 5–10 times per week in center's outpatient department and ward.

As for 5 December 2022, the patient had been receiving acupuncture for more than 17 months. Now he is able to walk without the cane, regain language function, and carry out daily communication with people independently, which has greatly improved his quality of life, but it is still difficult to lift the upper eyelid with right and left eyelid levator muscles due to a lack of strength in these muscles. In Supplementary Videos 2–4, you can see the effect of acupuncture on the speech and motor skills of the patient.

Acupuncture treatment

An acupuncturist with 20 years of experience in acupuncture provided him with acupuncture treatment based on the principle of "XingNaoKaiQiao." Patients are treated with matching acupoints based on their specific symptoms, such as such as limb weakness and language dysfunction, depending on the degree of their symptoms at each visit. As the patient lay supine, disposable filiform needles were inserted into the acupoints after skin disinfection. After manipulation, the needle was retained for 30 min each time. Table 1 shows the commonly used acupoints and their location, along with acupuncture manipulations. Figure 3 illustrates the acupoints for acupuncture.

Discussion

When an electrical accident occurs, the severity of tissue damage is closely related to characteristics of the power supply (low voltage <1,000 V or high voltage >1,000 V), the current type (alternating current or direct current), the propagation path of the current (Through chest or not), and the duration of contact (Bailey et al., 2007; Ahmed et al., 2021). Unlike lightning or direct current, patients always were exposed to alternating current for longer periods of time. There is a tendency for electric currents of 50 Hz to cause tonic muscle spasms [the "not let go" reaction (Rådman et al., 2016)], making the patient unable to release the power source without the assistance of someone else.

Annually, more than 3,000 people are admitted to burn centers in the United States because of electrical burns (Spies and Trohman, 2006). Burns caused by electric shock account for <5% of all burn center admissions, but they are associated with significant morbidity and mortality (Shih et al., 2017). Worldwide, nerve injuries caused by low-voltage electricity are probably much more common than we realize. Many patients do not report or consult physicians in the first moments after an electrical injury because most neurologic symptoms are mild and have a delayed onset. According to case reports, a 3-year-old child developed lower motor neuron (LMN) facial nerve palsy 1 day after experiencing a low-voltage electrical shock, and a 72-year-old man developed progressive weakness in both upper limbs 9 months after receiving a high-voltage electric shock, however, no one initially believed

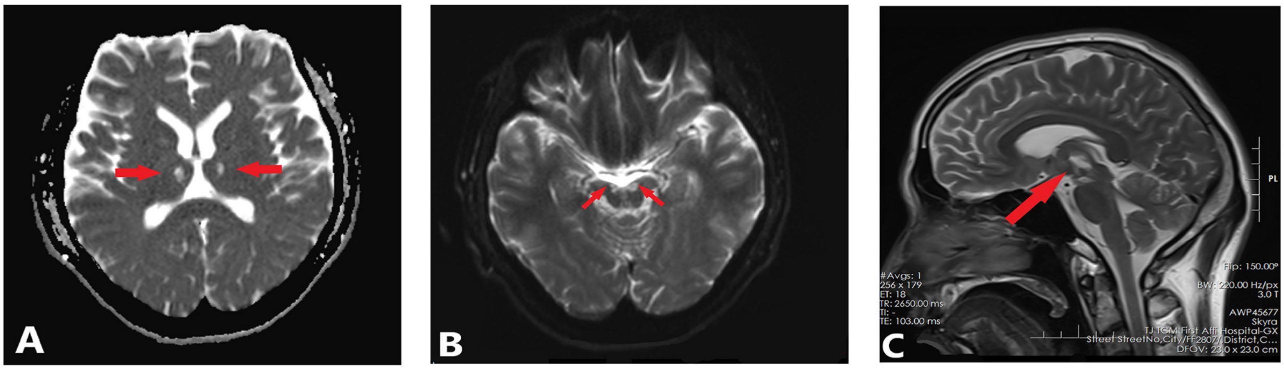

FIGURE 2
MRI revealed abnormal signals in the midbrain and thalamus. **(A)** The axial scanning of MRI revealed bilateral thalamic infarcts (DWI, diffusion-weighted imaging). **(B)** The axial scanning of MRI revealed Mesencephalic infarction (DWI, diffusion-weighted imaging). **(C)** The sagittal scanning of MRI revealed Mesencephalic infarction(T2-weighted MR images).

electric shock had been involved (Tashiro et al., 2000; Reddy et al., 2020). Besides consultation delay, missed diagnosis is also one of the reasons for the small number of nerve injury reports after electric shocks. In most cases, electrical damage to tissue cannot be detected by visual inspection or physical examination, and its sequelae are often apparent only after a certain period of time. Due to this, there is a high risk that nerve fiber damage will be missed when the overall burn appearance is not severe. An animal study has shown that electric shocks most damage myelinated nerve fibers that are large and fast-conducting (Abramov et al., 1996). Electron microscopy has also shown significant myelinated nerve fiber degeneration in peripheral nerves exposed to low-voltage electric shocks (Yvon et al., 2018). There is persistent severe neurological impairment in 50–73% of patients with low-voltage electrical injuries according to follow-up studies (Grube et al., 1990; Hussmann et al., 1995; Singerman et al., 2008; Tamam et al., 2013). Thus it can be seen that an electric shock-induced nerve injury is characterized by high incidence, delayed onset, and concealment. Therefore, it is necessary to conduct nerve sensory tests in patients with electrical injury in time to verify whether there is potential damage to the nervous system. Furthermore, T1 and T2 weighted images of MRI can assess the specificity of brain tissue as well as neural parenchymal changes in the brain (Chandrasekhar et al., 2018), and diffusion tensor imaging (DTI) is an effective technical tool to evaluate nerve tract damage (Devale et al., 2017). Unfortunately, the patient in our case report did not undergo DTI. Despite the fact that most neurological deficits can be reversed, a small number of progressive and permanent deficits pose the greatest risk of disability. As we all know, "prevention" is more important than "treatment," in addition to improving awareness of safe electricity use in daily life and enhancing emergency power outage measures, there is much more meaning in early detection of nerve damage symptoms and early application of neurotrophic and anti-inflammatory agents than in taking no measures to intervene the delayed symptoms.

In the published literatures, we found a case report of ischemic stroke after electrical injury in 2010. We compare the two cases in Table 2. In the 2010 case report, a patient underwent a low-voltage alternating current shock lasting 5 min and denied any head contusion, but developed an acute infarction in the right frontotemporal area involving the right basal ganglia and corona radiata. It is speculated by the authors of the 2010 case report that the ischemic stroke was caused by vasospasm or embolism of the supplying brain (Huan-Jui et al., 2010). We also thought that there is a high possibility of infarction of the apex of the brain stem caused by vasospasm due to the fact that our patient had no history of epilepsy or heart disease, and there was a similar electric shock experience with the above case (Unwitnessed continuous alternating current shock), in addition, there is radiographic evidence of vascular stenosis (The right vertebral artery is thin, the flow voids signal in the left vertebral artery has decreased, local stenosis of the P1 segment of bilateral posterior cerebral arteries, the C5 segment of bilateral internal carotid artery was poorly visualized). Further, the patient in 2021 case report has aphasia, cerebellar ataxia, visual field defects, oculomotor nerve palsy, and other manifestations of top of the basilar syndrome (Caplan, 1980). Of course, the presence of vasospasm needs to be confirmed further by transcranial Doppler (TCD). Based on the case report from 2010, it was necessary to use vascular spasmolytics (intravascular injection of nimodipine) and microcatheters to treat vasospasm and stenosis in the acute stage of electric injury, and at follow-up, the patient's dysarthria had improved after professional rehabilitation, but daily walking did not get rid of the quadricane (Huan-Jui et al., 2010). As a result of this case report, the patient's speech function improved almost perfectly without language training, and he did not need to use a walker to perform daily activities. A recent follow-up showed that the patient had recovered well, his weakness had significantly improved, and he can now live on his own at home. He described his condition as being between 70 and 80% recovered in his own words. Therefore, it is acceptable to include acupuncture as part of the rehabilitation treatment for patients who have suffered electric injuries to their nervous system. Acupuncture is widely used for treating neurological disorders, and has the advantages of being easy to operate, having few side effects, being safe, and so on. In response to questions about his current medication, the patient revealed that he does not currently take any medication orally due to potential side effects, which also indirectly proves that acupuncture is easily accepted by patients as a green treatment.

TABLE 1 Characteristics of acupuncture.

XingNaoKaiQiao acupuncture therapy	Acupoint selection	Acupoint location	Acupuncture manipulation
Mainpoints	Neiguan (Bilateral PC6)	In the volar aspect of the forearm, 2 cun above the wrist crease, between the tendons of radial wrist flexor and palmaris longus.	Puncture perpendicularly for 0.5–1 cun, using combinative reducing method (lifting-thrusting and twirling-rotating) for 1 min.
	Shuigou (Unilateral GV26)	In the face, at the junction of the upper 1/3 and middle 1/3 of the philtrum groovet.	Puncture obliquely upwards to the nasal septum for 0.3–0.5 cun with heavy bird-pecking method until the patient's eyeballs are moistened or tears flow.
	Sanyinjiao (SP6, bilateral)	In crus inside, 3 cun above the tip of the medial malleolus, at the posterior border of the tibia.	Puncture obliquely for 1∼1.5 cun, at the angle of 45° with the skin surface along the posterior border of the medial aspect of the tibia, with heavy insertion and light lifting manipulation to make the affected leg twitch three times.
Auxiliary points	Xiajiquan (Lower HT1, bilateral)	At the inner side of the upper arm, 1∼2 cun down from HT1, avoid axillary hair, on the muscles.	Puncture perpendicularly for 1∼1.5 cun with light insertion and heavy lifting manipulation to make the affected arm twitch three times.
	Chize (LU5, bilateral)	In the elbow, at the mid point of cubital crease, and the radialis depression of the biceps tendon.	Puncture perpendicularly for 1 cun, then lift and thrust needle until the needling feeling radiates from the elbow joint to the fingers.
	Weizhong (BL40, bilateral)	In the posterior region of knee, at the midpoint of popliteal crease.	The doctor lift the affected leg with one hand and against the knee joint with the elbow in order to straighten the affected limb and fully expose the popliteal space. Take the needle with the other hand, puncture perpendicularly or obliquely with the needle tip outward for 1∼1.5 cun, with light insertion and heavy lifting manipulation to make the affected leg twitch three times.
	Lian Quan (RN23, unilateral)	Above the prominentia laryngea, at the depression of superior border of hyoid bone.	Puncture perpendicularly for 1 cun, then twirling manipulation with the frequency of 60 times/min for 1 min.
	Yintang (EX-HN3, unilateral)	On the forehead, the midpoint between the glabella.	Puncture horizontally for 0.3∼0.5 cun with light bird-pecking method
	Fengchi (GB20, bilateral)	In the posterior cervical region, below the occipital bone, in the depression between the superior end of the sternocleidomastoid and the superior end of the trapezius.	Puncture perpendicularly for 1∼1.5 cun, then twirling manipulation with the frequency of 60 times/min for 1 min.
	Wangu (GB12, bilateral)	In the head, in the posteroinferior depression of the retroauricular mastoid.	Puncture perpendicularly for 1∼1.5 cun, then twirling manipulation with the frequency of 60 times/min for 1 min.
	Tianzhu (BL10, bilateral)	In the nape, in the hairline depression of the outer edge of the trapezius.	Puncture perpendicularly for 1∼1.5 cun, then twirling manipulation with the frequency of 60 times/min for 1 min.
	Zusanli (ST36, bilateral)	On the anterolateral side of the calf, when the lateral knee is 3 inches below, 1cun beyond the anterior border of the tibia.	Puncture perpendicularly for 1.5 cun without manipulation
	Taichong (LR3, bilateral)	On the dorsal side of the foot, in the depression preceding the junction of the first and second metatarsal bones.	Puncture perpendicularly for 1 cun without manipulation
	Yinlingquan (SP9, bilateral)	In the depression of the lower margin of the medial tibial condyle.	Puncture perpendicularly for 1.5∼2 cun without manipulation
	Yangbai (GB14, bilateral)	On the forehead, just above the pupil, 1 cun above the eyebrow.	Puncture horizontally for 0.3∼0.5 cun without manipulation

It was recommended to the world in 1979 by the World Health Organization that 43 indications of acupuncture be used, including those related to neuromuscular conditions, and acupuncture was approved for clinical use during stroke rehabilitation by the National Institutes of Health in 1997 (Liu, 2022). Study has shown that acupuncture has a positive effect on the recovery of cerebral perfusion, reducing degree of neurological damage, and improving prognosis for neurological function after stroke, which may related to the fact that acupuncture promotes blood vessel regeneration through the regulation of hemodynamics and the release of vasoactive substances (Wang et al., 2022). Moreover, acupuncture therapy promotes synaptic plasticity and

FIGURE 3
The selected acupoints.

TABLE 2 Two cases are compared based on their characteristics.

	Case in 2010	Case in 2021
Age	50	29
Gender	Male	Male
Type of current	60-Hz, 110-V alternating current supply	50-Hz, 220-V alternating current supply
Previous health status	Without any systemic disease or drug history	Rhinitis (In addition: stroke history was nonexistent)
Duration of current	More than 5 min	Unknown
head contusion	Denied any head contusion happened during fall down	No history of falling
laboratory investigations	All results within the normal limits	C-reactive protein, alanine aminotransferase, leukocytes, neutrophils, myoglobin, creatine kinase, and creatine kinase isoenzyme all increased
electrocardiogram	Normal	Normal
symptom	Retain consciousness, left limb weakness, glossolalia	Loss of consciousness, urinary incontinence
MRI	An acute infarction in the right frontotemporal area involving the right basal ganglia and corona radiata	Bilateral thalamic and midbrain infarction
MRA	Segmental narrowing of the siphon of the right internal carotid artery (ICA) and the M1 segment of right middle cerebral artery (MCA)	The left vertebral artery's flow void signal was decreased, and the right vertebral artery was thin, the localized stenosis of P1 segment of bilateral posterior cerebral arteries, a poor local visualization of the C5 segment of bilateral internal carotid arteries, localized stenosis in the clinoid segment of the bilateral internal carotid artery, localized stenosis in the A2 segment of the bilateral anterior cerebral artery, and slender right vertebral artery.
Endovascular treatment in the acute phase	Intravascular injection of nimodipine and microcatheter manipulation	Intravenous drip nalmefene hydrochloride injection, Xingnaojing injection, glycerol fructose and sodium chloride injection, furosemide injection
rehabilitation program	Physical, occupational and speech therapy	Acupuncture
Remaining symptoms during follow-up	This patient can walk with quadricane for daily activities and dysarthria improved in 2 months.	19 months after electrical trauma: weakness of the left and right levator palpebrae superioris, almost perfect speech and freedom from crutches, but emotional abnormalities

facilitates nerve repair through different ways (Qing et al., 2016). According to some studies, acupuncture has also been shown to be effective in regulating vasomotor contraction and preventing cerebral vasospasm (Ko et al., 2013; Li et al., 2022). However, it is unfortunate that the case report falls under Level IIIb of the evidence scale developed by the Center for Evidence-based Medicine at the University of Oxford and there are no systematic reviews or randomized controlled trials (RCTs) reports on acupuncture treatment for nerve sequelae resulting from electric injuries as far as. As a result, there is not enough evidence to support the use of acupuncture to treat nerve sequelae after electrical injury, and it is still difficult to promote its application. In spite of this, by sharing this case, we hope to inform colleagues that the use of acupuncture as an effective method in the management of neurological sequelae resulting from electrical trauma deserves further investigation.

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Need to add that, as part of our communication with the patient and his family, the family mentioned that the patient often cried without reason, had a low mood, and was experiencing a certain degree of cognitive decline. But there were no descriptions or treatments for this in his medical or medication history. Review of the studies found that survivors often suffer from psychological problems after electric shock, and long-term neuropsychological effects are common, mainly including post-traumatic stress disorder (PTSD), depression, insomnia, anxiety, cognitive impairment, and personality changes (Haq et al., 2008; Ramati et al., 2009; Andrews and Reisner, 2017; Fadhilah and Amin, 2019). There is a possibility that the mechanism involves the excessive release of NO, cortisol, oxygen free radicals, and glutamate by current stimulation on a large scale, moreover, after stroke, calcium overload, inflammation, oxidative stress, and other cascade reactions will occur in the local brain tissue as a result of ischemia and hypoxia. Upon complex interactions, these pathological factors or links produce cytotoxic effects on the vascular wall and neuronal tissue, ultimately leading to the emergence of neuropsychological symptoms (Andrews and Reisner, 2017; Rubino et al., 2022). Hence, it is important to pay attention to the mental state of electric shocks survivors. There are already some evidence-based evidence that acupuncture can alleviate post-stroke depression and cognitive impairment after stroke (Zhang et al., 2010; Zhou et al., 2020; Xie et al., 2022). However, due to the lack of attention and records on the psychological symptoms of patients in the early stage, it is difficult for us to make a corresponding assessment of whether acupuncture can have a positive effect on the psychological state of patients at this moment. In future clinical treatments, we will continue to track anxiety and depression levels and cognitive ability of patient with electric shock injury, and use neuropsychological scales to measure whether acupuncture can improve their mental state and cognitive abilities.

Conclusion

A variety of mechanisms may contribute to ischemic stroke caused by an electrical injury, one of which may be severe muscle spasm and sympathetic nerve excitation, resulting in vascular wall damage leading to vasospasm and thrombosis or embolism, another may be direct potential damage to the nervous system caused by the current. For doctors and electrical trauma patients, early professional neurological function tests should be performed to detect potential signs of neurological injury. Moreover, patients who have experienced electrical trauma may present with abnormal mental states, and managing PTSD and complex electrical trauma can be challenging for clinicians. Therefore, clinicians should pay extra attention to this issue and provide timely intervention. As shown in this case report, acupuncture combined with rehabilitation has a positive effect on the recovery from severe neurological injuries after electric shock, and is an effective, economical and safe auxiliary treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YD and ZM were responsible for the entire manuscript. HJ, YZ, and JZ conceived, designed, wrote, and revised the manuscript. BL, WZ, CL, and SD revised the manuscript and discussed interpretation. All authors approve the final manuscript prior to submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1139537/full#supplementary-material

References

Abramov, G. S., Bier, M., Capelli-Schellpfeffer, M., and Lee, R. C. (1996). Alteration in sensory nerve function following electrical shock. *Burns* 22, 602–606. doi: 10.1016/S0305-4179(96)00055-1

Ahmed, J., Stenkula, C., Omar, S., Ghanima, J., Bremtun, F. F., Bergan, J., et al. (2021). Patient outcomes after electrical injury: a retrospective study. *Scand. J. Trauma Resusc. Emerg. Med.* 29, 114. doi: 10.1186/s13049-021-00920-3

Andrews, C. J., and Reisner, A. D. (2017). Neurological and neuropsychological consequences of electrical and lightning shock: review and theories of causation. *Neural Regen. Res.* 12, 677–686. doi: 10.4103/1673-5374.206636

Bailey, B., Gaudreault, P., and Thivierge, R. L. (2007). Cardiac monitoring of high-risk patients after an electrical injury: a prospective multicentre study. *Emerg. Med. J.* 24, 348–352. doi: 10.1136/emj.2006.044677

Caplan, L. R. (1980). "Top of the basilar" syndrome. *Neurology* 30, 72–79. doi: 10.1212/WNL.30.1.72

Chandrasekhar, D. P., Noone, M. L., Babu, S. P. H., and Bose, V. T. C. (2018). Magnetic resonance imaging findings in brain resulting from high-voltage electrical shock injury of the scalp. *Indian J. Radiol. Imag.* 28, 312–314. doi: 10.4103/ijri.IJRI_368_17

Cherington, M. (2003). Neurologic manifestations of lightning strikes. *Neurology* 60, 182–185. doi: 10.1212/01.WNL.0000033801.15204.B5

Cherington, M. (2005). Spectrum of neurologic complications of lightning injuries. *NeuroRehabilitation* 20, 3–8. doi: 10.3233/NRE-2005-20102

Devale, M. M., Kadakia, G. J., Jain, V. G., and Munot, R. P. (2017). Direct electrical injury to brachial plexus. *Indian J. Plast. Surg.* 50, 217–219. doi: 10.4103/ijps.IJPS_177_16

Fadhilah, I., and Amin, M. M. (2019). Schizoaffective disorder that is induced by electrical voltage that is treated with risperidone. *Open Access. Maced. J. Med. Sci.* 7, 2667–2670. doi: 10.3889/oamjms.2019.399

Grube, B. J., Heimbach, D. M., Engrav, L. H., and Copass, M. K. (1990). Neurologic consequences of electrical burns. *J. Trauma* 30, 254–258. doi: 10.1097/00005373-199003000-00002

Hao, J. D. (2005). A case report of acupuncture at thirteen Gui points for the treatment of coma caused by electric injury. *Chin. Acupunct. Moxibust.* 7, 473.

Haq, M. Z., Prakash, R., Soy, A., Gupta, A., and Akhtar, S. (2008). Resolution of low voltage electrical injury induced psychosis with olanzapine. *Brain Inj.* 22, 361–364. doi: 10.1080/02699050801978955

Huang, S. S. (1957). A case of muscle spastic paralysis after electric injury was cured by acupuncture. *People's Milit. Surg.* 12, 73.

Huan-Jui, Y., Chih-Yang, L., Huei-Yu, L., and Po-Chih, C. (2010). Acute ischemic stroke in low-voltage electrical injury: a case report. *Surg. Neurol. Int.* 1, 83. doi: 10.4103/2152-7806.74093

Hussmann, J., Kucan, J. O., Russell, R. C., Bradley, T., and Zamboni, W. A. (1995). Electrical injuries: morbidity, outcome and treatment rationale. *Burns* 21, 530–535. doi: 10.1016/0305-4179(95)00037-C

Ko, C. N., Lee, I. W., Cho, S. Y., Lee, S. H., Park, S. U., Koh, J. S., et al. (2013). Acupuncture for cerebral vasospasm after subarachnoid hemorrhage: a retrospective case-control study. *J. Altern. Compl. Med.* 19, 471–473. doi: 10.1089/acm.2012.0076

Li, J., He, Y., Du, Y. H., Zhang, M., Georgi, R., Kolberg, B., et al. (2022). Effect of electro-acupuncture on vasomotor symptoms in rats with acute cerebral infarction based on phosphatidylinositol system. *Chin. J. Integr. Med.* 28, 145–152. doi: 10.1007/s11655-021-3341-6

Liu, C. Z. (2022). Reliability of clinical evidence for acupuncture: status quo and reflection. *J. Beijing Univ. Tradit. Chin. Med.* 45, 1018–1023.

Lu, L., Zhang, Y., Tang, X., Ge, S., Wen, H., Zeng, J., et al. (2022). Evidence on acupuncture therapies is underused in clinical practice and health policy. *BMJ* 376, e067475. doi: 10.1136/bmj-2021-067475

Qing, P., Chai, T. Q., Ding, H. M., Zhao, C. H., and Hu, J. (2016). Effect of electroacupuncture combined with rehabilitation training on neurological function and expression of neuronal growth associated protein 43 and synaptophysin in rats with focal cerebral ischemia/reperfusion injury. *Acupunct. Res.* 41, 314–320. doi: 10.13702/j.1000-0607.2016.04.006

Rådman, L., Nilsagård, Y., Jakobsson, K., Ek, Å., and Gunnarsson, L. G. (2016). Electrical injury in relation to voltage, "no-let-go" phenomenon, symptoms and perceived safety culture: a survey of Swedish male electricians. *Int. Arch. Occup. Environ. Health* 89, 261–270. doi: 10.1007/s00420-015-1069-3

Ramati, A., Rubin, L. H., Wicklund, A., Pliskin, N. H., Ammar, A. N., Fink, J. W., et al. (2009). Psychiatric morbidity following electrical injury and its effects on cognitive functioning. *Gen. Hosp. Psychiatry* 31, 360–366. doi: 10.1016/j.genhosppsych.2009.03.010

Reddy, D. P., Earan, S. K., and Kuppusamy, K. (2020). Electrical injury causing facial nerve palsy in a toddler. *Indian Pediatr.* 57, 76–77. doi: 10.1007/s13312-020-1713-1

Rubino, A., Bernardo, P., Russo, C., Tucci, C., D'Amato, L., Piccolo, V., et al. (2022). Immediate and progressive neurological damage after electrical injury: a pediatric case report. *Brain Dev.* 45, 87–91. doi: 10.1016/j.braindev.2022.08.007

Shao, C. J. (1997). Acupuncture for aphasia. *J. Clin. Acupunct. Moxibust.* Z1, 79–80. doi: 10.19917/j.cnki.1005-0779.1997.z1.075

Shih, J. G., Shahrokhi, S., and Jeschke, M. G. (2017). Review of adult electrical burn injury outcomes worldwide: an analysis of low-voltage vs. high-voltage electrical injury. *J. Burn Care Res.* 38, e293–e298. doi: 10.1097/BCR.0000000000000373

Singerman, J., Gomez, M., and Fish, J. S. (2008). Long-term sequelae of low-voltage electrical injury. *J. Burn Care Res.* 29, 773–777. doi: 10.1097/BCR.0b013e318184815d

Spies, C., and Trohman, R. G. (2006). Narrative review: electrocution and life-threatening electrical injuries. *Ann. Intern. Med.* 145, 531–537. doi: 10.7326/0003-4819-145-7-200610030-00011

Tamam, Y., Tamam, C., Tamam, B., Ustundag, M., Orak, M., and Tasdemir, N. (2013). Peripheral neuropathy after burn injury. *Eur. Rev. Med. Pharmacol. Sci.* 17, 107–111.

Tashiro, K., Inoue, I., Ohyagi, Y., Osoegawa, M., Fujimoto, M., Furuya, H., et al. (2000). A case of mortor neuron syndrome with onset 9 months after electrical injury. *Rinsho Shinkeigaku* 40, 732–735.

Wang, L., Su, X. T., Cao, Y., Yang, N. N., Hao, X. W., Li, H. P., et al. (2022). Potential mechanisms of acupuncture in enhancing cerebral perfusion of ischemic stroke. *Front. Neurol.* 13, 1030747. doi: 10.3389/fneur.2022.1030747

Warenits, A. M., Aman, M., Zanon, C., Klimitz, F., Kammerlander, A. A., Laggner, A., et al. (2020). International multi-center analysis of in-hospital morbidity and mortality of low-voltage electrical injuries. *Front. Med.* 7, 590758. doi: 10.3389/fmed.2020.590758

Xie, J., Geng, X., Fan, F., Fu, X., He, S., and Li, T. (2022). The efficacy of therapies for post-stroke depression in aging: an umbrella review. *Front. Aging Neurosci.* 14, 993250. doi: 10.3389/fnagi.2022.993250

Xu, M., Li, D., and Zhang, S. (2018). Acupuncture for acute stroke. *Cochrane Database Syst. Rev.* 3, Cd003317. doi: 10.1002/14651858.CD003317.pub3

Yvon, A., Faroni, A., Reid, A. J., and Lees, V. C. (2018). Selective fiber degeneration in the peripheral nerve of a patient with severe complex regional pain syndrome. *Front. Neurosci.* 12, 207. doi: 10.3389/fnins.2018.00207

Zhang, W., Lou, B. D., Li, J. X., Shi, W. Y., Liu, X. J., Tang, J., et al. (2021). Clinical practice guidelines of Chinese medicine rehabilitation for ischemic stroke (cerebral infarction). *Rehabil. Med.* 31, 437–447. doi: 10.3724/SP.J.1329.2021.06001

Zhang, Z. J., Chen, H. Y., Yip, K. C., Ng, R., and Wong, V. T. (2010). The effectiveness and safety of acupuncture therapy in depressive disorders: systematic review and meta-analysis. *J. Affect. Disord.* 124, 9–21. doi: 10.1016/j.jad.2009.07.005

Zheng, Y. G. (1986). Acupuncture rescue electroshock coma. *Shanghai J. Acupunct. Moxibust.* 4, 42. doi: 10.13460/j.issn.1005-0957.1986.04.026

Zhou, L., Wang, Y., Qiao, J., Wang, Q. M., and Luo, X. (2020). Acupuncture for improving cognitive impairment after stroke: a meta-analysis of randomized controlled trials. *Front. Psychol.* 11, 549265. doi: 10.3389/fpsyg.2020.549265

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Electroacupuncture and manual acupuncture at LR3 and ST36 have attenuating effects on hypertension and subsequent cognitive dysfunction in spontaneously hypertensive rats: A preliminary resting-state functional magnetic resonance imaging study

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Introduction: Chronic hypertension may have a contributory role toward cognitive impairment. Acupuncture exerts protective effects on cognitive functions while controlling the blood pressure. However, the neural mechanism underlying the dual attenuating effect of acupuncture remains unclear. In this study, we investigated the effects of electroacupuncture (EA) and manual acupuncture (MA) on the functional activity of the brain regions of spontaneously hypertensive rats (SHRs) by through resting-state functional magnetic resonance imaging (rs-fMRI). We also evaluated the differences in these functional activities between the EA and MA groups.

Methods: We randomly assigned 30 SHRs into the EA, MA, and model (SHR) groups. Wistar Kyoto rats ($n = 10$) were used as normal control (WKY). The interventions were administered once every alternate day for 12 weeks. The systolic blood pressure of all rats was recorded every 2 weeks until the end of the intervention. After the intervention, rs-fMRI scanning was performed to access the whole brain data of rats randomly selected from each group evenly. The amplitude of low frequency fluctuation (ALFF) analysis, regional homogeneity (ReHo) analysis, and functional connectivity (FC) analysis were also conducted. The Morris water maze (MWM) test was conducted to evaluate the learning and memory of the rats. Hematoxylin-eosin staining and Nissl staining were performed to observe histopathological changes in the key brain regions.

Results: We demonstrated that, when compared with the SHR group, the EA and MA groups had significantly lower blood pressure and better performance for behavioral test indices, and that the effect of EA was better than that of MA.

ALFF and ReHo analyses revealed enhancement of the neuronal activity of some functionally impaired brain areas in the EA and MA groups. The main callback brain regions included the hypothalamus, entorhinal cortex, brain stem, prelimbic cortex, cingulate cortex, corpus callosum, and cerebellum. The FC analysis demonstrated that EA and MA enhanced the functional connectivity between the seeds and brain regions such as the brain stem, entorhinal cortex, hippocampus, prelimbic cortex, and cerebellum. The pathological test of the entorhinal cortex also verified the protective effect of acupuncture on the neuronal functional activity.

Discussion: Our findings suggested that EA and MA exhibited attenuating effects on hypertension and cognitive dysfunction by enhancing the functional activities in the corresponding brain regions. Moreover, EA activated more callback brain regions and functional connectivity than MA, which may explain why the effect of EA was better than that of MA.

KEYWORDS

spontaneously hypertensive rats, cognitive functions, electroacupuncture, manual acupuncture, rs-fMRI

1. Introduction

Hypertension, a common chronic disease prevalent worldwide, has been reported as one of the main risk factors for cardiovascular and cerebrovascular conditions such as coronary heart disease, stroke, and cognitive impairment (GBD 2015 Risk Factors Collaborators, 2016), and its prevalence keeps growing (Mills et al., 2020). Globally, the total number of adult patients with hypertension is estimated to increase to 1.56 billion by 2025 (Kearney et al., 2005), implying a severe challenge to public health. Past studies have reported that chronic hypertension may induce cognitive impairment or even cognitive dysfunction (Gottesman et al., 2014; Mills et al., 2016; Iadecola and Gottesman, 2019), mainly affecting learning and memory, attention, and executive function (Xue et al., 2019). Chronic hypertension also plays a contributory role toward the increase in the morbidity rate of vascular dementia and Alzheimer's disease (Carnevale et al., 2020; García-Alberca et al., 2020), thereby bringing a huge economic burden to families and society. Therefore, investigating the pathogenesis and prevention of cognitive impairment followed by chronic hypertension is of great social significance.

Chronic hypertension may also damage the structural and functional integrity of brain microcirculation, leading to cerebral microvascular obstruction, impaired neurovascular coupling, and damage to the cerebral blood supply. In addition, hypertension may induce conditions associated with the occurrence of cognitive impairment, such as blood–brain barrier disruption, neuroinflammation, amyloid lesions, and white matter lesions (Ungvari et al., 2021). Although the pathogenesis of cognitive impairment followed by hypertension remains unclear, past studies have substantiated that these conditions can be prevented and treated (Walker et al., 2017; van Eersel et al., 2019).

Acupuncture, a type of complementary and alternative medicine that originated in China, has been incorporated as a complementary therapy in the clinical practice of hypertension (Chen et al., 2018). Past studies by our team (Wu et al., 2021; Yang et al., 2022a,b) have confirmed that acupuncture controls blood pressure in hypertensive patients and spontaneously hypertensive rats (SHRs) exhibiting an ameliorating effect on hypertensive target organs such as the brain, heart, and kidneys. Other studies (Lanari et al., 2007; Lai et al., 2012; Kim et al., 2013) have reported that acupuncture enhanced the recovery of damaged brain tissues by inhibiting neuronal apoptosis, enhancing neuronal plasticity, improving the cerebral blood flow, and regulating the brain metabolism. Although the action mechanism remains unclear, these studies have indicated that acupuncture may attenuate cognitive impairment followed by hypertension.

Resting-state functional magnetic resonance imaging (rs-fMRI) presents functional information about the brain according to the changes in the blood flow and oxygen cooperation in different brain regions in the resting state (Yousaf et al., 2018). In recent years, rs-fMRI has gradually been adopted in acupuncture studies for assessing acupuncture-induced changes in the brain activity signals, which are of great significance to further reveal the neuroimaging mechanism of acupuncture effect (Zhang et al., 2013; Wang et al., 2016). The commonly used rs-fMRI analyses include the amplitude of low-frequency fluctuation (ALFF), regional homogeneity (ReHo), and functional connectivity (FC) analyses. ALFF and ReHo analyses can directly reflect neuron spontaneous activity without the influence of model-generated errors (Liu et al., 2014). Specifically, ALFF analysis reflects the spontaneous activity level of each voxel from the perspective of energy (Liang et al., 2014), while ReHo analysis evaluates the synchronization and coordination of neuronal activities in the local brain regions in time (Liu et al., 2008). Increased ALFF and ReHo values indicate neuronal activation in the corresponding brain areas (Li et al., 2021). FC analysis reflects the functional connectivity between different brain regions (Hutchison et al., 2013). By conducting ALFF and ReHo analyses, some studies have reported that acupuncture exerts an antihypertensive effect to an extent by activating the blood pressure-regulating brain regions (Zhang et al., 2019, 2021). Furthermore, through FC analysis and using the key brain areas for blood pressure regulation as seeds, Zheng et al. (2016) found that acupuncture enhanced the functional connectivity between the hypothalamus and blood pressure-regulating brain regions.

Electroacupuncture (EA) and manual acupuncture (MA) are different in various aspects, such as action mechanisms, targets, and clinical efficacy (Stener-Victorin et al., 2006; Zhao, 2008; Johansson et al., 2013). Based on our understanding, only a few studies have adopted rs-fMRI in their analysis of the protective effects of EA and MA on the cognitive functions in the hypertensive model. Here, we selected SHRs to evaluate the attenuative effect of acupuncture methods on hypertension and cognitive impairment through the blood pressure analysis and the Morris water maze (MWM) test. rs-fMRI was performed for analyzing brain signal changes in SHRs and for comparing the brain functional activity between the EA and MA groups. We identified the neuroimaging mechanisms of EA and MA that affected blood pressure control and cognitive function protection in SHRs, thereby providing insights to identifying the target brain regions in future studies.

2. Materials and methods

2.1. Animals

A total of 30 male SHRs (age: 14 weeks, weight: 280 ± 20 g) and 10 male Wistar–Kyoto (WKY) rats (age: 14 weeks, weight: 280 ± 20 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., (SCXK [Beijing] 2016-0006). All animals were reared at the laboratory of the Beijing University of Chinese Medicine (SPF-grade), and the rearing conditions were uniformly maintained at the laboratory. All rats were adaptively fed for 1 week before the experiment so as to reduce the influence of the rearing environment on the experimental outcomes. All experiments were approved by the Institutional Animal Care and Use Committee of the Beijing University of Chinese Medicine (ethics approval number: BUCM-4-2021041001-2093).

2.2. Experimental procedures

The experimental process is depicted in **Figure 1A**. Briefly, after 1 week of adaptive feeding to all rats, 30 SHRs were randomly assigned to the model (SHR), EA, and MA groups, with 10 rats/group. Ten WKY rats served as the blank control (WKY). The rats were intervened every alternate day for 12 weeks. The blood pressure of all rats was recorded every 2 weeks during the intervention period. After the intervention, eight rats from each group were randomly subjected to rs-fMRI scanning. Next, the MWM test was performed to all rats. Finally, the rats were sacrificed for obtaining their brain tissues, and their brain slices were stained with hematoxylin–eosin (HE) and Nissl.

2.3. Blood pressure measurement

The blood pressure was measured and recorded at a fixed time (08:00 to 11:00) on days 0, 14, 28, 42, 56, 70, and 84 of the intervention courses. At room temperature (23 ± 2°C), the rats (non-anesthetized) were placed in the blood pressure-measurement rat sleeve (thermostat used to maintain the temperature at 36°C) by two experienced technicians and for a 10-min preheat. The blood pressure was measured in the tail artery of the rats using an indirect blood pressure meter (BP-2010E, Softron Biotechnology, Beijing, China). The systolic blood pressure of each rat was measured thrice and the average value was used as the measurement outcome.

2.4. Interventional methods

Interventions were initiated after measurement and the baseline blood pressure of the rats was recorded. The rats in the EA and MA groups (non-anesthetized) were fixed on a sterilized rat plate. For the rats in the two groups, the same acupoints were selected (**Figure 1B**): Taichong (LR3), which is located at the anterior aspect of the depression between the first and second metatarsal bones, and Zusanli (ST36), which is located at 2-mm lateral to the anterior tubercle of the tibia and 5-mm below the capitulum fibulae under the knee joint (Ma et al., 2020). The sterilized disposable stainless-steel needles (0.18 × 13 mm, ZHONGYANTAIHE, Beijing, China) were inserted into the bilateral LR3 of the rat 2-mm deep at a slope of 30 degrees, and the perpendicular needling was applied at ST36 up to a 5-mm depth (Tian et al., 2013). In the EA group the bilateral acupoints were connected with an acupoint nerve stimulator (Gensun Medical Technology, Jiangsu, China) for current stimulation with an intensity of 1 mA, 2 Hz (Zhang et al., 2009). In the MA group, needles on the bilateral acupoints were bidirectionally rotated for manual stimulation. Each needle was rotated at the speed of 180°/s within 90°. The manipulation was applied every 5 min for 15 s each time (Ding et al., 2019). The EA and MA groups were treated for 20 min each time, the SHR and WKY groups were not treated but were subjected to the same grasping and fixation stimulation similar to that for the EA and MA groups for 20 min each. The interventions were performed every alternate day from 13:00 to 16:00 over 12 weeks.

2.5. The MWM test

A day before the formal experiment, the rats were placed in a pool (no platform) for adaptive swimming (60 s) to familiarize themselves with the environment. The MWM experiment was conducted for six consecutive days, from 8:00 to 12:00. We then divided the cylindrical pool (160-cm diameter, 50-cm deep) into four quadrants. In the acquired training group, a circular platform (10-cm diameter) was placed 1–2 cm below the horizontal plane of the target quadrant, and the time taken for the rats to enter the water from different quadrants to find the platform (escape latency) was recorded. The time limit was set to 60 s. A timeout was recorded as 60 s, after which the rats were placed on the platform for 10 s to familiarize themselves with the surrounding environment. Each rat was trained four times a day at an interval of 30 min for five consecutive days. On the 6th day, a space probe trial was performed, and the platform was removed from the pool. We then recorded

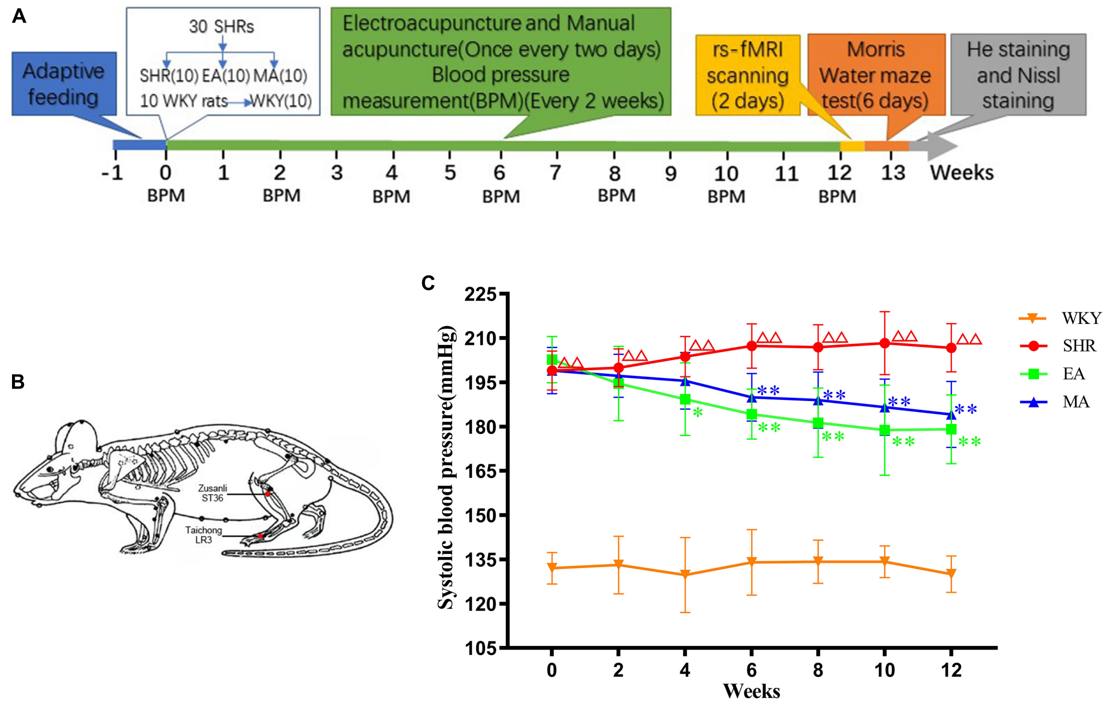

FIGURE 1
Electroacupuncture and manual acupuncture attenuate hypertension in spontaneously hypertensive rats (SHRs). **(A)** Experimental procedures. **(B)** The location of acupoints in SHRs. Red dots indicate the location of Zusanli (ST36) and Taichong (LR3). **(C)** Systolic blood pressure values in each group. The values are presented as the mean ± SEM ($n = 10$ rats/group). $^{\Delta\Delta}p < 0.01$ vs. Wistar–Kyoto (WKY) group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. SHR group. WKY, normal-control group; SHR, model group; EA, electroacupuncture-stimulation group; MA, manual acupuncture-stimulation group.

and observed the time spent by the rats in the target quadrant within the 60-s period, the number of platform crossings, and the swimming traces.

2.6. MRI acquisition and data processing

2.6.1. Anesthesia and fixation of rats

Eight rats from each group were randomly subjected to rs-fMRI scanning. Briefly, the rats were injected dexmedetomidine hydrochloride (100 μg/mL) into the interior lateral thigh muscle to prepare them before scanning using a dose of 0.02 mL per 100 g·bw. The rats were then anesthetized with a 5% isoflurane and 95% oxygen mixture for induction. During the scanning process, the rats were kept prone on the rat-specific scanning bed, fixed to the head through the hook and ear rods, with a 2% isoflurane and 98% oxygen mixture used for maintaining the anesthetic state. The T2-weighted sequence was scanned first, followed by the BOLD sequence. The body temperature, respiratory rate, and heart rate of the rats were monitored in real-time by a physiological detector (Model 1025, Small Animal Instruments Inc., Stony Brook, NY, USA) during the scanning process.

2.6.2. Scan sequence and parameter setting

All rats were scanned with the 7.0T Bruker animal *in vivo* MRI scanner (PharmaScan 70/16 US, Bruker, Germany) with a special cranial surface coil for small animals. The T2-weighted images were acquired by using the T2_TurboRARE sequence with the following parameters: repetition time (TR) = 5,500 ms, echo time (TE) = 33 ms, percent phase field view = 100, slice thickness = 0.5 mm, acquisition matrix = 256 × 256, flip angle = 90°. The images of resting-state blood oxygen level-dependent (BOLD) were acquired with the T2Star_FID_EPI sequence using the following parameters: TR = 2,000 ms, TE = 11 ms, number of averages = 1, percent phase field view = 80, slice thickness = 0.5 mm, acquisition matrix = 80 × 48, and flip angle = 90°.

2.6.3. Data preprocessing and parameters calculation

SPM12 package, REST package, FC toolkit, and DPABI package on the MATLAB platform were used for data preprocessing and the calculation of indicators. (1) Format conversion: The original functional image and T2 image were converted from the DICOM format to the NIFTI format. (2) The removal of the starting time point: the BOLD signal is often unstable at the beginning of functional image acquisition; therefore, the first 10 time points have to be excluded. (3) Voxel augment: The SPM package is designed based on the actual size of the human brain. The mouse brain is much smaller than the human brain; therefore, the collected rat MRI was enlarged 10 times to adapt to the operation of the package. (4) Slice timing: The data of all voxels were adjusted to the standard time so as to achieve theoretical consistency of the acquisition time of BOLD signals for all voxels at a time point. (5) Realign: The little movement of each rat's head was aligned between different time points in the scan. The criterion for exclusion was set as one voxel size movement (3 × 3 × 3 mm after amplification).

(6) Reorientation: the average functional phase was corrected for origin, and the origin correction matrix of the average functional phase was applied to the functional phase file. The T2 phase was also corrected for the origin. (7) Normalization: all subject brains were registered to a unified standard space to resolve the problems of the difference in the brain shape between different rats and the inconsistency in the head spatial position during scanning so as to facilitate subsequent statistical analyses. (8) Smooth: the high-frequency noise generated by image deformation during spatial standardization was reduced and the statistical effectiveness was improved. The size of the smoothing nucleus was 2–3 times the size of the enlarged voxel. (9) Indicator calculation: The REST package and the SPM12 package were used in the calculation of the ALFF and ReHo values. The ALFF data calculation was performed in the 0.01–0.08-Hz frequency band after the spatial-smoothing process. The ReHo data calculation was performed as per the spatial normalization process, and the noise in the frequency band < 0.01 Hz (low frequency) and > 0.08 Hz (high frequency) was removed. The FC package, the SPM12 package, and the DPABI package were employed in the calculation of the FC values performed after the spatial smoothing process.

2.7. Histopathology

Six rats were randomly selected from each group. From them, samples of three rats were subjected to HE staining, and the other three to Nissl stanning. These rats received deep anesthesia and precooled normal saline and 4% paraformaldehyde were perfused through the cardiac veins. The brain tissues were collected and fixed in a 4% paraformaldehyde solution. The tissue was dehydrated, subjected to transparent treatment in xylene, embedded in paraffin wax, sliced into 4 μm slices, flattened, and stored after baking.

HE staining: The sections were dewaxed, covered with water, stained with hematoxylin for 3–8 min, and differentiated in the hydrochloric acid alcohol solution. Then, the slices were stained with eosin for 1–3 min, dehydrated in graded ethanol, treated with xylene, and sealed with neutral resin. The sections were observed with a panoramic scanner (Pannoramic MIDI, 3D HISTECH, Budapest, Hungary) and the relevant images were collected.

Nissl staining: The sections were dewaxed and covered with water, stained with Nissl stain for 10–30 min, differentiated in the Nissl differentiation fluid for 1–3 min, dehydrated in graded ethanol, treated with xylene, and sealed with neutral resin. The sections were observed in a panoramic scanner (Pannoramic MIDI, 3D HISTECH, Budapest, Hungary) and the relevant images were collected. We then randomly selected five fields (Scale bar = 100 μm) in the entorhinal cortex of each brain section. The number of nissl-positive neurons was calculated with the ImageJ software (version 1.8, National Institute of Health, Bethesda, MD, USA), and the average number of the cells in the five fields was counted.

2.8. Statistical analyses

The experimental data were analyzed by the SPSS 20.0 software (IBM, Armonk, NY, USA). The normality of the data was tested, followed by the homogeneity test. The experimental data on the systolic blood pressure and escape latency in the MWM test was subjected to a two-way repeated measurement analysis of variance. The experimental data on the time spent in the target quadrant, the number of platform crossings, and the number of Nissl-positive neurons were analyzed by one-way analysis of variance. Fisher's least-significant difference (LSD) test was performed for pairwise comparison between the groups, and $p < 0.05$ was considered to indicate statistical significance. Line charts and histograms in the experiment were drawn with GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

The rs-fMRI data was modeled in the general linear model. The data were compared between groups by one-way analysis of variance, followed by a *post hoc* two-sample *t*-test. The regions with significant changes in the ALFF and ReHo values were considered as uncorrected $p < 0.005$ with cluster-extent > 5 voxels. The regions with significant changes in the FC values were considered as uncorrected $p < 0.005$ with cluster-extent > 2 voxels.

We performed Pearson's (data conform to the normal distribution) or Spearman's (data did not conform to the normal distribution) correlation analyses between the behavioral test indices (the escape latency and the time spent in the target quadrant) and ALLF/ReHo values in the regions with significant changes in the EA/MA group. We consider $p < 0.05$ as a threshold of statistical significance.

3. Results

3.1. Effects of EA and MA on blood pressure in SHRs

We analyzed systolic blood pressure values of SHRs to investigate the attenuating effects of EA and MA on hypertension. The results demonstrated significant treatment effect ($F = 582.178$, $p < 0.001$), time effect ($F = 3.548$, $p < 0.01$), and interaction of time and treatment effect ($F = 4.301$, $p < 0.001$), indicating significant differences in the systolic blood pressure values of each group in different periods. When compared with the WKY group, the systolic blood pressure of the SHR group gradually increased and was stable at a higher level within 0–12 weeks of intervention ($p < 0.01$). Compared with the SHR group, the systolic blood pressure of the EA and MA groups began to decrease at the 4th and 6th week of measurement, respectively, ($p < 0.05$, $p < 0.01$). Specifically, when compared with the SHR group, the systolic blood pressure of both the EA and MA groups decreased consecutively from the 6th week to the 12th week of intervention (both $p < 0.01$). Thus, both EA and MA can reduce systolic blood pressure in SHRs, and the acupuncture effect in lowering blood pressure has a cumulative time effect. Moreover, EA exhibited a rapid onset in lowering the blood pressure when compared with MA (**Figure 1C**).

3.2. Effects of EA and MA on cognitive functions of SHRs

Morris water maze (MWM) reflected the spatial learning and memory function of rats. In the acquired training experiment

(Figure 2A), the escape latency had a significant treatment effect ($F = 24.113$, $p < 0.001$) and time effect ($F = 59.745$, $p < 0.001$). The SHR group required more time to find the hidden platform than the WKY group ($p < 0.01$). On the contrary, the EA and MA groups required a significantly shorter time (both $p < 0.01$) to identify the platform when compared to the SHR group. In the probe trial (Figures 2B–D), time spent in the target quadrant and the number of platform crossings were significantly reduced in the SHR group when compared with the WKY group (both $p < 0.01$). Time spent in the target quadrant in the EA group was significantly increased when compared with that in the SHR group ($p < 0.01$). No significant difference was observed between the MA and SHR groups ($p > 0.05$). When compared with the SHR group, the number of platform crossings was significantly increased in the EA and MA groups (both $p < 0.01$). Specifically, the number of platform crossings was higher in the EA group than in the MA group ($p < 0.01$). Thus, chronic hypertension can cause impairment of learning and memory function. EA and MA exhibited different degrees of attenuating effects on cognitive impairment followed by hypertension, and comprehensive analysis revealed that the effect of EA was better than that of MA.

3.3. Effects of EA and MA on brain regions regulating blood pressure and cognitive functions of SHRs

Data accessed through rs-fMRI were subjected to ALFF and ReHo analyses. ALFF (Supplementary Table 1) and ReHo (Supplementary Table 2) values of some brain regions were significantly lower in the SHR group than in the WKY group (Figures 3A, D), indicating that chronic hypertension decreased the functional activity in some brain areas. ALFF (Figures 3B, C; Supplementary Tables 3, 4) and ReHo (Figures 3E, F; Supplementary Tables 5, 6) values of some brain regions were significantly higher in the EA and MA groups than in the SHR group, demonstrating that EA and MA activated these brain areas and enhanced the functional activity of local neurons. After ALFF and ReHo analyses, we performed an intersection between the brain regions of decreased ALFF/ReHo values in the SHR group (compared to the WKY group) and the brain regions of increased ALFF/ReHo values in the EA group (compared to the SHR group) to obtain reversal brain regions of the EA group, that is, the callback brain regions of the EA group (Figure 3G). These regions included the right hypothalamus region, right brainstem, right entorhinal cortex, right prelimbic cortex, right basal forebrain region, bilateral cerebellum, right olfactory bulb, and right primary somatosensory cortex. In the same manner, we obtained the callback brain regions of the MA group. These regions included the right hypothalamus region, right entorhinal cortex, right cingulate cortex, right corpus callosum, right striatum, and bilateral olfactory bulb. The EA group activated more callback brain regions than the MA group, and most of these regions were involved in regulating blood pressure and cognitive functions. In addition, the brain areas regulating the sensation and olfaction were activated. These areas are often considered the key targets for acupuncture intervention.

3.4. Effects of EA and MA on functional connectivity between seeds and brain regions regulating blood pressure and cognitive functions of SHRs

Based on the results of ALFF and ReHo analyses, we performed another intersection between the callback regions of the EA group and the MA group, in which the right hypothalamus (HHA. R) and the right entorhinal cortex (Ent. R) were identified, indicating a critical role of the functional activity of the two brain regions. Therefore, the right hypothalamus (HHA. R) and right entorhinal cortex (Ent. R) were selected as seeds for FC analysis. When compared with the WKY group, the functional connectivity of HHA.R (Supplementary Table 7) and Ent. R (Supplementary Table 8) in the SHR group with other brain regions was impaired, indicating that chronic hypertension damaged functional activity in brain regions as well as the functional connectivity between them. EA and MA exerted reversal effects as they enhanced functional connectivity between the seeds and some connected brain regions (Table 1). Most brain areas were involved in blood pressure and cognitive function regulation. In addition, the reversal of functional connectivity in the EA group partially coincided with that in the MA group, and the number of callback brain regions functionally connected with seed points was higher in the EA group than in the MA group.

3.5. Relationship between the ALLF/ReHo value of right entorhinal cortex and behavioral test indices in MWM test

The results of Pearson's correlation analysis (Figures 4A–C) revealed significant correlations between the ReHo value of Ent. R in the MA group and the escape latency ($r = -0.752$, $p = 0.031$), the ReHo value of Ent. R in the EA group and the escape latency ($r = 0.738$, $p = 0.037$), and the ALFF value of Ent. R in the EA group and the time spent in the target quadrant ($r = 0.776$, $p = 0.024$). Specifically, the ReHo value of Ent. R in the MA/EA group was negatively correlated with the escape latency; the ALFF value of Ent. R in the EA group was positively correlated with the time spent in the target quadrant. These results demonstrated that under the intervention of the EA and the MA, the stronger the functional activity of entorhinal cortex, the better was the performance of MWM test of the rats.

3.6. Effects of EA and MA on neuronal injury in entorhinal cortex in SHRs

Through HE staining, histopathological changes in the entorhinal cortex were evaluated. Neurons in the WKY group were histologically normal; they were closely arranged and orderly and exhibited a regular morphology, with uniform size. In contrast, neurons in the SHR group were disordered, with increased intercellular space. Furthermore, neuronal injury and apoptosis occurred. However, when compared with the SHR group, the EA and MA groups improved these abnormalities. The EA group

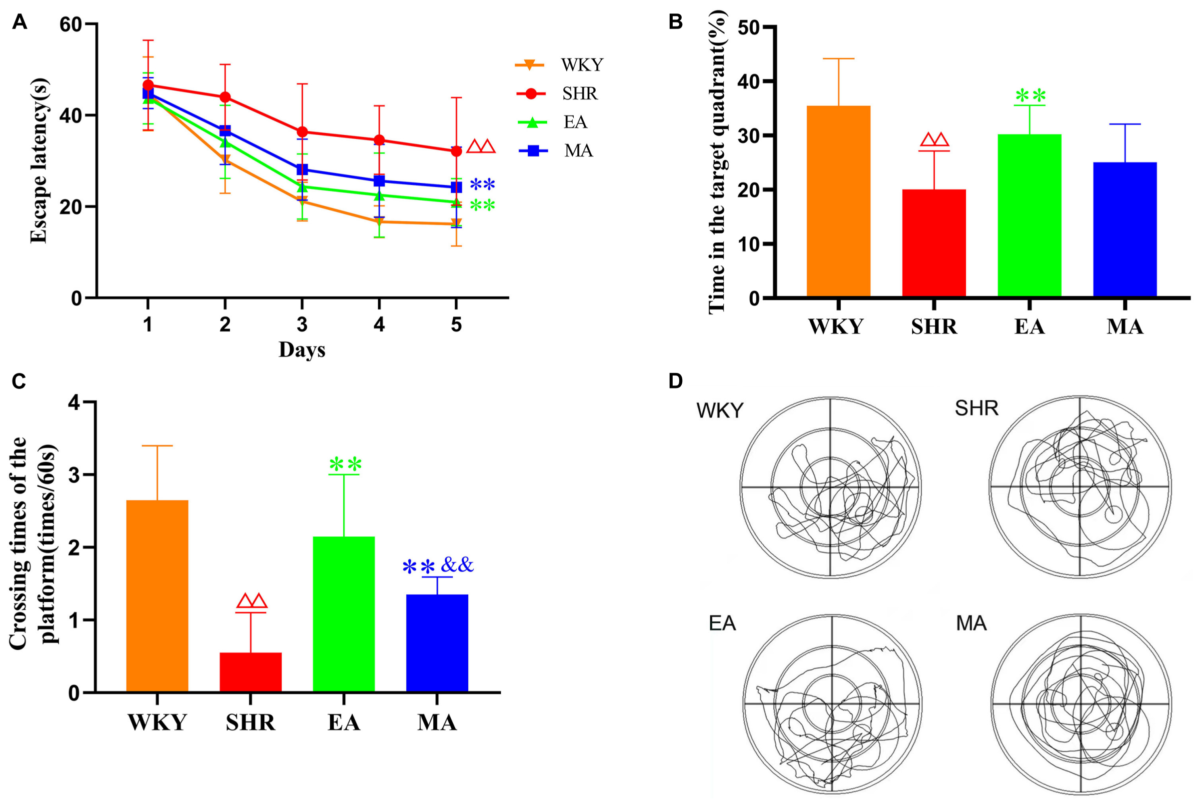

FIGURE 2
Electroacupuncture and manual acupuncture protect the cognitive functions of spontaneously hypertensive rats (SHRs). The Morris water maze test was performed in the Wistar–Kyoto (WKY), SHR, EA, and MA groups. **(A)** Escape latency. **(B)** Time spent in the target quadrant. **(C)** The number of platform crossings. The values are presented as the mean ± SEM ($n = 10$ rats/group). **(D)** Typical swimming traces in the probe trial. $^{\Delta\Delta}p < 0.01$ vs. WKY group; $^{**}p < 0.01$ vs. SHR group; $^{\&\&}p < 0.01$ vs. EA group. WKY, normal control group; SHR, model group; EA, electroacupuncture group; MA, manual acupuncture group.

specifically exhibited a better improvement than the MA group (**Figure 5A**, 1st line). The number of Nissl-positive neurons in the entorhinal cortex was also evaluated using Nissl staining. The number of Nissl-positive neurons was significantly decreased in the SHR group when compared with that in the WKY group ($p < 0.01$). The number of Nissl-positive neurons was significantly increased in the EA and MA groups compared with the SHR group ($p < 0.01$). Moreover, the EA group had more Nissl-positive neurons than the MA group ($p < 0.01$) (**Figures 5A, B**).

4. Discussion

In this study, 14-weeks-old SHRs were selected as the experimental model. SHRs are internationally recognized animal models of hypertension (Tayebati et al., 2012). They are often used in studies on hypertensive brain injury as their symptoms are similar to those of humans, such as course-dependent arterial high blood pressure, brain atrophy, and brain neuron loss (Amenta et al., 2010; Fei et al., 2017; Gao et al., 2019). SHRs exhibit stable hypertensive symptoms at approximately 12 weeks of age and cognitive impairment at 26–28 weeks of age (Johnson et al., 2020). Clinical studies (Turana et al., 2019; de Menezes et al., 2021) have confirmed that ameliorating hypertension in the early stage is crucial for protecting cognitive functions. SHRs aged 14 weeks are in the early hypertension stage and exhibit no cognitive impairment. Therefore, 14-weeks-aged SHRs are suitable for investigating the attenuating effects of acupuncture in cognitive dysfunction. According to the traditional Chinese medicine theory, several acupoints control blood pressure. Of them, LR3 and ST36 are two commonly used acupoints that lower the blood pressure in clinical and animal studies (Lai et al., 2012; Zhang et al., 2018; Luo et al., 2019). Leung et al. (2016) reported that LR3 and ST36, when combined, are beneficial for protecting cognitive functions in SHRs.

In this study, the blood pressure of SHRs exhibited a slowly increasing trend within the experimental period, which is similar to hypertension development in humans (Philip et al., 2021). Both EA and MA could ameliorate blood pressure in SHRs, demonstrating consistency with the results of the previous reports of our team (Wu et al., 2021; Yang et al., 2022a). Moreover, EA has a faster onset of the blood pressure-lowering effect than MA. Interestingly, we found that blood pressure amelioration presented a cumulative effect with progressing EA and MA interventions. In fact, the long-term cardiovascular effect of an acupuncture therapy course is considerably better than that of one-time acupuncture (Longhurst and Tjen, 2013). This agrees with the result of Li et al. (2018). They reported the cumulative effect of acupuncture at LR3 on lowering blood pressure. Clinical and animal studies (Lu et al., 2017; Whelton and Williams, 2018) have proven that antihypertensive

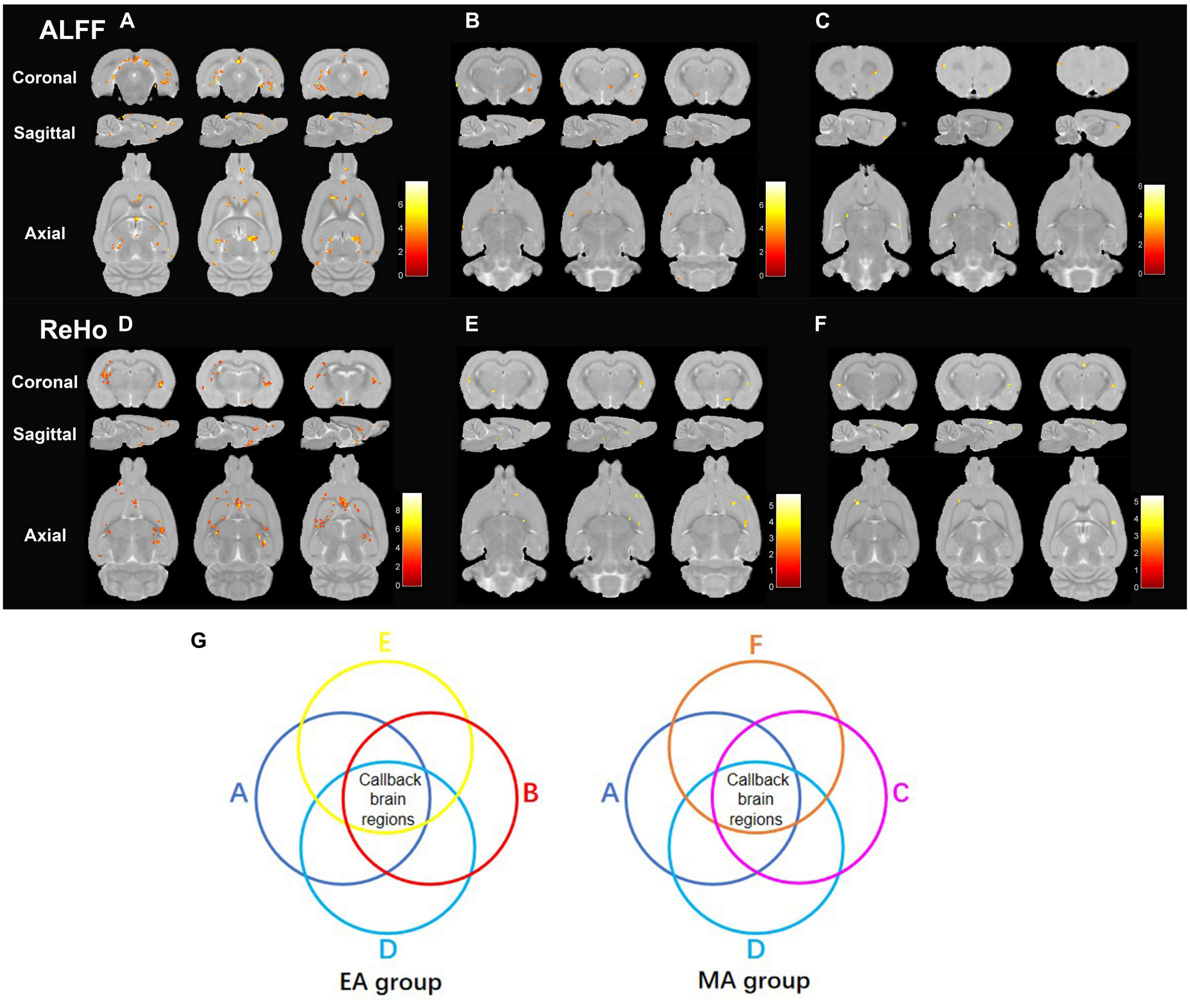

FIGURE 3
Electroacupuncture and manual acupuncture activate callback brain regions regulating blood pressure and cognitive functions of spontaneously hypertensive rats (SHRs). ALFF and ReHo analyses were performed in the Wistar–Kyoto (WKY), SHR, EA, and MA groups. **(A)** The brain regions with decreased ALFF values in SHR compared to that in WKY. **(B)** The brain regions with increased ALFF values in EA compared to that in SHR. **(C)** The brain regions with increased ALFF values in MA compared to that in SHR. **(D)** The brain regions with decreased ReHo values in SHR compared to that in WKY. **(E)** The brain regions with increased ReHo values in EA compared to that in SHR. **(F)** The brain regions with increased ReHo values in MA compared to that in SHR. Differential brain regions are displayed sequentially on the coronal plane, sagittal plane, and axial plane ($p < 0.005$, uncorrected, Cluster > 5). Color bars signify the *t* value of the group analysis (brighter color represents a higher *t* value). **(G)** The process of determining the callback brain regions. WKY, normal-control group; SHR, model group; EA, electroacupuncture group; MA, manual acupuncture group; ALFF, the amplitude of low-frequency fluctuation; ReHo, regional homogeneity.

treatment protects against cognitive impairment. The MWM test results indicated the attenuating effect of EA and MA on cognitive impairment in hypertensive rats, which may be related to the antihypertensive effect of acupuncture.

A past study has confirmed that acupuncture can exert therapeutic effect through a series of pathways of action (Lai et al., 2012). ALFF and ReHo analyses revealed that the callback brain regions of EA and MA are mainly involved in regulating blood pressure and cognitive functions. The callback brain regions shared by the two interventions are the hypothalamus and the entorhinal cortex. The functional activities of these two brain regions have crucial roles in lowering blood pressure and protecting cognitive functions. The hypothalamus is a key brain region associated with blood pressure regulation. Combined with the brainstem, the hypothalamus regulates sympathetic nerve activity (Farnham et al., 2008; Ye et al., 2013), which is often related to essential hypertension occurrence (Guyenet, 2006). Moreover, angiotensin secreted by astrocytes that is present in both the hypothalamus and brainstem (Bloch et al., 2015) plays a major role in blood pressure regulation. In addition, the hypothalamus regulates cardiovascular and cerebrovascular effects through oxidative stress pathways (Coleman et al., 2013), proinflammatory cytokine levels (Qi et al., 2013), and arginine vasopressin secretion (Yi et al., 2012). The entorhinal cortex is a key player in brain learning and memory during memory storage, consolidation, and reactivation (O'Neill et al., 2017; Gerlei et al., 2021). The entorhinal cortex selectively gatekeeps the cortical memory network by interacting with brain regions, such as the anterior cingulate cortex or hippocampus, selectively according to the age of memory (Takehara-Nishiuchi, 2014). Hales et al. (2021)

TABLE 1 The increased strengths of the functional connectivity (FC) in the electroacupuncture (EA), and the manual acupuncture (MA) groups in comparison with the spontaneously hypertensive rats (SHR) group.

Connected region	Group	Positive brain regions	Voxel size	*t* value	Peak MNI coordinate (mm)		
					X	*Y*	*Z*
Seed 1 HHA.R	EA group	Brainstem. R	2	3.53	8.00	−101.05	−14.80
		Entorhinal cortex.L	2	3.70	-52.00	−35.05	−11.80
		Molecular layer of the cerebellum.R	3	4.44	41.00	−125.05	18.20
	MA group	Brainstem. L	2	3.72	-13.00	−92.05	−32.80
		Molecular layer of the cerebellum.R	2	4.79	32.00	−125.05	27.20
		Basal forebrain region. R	2	4.41	14.00	24.95	−11.80
Seed 2 Ent.R	EA group	Cornu ammonis 1.R	2	4.10	23.00	−47.05	51.20
		PreLimbic cortex.R	2	4.38	16.00	33.95	15.20
		Dentate gyrus.R	4	3.24	32.00	−59.05	36.20
		Brainstem. R	2	3.97	20.00	−125.05	−26.80
		Corpus callosum. R	2	4.10	23.00	−47.05	51.20
		Striatum.R	3	3.14	23.00	12.95	21.20
		Granule cell level of the cerebellum.L	2	4.96	-64.00	−113.05	6.20
	MA group	Cornu ammonis 1.R	2	4.26	50.00	−56.05	6.20
		Dentate gyrus.R	2	4.26	50.00	−56.05	6.20
		Ectorhinal cortex.L	2	4.14	-58.00	−32.05	0.20
		Brainstem. L	3	3.83	-1.00	−50.05	−5.80
		Granule cell level of the cerebellum.L	2	3.34	-28.00	−122.05	12.20

HHA.R, hypothalamic region.R; Ent.R, entorhinal cortex.R; FC, functional connectivity; EA, electroacupuncture group; MA, manual acupuncture group; L, left; R, right. $p < 0.005$, uncorrected, Cluster > 2.

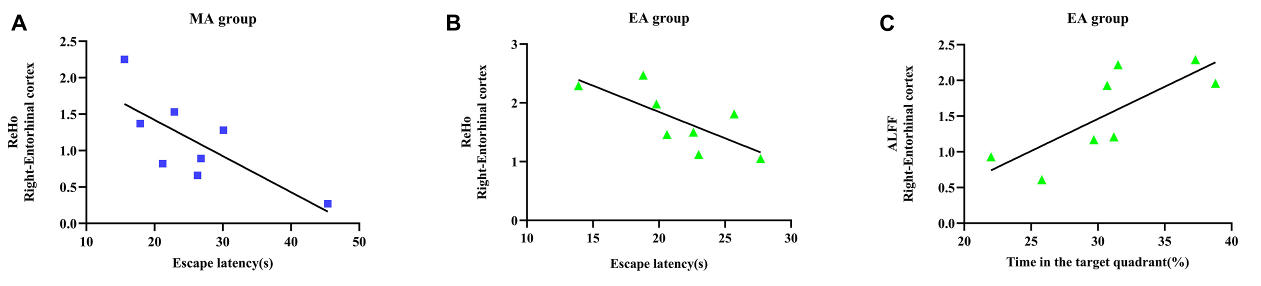

FIGURE 4
Relationship between functional activity of entorhinal cortex and behavioral test indices of Morris water maze (MWM) test. **(A)** Correlation analysis between the regional homogeneity (ReHo) value of entorhinal cortex and escape latency in the manual acupuncture (MA) group. **(B)** Correlation analysis between the ReHo value of entorhinal cortex and escape latency in the electroacupuncture (EA) group. **(C)** Correlation analysis between the amplitude of low-frequency fluctuation (ALFF) value and the time spent in the target quadrant in the EA group. $n = 8$ per group. Pearson's correlation analysis was used with $p < 0.05$ for statistical significance and r for correlation.

found that the performance of rats with entorhinal cortex lesions is unsatisfactory in remembering platform positions in the MWM test. Imaging studies (Zuo et al., 2018; Wang et al., 2020) have revealed that the functional connectivity between the entorhinal cortex and basal forebrain regions is impaired in cognitive dysfunction patients. In addition, other callback brain regions such as the prelimbic cortex (da Silva et al., 2020), cingulate cortex (Rolls, 2019), corpus callosum (Edwards et al., 2014), cerebellum (Argyropoulos et al., 2020), and striatum (Provost et al., 2015) are associated with advance cognitive activities such as learning, memory, and emotion. Some fMRI studies (Wen et al., 2018; Wei et al., 2020) have reported that EA and MA exert a therapeutic

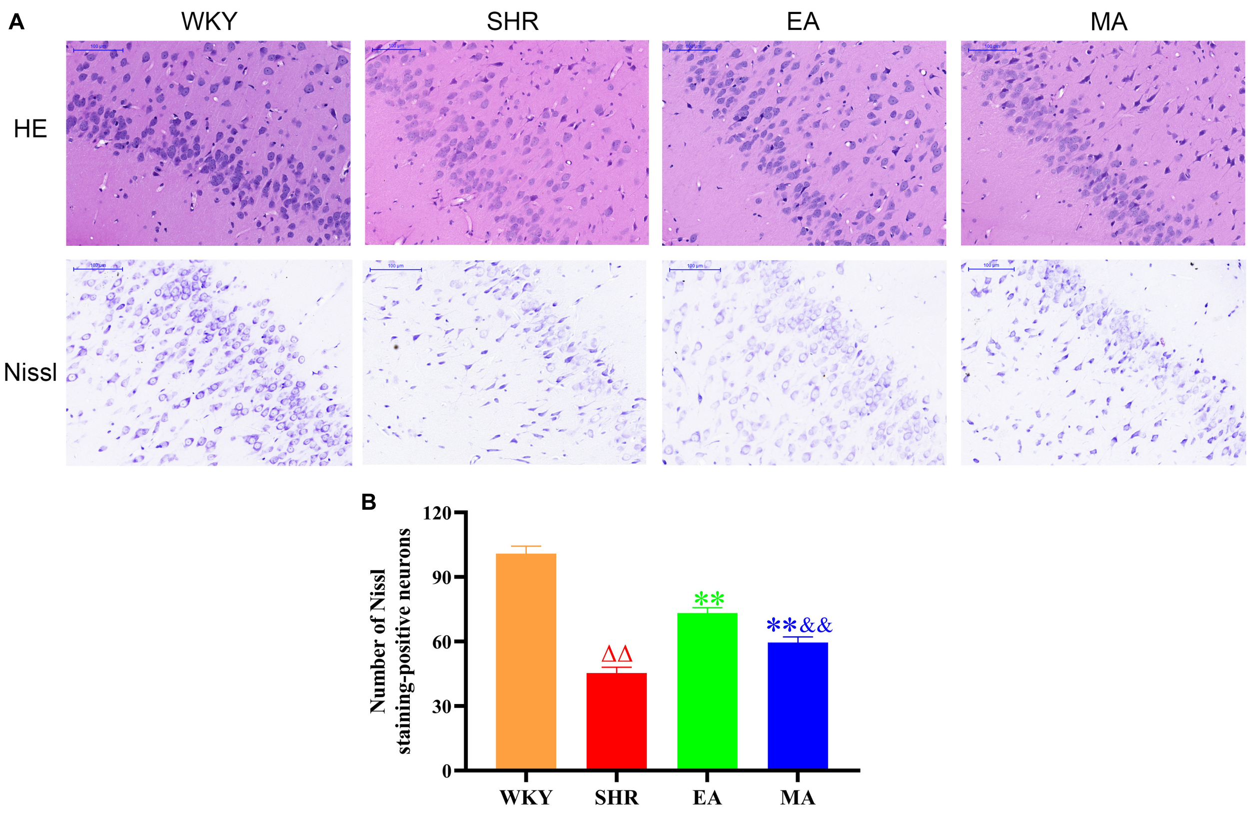

FIGURE 5
Electroacupuncture and manual acupuncture ameliorate neuronal injury in entorhinal cortex in spontaneously hypertensive rats (SHRs). The results of HE staining and Nissl staining in the entorhinal cortex. **(A)** Representative images of HE staining and Nissl staining in the entorhinal cortex of the Wistar–Kyoto (WKY), SHR, EA, and MA groups. Scale bar = 100 μm. **(B)** Quantitative evaluation of Nissl staining-positive neurons. Scale bar = 100 μm. The values are expressed as the means ± SEM (n = 3 rats/group). $^{\Delta\Delta}p < 0.01$ vs. WKY group; $^{**}p < 0.01$ vs. SHR group; $^{\&\&}p < 0.01$ vs. EA group. WKY, normal control group; SHR, model group; EA, electroacupuncture group; MA, manual acupuncture group.

role by activating the functional activities of the brain regions such as the prelimbic cortex, cingulate cortex, and cerebellum. A past study also substantiated the regulatory effect of acupuncture on the functional activities of the cerebral cortex and some subcortical regions (Cai et al., 2018). In this study, acupuncture activated the neuronal functional activity of the brain regions that regulate blood pressure and cognitive function, indicating that the activation may act as one of the neuroimaging mechanisms underlying the ameliorating effect of acupuncture on hypertension and cognitive impairment. In addition, the brain regions regulating sensation and olfaction were activated, which may have positive effects on attenuating the occurrence and development of hypertensive brain injury.

Based on the results of the previously mentioned analysis, the hypothalamus and entorhinal cortex were selected as seeds in the FC analysis. According to the results, acupuncture enhanced the functional connectivity between the seeds and brain regions such as the brainstem, entorhinal cortex, hippocampus, prelimbic cortex, and cerebellum. Chen et al. (2013) reported that acupuncture has a regulatory role in the cardiovascular system as it affects complex brain networks such as the cerebral cortex, hypothalamus, and brainstem, which is similar to the results of our analysis. Interestingly, EA enhanced the functional connectivity between the hypothalamus, the selected seed point, and the entorhinal cortex, thereby suggesting a close relationship between blood pressure and cognitive functions. In addition, when the entorhinal cortex was used as the seed point, functional connectivity between the seed points (both in the EA and MA groups) and hippocampus (CA1 area and dentate gyrus) were enhanced. Projection neurons in the entorhinal cortex directly formed excitatory synapses on the pyramidal cells of the CA1 area for impulse transmission (Li et al., 2017). Deep brain stimulation of the entorhinal cortex increases dentate gyrus neurogenesis (Jiang et al., 2022). An MRI study (Lin et al., 2022) proved that EA improves cognitive function by increasing the functional connectivity between the entorhinal cortex and hippocampus in mice. Thus, the neurofunctional connection among the hypothalamus, entorhinal cortex, and hippocampus may play a critical role in regulating blood pressure and cognitive functions.

The hippocampus is crucial for learning and memory (Lisman et al., 2017). When compared with the WKY group, ALFF and ReHo values of the hippocampus in the SHR group decreased, suggesting that chronic hypertension damaged the functional activity of hippocampal neurons. However, the hippocampus is not one of the EA- and MA-activated callback brain areas. In fact, a lot of information from the cortex is projected from the entorhinal cortex to the hippocampus (Jiang et al., 2022). A study reported that entorhinal cortical atrophy in Parkinson's disease patients with dementia occurs earlier than hippocampal atrophy (Goldman et al., 2012). Therefore, acupuncture may activate the entorhinal cortex earlier than the hippocampus. The FC analysis revealed that the functional connectivity between the entorhinal cortex and hippocampus was enhanced. In this study, there was a significant correlation between the functional activities of the entorhinal cortex in the MA/EA group and the indices of MWM test. It not only explains the curative effect of acupuncture, but also further illustrates the importance of the entorhinal cortex in regulating the cognitive functions. Histopathologically, acupuncture exhibited an attenuating effect on neuronal damage and apoptosis in the entorhinal cortex. This finding illustrates the synergy of acupuncture in protecting neuronal structure and functional activities.

Electroacupuncture (EA) was superior to MA in lowering blood pressure and protecting cognitive functions in SHRs. ALFF and ReHo analyses revealed that the callback brain regions activated by the EA and MA groups were duplicated but not identical, and that the total number of callback brain regions activated was higher in the EA group than in the MA group. Similarly, FC analysis demonstrated that a higher number of callback brain regions in the EA group were involved in functional connectivity enhancement when compared with the MA group. In addition, histopathological analysis revealed that EA had a better attenuating effect on entorhinal cortex neuronal injury and apoptosis than MA. In other words, EA activated more callback brain regions and functional connectivity than MA, which may explain the better effect of the EA group relative to that of the MA group.

This study has several limitations. First, as a neuroimaging study, this experiment mainly focused on macro changes in the brain and explored the effect of acupuncture on the brain region functions. The molecular mechanisms were not discussed. Future studies will adopt proteomics and genomics to study the central mechanism of the target brain regions. Second, anesthesia was required during the rs-fMRI scan in rats. Although isoflurane-dexmedetomidine hydrochloride is deemed suitable as an anesthetic method for rs-fMRI (Paasonen et al., 2018), it may affect the rs-fMRI scanning data. Third, only the systolic blood pressure of SHRs was analyzed. In the future, we plan to analyze the diastolic blood pressure data of SHRs to further contribute to the blood pressure research.

5. Conclusion

We here confirmed that EA and MA exerted attenuating effects on hypertension and cognitive dysfunction in SHRs, and that the effect of EA is better than that of MA. Neuroimaging analysis revealed that acupuncture enhances the neuronal activity and functional connectivity in the brain regions involved in the regulation of blood pressure and cognitive functions. Specifically, EA activated more callback brain regions and functional connectivity than MA.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

This animal study was reviewed and approved by Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine.

Author contributions

J-PL participated in the acupuncture intervention, the aggregation of the results of statistical analysis, and drafted the manuscript. Y-YL and K-ZY have performed the measurement and record of the blood pressure of the rats. X-LL, JS, and YG took part in the behavioral experiment and pathological staining of the rats. B-NY took part in the imaging processing. ZT and X-YG took part of the work in statistical analyses. YT participated in the translation, revision, and proofreading of the manuscript. Q-GL, MX, and S-FS responsible for experimental designing and supervision. All authors have reviewed and approved the manuscript.

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Conflict of interest

YT was employed by Beijing Tong Ren Tang International Natural-Pharm Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1129688/full#supplementary-material

References

Amenta, F., Tayebati, S. K., and Tomassoni, D. (2010). Spontaneously hypertensive rat neuroanatomy: Applications to pharmacological research. *Ital. J. Anat. Embryol.* 115, 13–17.

Argyropoulos, G. P. D., van Dun, K., Adamaszek, M., Leggio, M., Manto, M., Masciullo, M., et al. (2020). The cerebellar cognitive affective/schmahmann syndrome: A task force paper. *Cerebellum* 19, 102–125. doi: 10.1007/s12311-019-01068-8

Bloch, S., Obari, D., and Girouard, H. (2015). Angiotensin and neurovascular coupling: Beyond hypertension. *Microcirculation* 22, 159–167. doi: 10.1111/micc.12193

Cai, R. L., Shen, G. M., Wang, H., and Guan, Y. Y. (2018). Brain functional connectivity network studies of acupuncture: A systematic review on resting-state fMRI. *J. Integr. Med.* 16, 26–33. doi: 10.1016/j.joim.2017.12.002

Carnevale, L., Maffei, A., Landolfi, A., Grillea, G., Carnevale, D., and Lembo, G. (2020). Brain functional magnetic resonance imaging highlights altered connections and functional networks in patients with hypertension. *Hypertension* 76, 1480–1490. doi: 10.1161/hypertensionaha.120.15296

Chen, H., Dai, J., Zhang, X., Wang, K., Huang, S., Cao, Q., et al. (2013). Hypothalamus-related resting brain network underlying short-term acupuncture treatment in primary hypertension. *Evid. Based Complement Alternat. Med.* 2013:808971. doi: 10.1155/2013/808971

Chen, H., Shen, F. E., Tan, X. D., Jiang, W. B., and Gu, Y. H. (2018). Efficacy and safety of acupuncture for essential hypertension: A meta-analysis. *Med. Sci. Monit.* 24, 2946–2969. doi: 10.12659/msm.909995

Coleman, C. G., Wang, G., Faraco, G., Marques Lopes, J., Waters, E. M., Milner, T. A., et al. (2013). Membrane trafficking of NADPH oxidase p47(phox) in paraventricular hypothalamic neurons parallels local free radical production in angiotensin II slow-pressor hypertension. *J. Neurosci.* 33, 4308–4316. doi: 10.1523/jneurosci.3061-12.2013

da Silva, T. R., Raymundi, A. M., Bertoglio, L. J., Andreatini, R., and Stern, C. A. (2020). Role of prelimbic cortex PKC and PKMζ in fear memory reconsolidation and persistence following reactivation. *Sci. Rep.* 10:4076. doi: 10.1038/s41598-020-60046-x

de Menezes, S. T., Giatti, L., Brant, L. C. C., Griep, R. H., Schmidt, M. I., Duncan, B. B., et al. (2021). Hypertension, prehypertension, and hypertension control: Association with decline in cognitive performance in the ELSA-Brasil Cohort. *Hypertension* 77, 672–681. doi: 10.1161/hypertensionaha.120.16080

Ding, N., Jiang, J., Xu, A., Tang, Y., and Li, Z. (2019). Manual acupuncture regulates behavior and cerebral blood flow in the SAMP8 mouse model of Alzheimer's disease. *Front. Neurosci.* 13:37. doi: 10.3389/fnins.2019.00037

Edwards, T. J., Sherr, E. H., Barkovich, A. J., and Richards, L. J. (2014). Clinical, genetic and imaging findings identify new causes for corpus callosum development syndromes. *Brain* 137, 1579–1613. doi: 10.1093/brain/awt358

Farnham, M. M., Li, Q., Goodchild, A. K., and Pilowsky, P. M. (2008). PACAP is expressed in sympathoexcitatory bulbospinal C1 neurons of the brain stem and increases sympathetic nerve activity in vivo. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R1304–R1311. doi: 10.1152/ajpregu.00753.2007

Fei, Y. L., Lv, H. J., Li, Y. B., Liu, J., Qian, Y. H., Yang, W. N., et al. (2017). Tongxinluo improves cognition by decreasing β-amyloid in spontaneous hypertensive rats. *Brain Res.* 1663, 151–160. doi: 10.1016/j.brainres.2017.03.005

Gao, F., Jing, Y., Zang, P., Hu, X., Gu, C., Wu, R., et al. (2019). Vascular cognitive impairment caused by cerebral small vessel disease is associated with the TLR4 in the hippocampus. *J. Alzheimers Dis.* 70, 563–572. doi: 10.3233/jad-190240

García-Alberca, J. M., Mendoza, S., Gris, E., Royo, J. L., Cruz-Gamero, J. M., and García-Casares, N. (2020). White matter lesions and temporal atrophy are associated with cognitive and neuropsychiatric symptoms in patients with hypertension and Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 35, 1292–1300. doi: 10.1002/gps.5366

GBD 2015 Risk Factors Collaborators (2016). Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: A systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388, 1659–1724. doi: 10.1016/s0140-6736(16)31679-8

Gerlei, K. Z., Brown, C. M., Sürmeli, G., and Nolan, M. F. (2021). Deep entorhinal cortex: From circuit organization to spatial cognition and memory. *Trends Neurosci.* 44, 876–887. doi: 10.1016/j.tins.2021.08.003

Goldman, J. G., Stebbins, G. T., Bernard, B., Stoub, T. R., Goetz, C. G., and deToledo-Morrell, L. (2012). Entorhinal cortex atrophy differentiates Parkinson's disease patients with and without dementia. *Mov. Disord.* 27, 727–734. doi: 10.1002/mds.24938

Gottesman, R. F., Schneider, A. L., Albert, M., Alonso, A., Bandeen-Roche, K., Coker, L., et al. (2014). Midlife hypertension and 20-year cognitive change: The atherosclerosis risk in communities neurocognitive study. *JAMA Neurol.* 71, 1218–1227. doi: 10.1001/jamaneurol.2014.1646

Guyenet, P. G. (2006). The sympathetic control of blood pressure. *Nat. Rev. Neurosci.* 7, 335–346. doi: 10.1038/nrn1902

Hales, J. B., Reitz, N. T., Vincze, J. L., Ocampo, A. C., Leutgeb, S., and Clark, R. E. (2021). A role for medial entorhinal cortex in spatial and nonspatial forms of memory in rats. *Behav. Brain Res.* 407:113259. doi: 10.1016/j.bbr.2021.113259

Hutchison, R. M., Womelsdorf, T., Allen, E. A., Bandettini, P. A., Calhoun, V. D., Corbetta, M., et al. (2013). Dynamic functional connectivity: Promise, issues, and interpretations. *Neuroimage* 80, 360–378. doi: 10.1016/j.neuroimage.2013.05.079

Iadecola, C., and Gottesman, R. F. (2019). Neurovascular and cognitive dysfunction in hypertension. *Circ. Res.* 124, 1025–1044. doi: 10.1161/circresaha.118.313260

Jiang, Y., Liu, D. F., Zhang, X., Liu, H. G., Zhang, C., and Zhang, J. G. (2022). Modulation of the rat hippocampal-cortex network and episodic-like memory performance following entorhinal cortex stimulation. *CNS Neurosci. Ther.* 28, 448–457. doi: 10.1111/cns.13795

Johansson, J., Mannerås-Holm, L., Shao, R., Olsson, A., Lönn, M., Billig, H., et al. (2013). Electrical vs manual acupuncture stimulation in a rat model of polycystic ovary syndrome: Different effects on muscle and fat tissue insulin signaling. *PLoS One* 8:e54357. doi: 10.1371/journal.pone.0054357

Johnson, A. C., Miller, J. E., and Cipolla, M. J. (2020). Memory impairment in spontaneously hypertensive rats is associated with hippocampal hypoperfusion and hippocampal vascular dysfunction. *J. Cereb. Blood Flow Metab.* 40, 845–859. doi: 10.1177/0271678x19848510

Kearney, P. M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P. K., and He, J. (2005). Global burden of hypertension: Analysis of worldwide data. *Lancet* 365, 217–223. doi: 10.1016/s0140-6736(05)17741-1

Kim, J. H., Choi, K. H., Jang, Y. J., Bae, S. S., Shin, B. C., Choi, B. T., et al. (2013). Electroacupuncture acutely improves cerebral blood flow and attenuates moderate ischemic injury via an endothelial mechanism in mice. *PLoS One* 8:e56736. doi: 10.1371/journal.pone.0056736

Lai, X., Wang, J., Nabar, N. R., Pan, S., Tang, C., Huang, Y., et al. (2012). Proteomic response to acupuncture treatment in spontaneously hypertensive rats. *PLoS One* 7:e44216. doi: 10.1371/journal.pone.0044216

Lanari, A., Silvestrelli, G., De Dominicis, P., Tomassoni, D., Amenta, F., and Parnetti, L. (2007). Arterial hypertension and cognitive dysfunction in physiologic and pathologic aging of the brain. *Am. J. Geriatr. Cardiol.* 16, 158–164. doi: 10.1111/j.1076-7460.2007.06502.x

Leung, S. B., Zhang, H., Lau, C. W., and Lin, Z. X. (2016). Attenuation of blood pressure in spontaneously hypertensive rats by acupuncture was associated with reduction oxidative stress and improvement from endothelial dysfunction. *Chin. Med.* 11:38. doi: 10.1186/s13020-016-0110-0

Li, J., Wang, Y., He, K., Peng, C., Wu, P., Li, C., et al. (2018). Effect of acupuncture at LR3 on cerebral glucose metabolism in a rat model of hypertension: A (18)F-FDG-PET study. *Evid. Based Complement Alternat. Med.* 2018:5712857. doi: 10.1155/2018/5712857

Li, L., Ma, J., Xu, J. G., Zheng, Y. L., Xie, Q., Rong, L., et al. (2021). Brain functional changes in patients with Crohn's disease: A resting-state fMRI study. *Brain Behav.* 11:e2243. doi: 10.1002/brb3.2243

Li, Y., Xu, J., Liu, Y., Zhu, J., Liu, N., Zeng, W., et al. (2017). A distinct entorhinal cortex to hippocampal CA1 direct circuit for olfactory associative learning. *Nat. Neurosci.* 20, 559–570. doi: 10.1038/nn.4517

Liang, P., Xiang, J., Liang, H., Qi, Z., Li, K., and Alzheimer's Disease NeuroImaging Initiative (2014). Altered amplitude of low-frequency fluctuations in early and late mild cognitive impairment and Alzheimer's disease. *Curr. Alzheimer Res.* 11, 389–398. doi: 10.2174/1567205011666140331225335

Lin, B., Zhang, L., Yin, X., Chen, X., Ruan, C., Wu, T., et al. (2022). Modulation of entorhinal cortex-hippocampus connectivity and recognition memory following electroacupuncture on 3×Tg-AD model: Evidence from multimodal MRI and electrophysiological recordings. *Front. Neurosci.* 16:968767. doi: 10.3389/fnins.2022.968767

Lisman, J., Buzsáki, G., Eichenbaum, H., Nadel, L., Ranganath, C., and Redish, A. D. (2017). Viewpoints: How the hippocampus contributes to memory, navigation and cognition. *Nat. Neurosci.* 20, 1434–1447. doi: 10.1038/nn.4661

Liu, X., Wang, S., Zhang, X., Wang, Z., Tian, X., and He, Y. (2014). Abnormal amplitude of low-frequency fluctuations of intrinsic brain activity in Alzheimer's disease. *J. Alzheimers Dis.* 40, 387–397. doi: 10.3233/jad-131322

Liu, Y., Wang, K., Yu, C., He, Y., Zhou, Y., Liang, M., et al. (2008). Regional homogeneity, functional connectivity and imaging markers of Alzheimer's disease: A review of resting-state fMRI studies. *Neuropsychologia* 46, 1648–1656. doi: 10.1016/j.neuropsychologia.2008.01.027

Longhurst, J. C., and Tjen, A. L. S. (2013). Acupuncture regulation of blood pressure: Two decades of research. *Int. Rev. Neurobiol.* 111, 257–271. doi: 10.1016/b978-0-12-411545-3.00013-4

Lu, J., Guo, Y., Guo, C. Q., Shi, X. M., Du, N. Y., Zhao, R. L., et al. (2017). Acupuncture with reinforcing and reducing twirling manipulation inhibits hippocampal neuronal apoptosis in spontaneously hypertensive rats. *Neural Regen. Res.* 12, 770–778. doi: 10.4103/1673-5374.206648

Luo, X., Huang, J., Yu, J., and Tang, C. (2019). Effect of taichong (LR 3) acupuncture in spontaneously hypertensive rats. *J. Tradit. Chin. Med.* 39, 74–80.

Ma, S. M., Wang, L., Su, X. T., Yang, N. N., Huang, J., Lin, L. L., et al. (2020). Acupuncture improves white matter perfusion and integrity in rat model of vascular dementia: An MRI-based imaging study. *Front. Aging Neurosci.* 12:582904. doi: 10.3389/fnagi.2020.582904

Mills, K. T., Bundy, J. D., Kelly, T. N., Reed, J. E., Kearney, P. M., Reynolds, K., et al. (2016). Global disparities of hypertension prevalence and control: A systematic analysis of population-based studies from 90 countries. *Circulation* 134, 441–450. doi: 10.1161/circulationaha.115.018912

Mills, K. T., Stefanescu, A., and He, J. (2020). The global epidemiology of hypertension. *Nat. Rev. Nephrol.* 16, 223–237. doi: 10.1038/s41581-019-0244-2

O'Neill, J., Boccara, C. N., Stella, F., Schoenenberger, P., and Csicsvari, J. (2017). Superficial layers of the medial entorhinal cortex replay independently of the hippocampus. *Science* 355, 184–188. doi: 10.1126/science.aag2787

Paasonen, J., Stenroos, P., Salo, R. A., Kiviniemi, V., and Gröhn, O. (2018). Functional connectivity under six anesthesia protocols and the awake condition in rat brain. *Neuroimage* 172, 9–20. doi: 10.1016/j.neuroimage.2018.01.014

Philip, R., Beaney, T., Appelbaum, N., Gonzalvez, C. R., Koldeweij, C., Golestaneh, A. K., et al. (2021). Variation in hypertension clinical practice guidelines: A global comparison. *BMC Med.* 19:117. doi: 10.1186/s12916-021-01963-0

Provost, J. S., Hanganu, A., and Monchi, O. (2015). Neuroimaging studies of the striatum in cognition Part I: Healthy individuals. *Front. Syst. Neurosci.* 9:140. doi: 10.3389/fnsys.2015.00140

Qi, J., Zhang, D. M., Suo, Y. P., Song, X. A., Yu, X. J., Elks, C., et al. (2013). Renin-angiotensin system modulates neurotransmitters in the paraventricular nucleus and contributes to angiotensin II-induced hypertensive response. *Cardiovasc. Toxicol.* 13, 48–54. doi: 10.1007/s12012-012-9184-9

Rolls, E. T. (2019). The cingulate cortex and limbic systems for emotion, action, and memory. *Brain Struct. Funct.* 224, 3001–3018. doi: 10.1007/s00429-019-01945-2

Stener-Victorin, E., Fujisawa, S., and Kurosawa, M. (2006). Ovarian blood flow responses to electroacupuncture stimulation depend on estrous cycle and on site and frequency of stimulation in anesthetized rats. *J. Appl. Physiol.* 101, 84–91. doi: 10.1152/japplphysiol.01593.2005

Takehara-Nishiuchi, K. (2014). Entorhinal cortex and consolidated memory. *Neurosci. Res.* 84, 27–33. doi: 10.1016/j.neures.2014.02.012

Tayebati, S. K., Tomassoni, D., and Amenta, F. (2012). Spontaneously hypertensive rat as a model of vascular brain disorder: Microanatomy, neurochemistry and behavior. *J. Neurol. Sci.* 322, 241–249. doi: 10.1016/j.jns.2012.05.047

Tian, G. H., Sun, K., Huang, P., Zhou, C. M., Yao, H. J., Huo, Z. J., et al. (2013). Long-term stimulation with electroacupuncture at DU20 and ST36 rescues hippocampal neuron through attenuating cerebral blood flow in spontaneously hypertensive rats. *Evid. Based Complement Alternat. Med.* 2013:482947. doi: 10.1155/2013/482947

Turana, Y., Tengkawan, J., Chia, Y. C., Hoshide, S., Shin, J., Chen, C. H., et al. (2019). Hypertension and dementia: A comprehensive review from the HOPE Asia network. *J. Clin. Hypertens.* 21, 1091–1098. doi: 10.1111/jch.13558

Ungvari, Z., Toth, P., Tarantini, S., Prodan, C. I., Sorond, F., Merkely, B., et al. (2021). Hypertension-induced cognitive impairment: From pathophysiology to public health. *Nat. Rev. Nephrol.* 17, 639–654. doi: 10.1038/s41581-021-00430-6

van Eersel, M. E. A., Joosten, H., Gansevoort, R. T., Slaets, J. P. J., and Izaks, G. J. (2019). Treatable vascular risk and cognitive performance in persons aged 35 years or older: Longitudinal study of six years. *J. Prev. Alzheimers Dis.* 6, 42–49. doi: 10.14283/jpad.2018.47

Walker, K. A., Power, M. C., and Gottesman, R. F. (2017). Defining the relationship between hypertension, cognitive decline, and dementia: A review. *Curr. Hypertens. Rep.* 19:24. doi: 10.1007/s11906-017-0724-3

Wang, D., Belden, A., Hanser, S. B., Geddes, M. R., and Loui, P. (2020). Resting-state connectivity of auditory and reward systems in Alzheimer's disease and mild cognitive impairment. *Front. Hum. Neurosci.* 14:280. doi: 10.3389/fnhum.2020.00280

Wang, Y., Zheng, Y., Qu, S., Zhang, J., Zhong, Z., Zhang, J., et al. (2016). Cerebral targeting of acupuncture at combined acupoints in treating essential hypertension: An Rs-fMRI study and curative effect evidence. *Evid. Based Complement Alternat. Med.* 2016:5392954. doi: 10.1155/2016/5392954

Wei, Y., Mei, L., Long, X., Wang, X., Diao, Y., Nguchu, B. A., et al. (2020). Functional MRI investigation of ultrasound stimulation at ST 36. *Evid. Based Complement Alternat. Med.* 2020:6794013. doi: 10.1155/2020/6794013

Wen, T., Zhang, X., Liang, S., Li, Z., Xing, X., Liu, W., et al. (2018). Electroacupuncture ameliorates cognitive impairment and spontaneous low-frequency brain activity in rats with ischemic stroke. *J. Stroke Cerebrovasc. Dis.* 27, 2596–2605. doi: 10.1016/j.jstrokecerebrovasdis.2018.05.021

Whelton, P. K., and Williams, B. (2018). The 2018 European society of cardiology/European society of hypertension and 2017 American college of cardiology/American heart association blood pressure guidelines: More similar than different. *JAMA* 320, 1749–1750. doi: 10.1001/jama.2018.16755

Wu, J., Zhang, X., Zhao, J., Xue, Y., Yu, P., Wu, X., et al. (2021). Clinical study on acupuncture treatment of hypertension with hyperactivity of liver yang. *Medicine* 100:e25668. doi: 10.1097/md.0000000000025668

Xue, H., Hou, P., Li, Y., Mao, X., Wu, L., and Liu, Y. (2019). Factors for predicting reversion from mild cognitive impairment to normal cognition: A meta-analysis. *Int. J. Geriatr. Psychiatry* 34, 1361–1368. doi: 10.1002/gps.5159

Yang, K., Lv, T., Wu, J., Zhang, X., Xue, Y., Yu, P., et al. (2022a). The protective effect of electroacupuncture on the renal cortex of SHRs: A metabonomic analysis. *Biomed. Chromatogr.* 36:e5338. doi: 10.1002/bmc.5338

Yang, K., Zhang, P., Lv, T., Wu, J., and Liu, Q. (2022b). Acupuncture at Taichong and Zusanli points exerts hypotensive effect in spontaneously hypertensive rats by metabolomic analysis. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 1207:123352. doi: 10.1016/j.jchromb.2022.123352

Ye, Z. Y., Li, D. P., and Pan, H. L. (2013). Regulation of hypothalamic presympathetic neurons and sympathetic outflow by group II metabotropic glutamate receptors in spontaneously hypertensive rats. *Hypertension* 62, 255–262. doi: 10.1161/hypertensionaha.113.01466

Yi, S. S., Kim, H. J., Do, S. G., Lee, Y. B., Ahn, H. J., Hwang, I. K., et al. (2012). Arginine vasopressin (AVP) expressional changes in the hypothalamic paraventricular and supraoptic nuclei of stroke-prone spontaneously hypertensive rats. *Anat. Cell Biol.* 45, 114–120. doi: 10.5115/acb.2012.45.2.114

Yousaf, T., Dervenoulas, G., and Politis, M. (2018). Advances in MRI methodology. *Int. Rev. Neurobiol.* 141, 31–76. doi: 10.1016/bs.irn.2018.08.008

Zhang, G., Qu, S., Zheng, Y., Chen, J., Deng, G., Yang, C., et al. (2013). Key regions of the cerebral network are altered after electroacupuncture at the Baihui (GV20) and Yintang acupuncture points in healthy volunteers: An analysis based on resting fcMRI. *Acupunct. Med.* 31, 383–388. doi: 10.1136/acupmed-2012-010301

Zhang, J., Cai, X., Wang, Y., Zheng, Y., Qu, S., Zhang, Z., et al. (2019). Different brain activation after acupuncture at combined acupoints and single acupoint in hypertension patients: An Rs-fMRI study based on ReHo analysis. *Evid. Based Complement Alternat. Med.* 2019:5262896. doi: 10.1155/2019/5262896

Zhang, J., Lyu, T., Yang, Y., Wang, Y., Zheng, Y., Qu, S., et al. (2021). Acupuncture at LR3 and KI3 shows a control effect on essential hypertension and targeted action on cerebral regions related to blood pressure regulation: A resting state functional magnetic resonance imaging study. *Acupunct. Med.* 39, 53–63. doi: 10.1177/0964528420920282

Zhang, J., Ng, D., and Sau, A. (2009). Effects of electrical stimulation of acupuncture points on blood pressure. *J. Chiropr. Med.* 8, 9–14. doi: 10.1016/j.jcm.2008.07.003

Zhang, Q., Tan, Y. Y., Liu, X. H., Yao, F. R., and Cao, D. Y. (2018). Electroacupuncture improves baroreflex and γ-aminobutyric acid type B receptor-mediated responses in the nucleus tractus solitarii of hypertensive rats. *Neural Plast.* 2018:8919347. doi: 10.1155/2018/8919347

Zhao, Z. Q. (2008). Neural mechanism underlying acupuncture analgesia. *Prog. Neurobiol.* 85, 355–375. doi: 10.1016/j.pneurobio.2008.05.004

Zheng, Y., Zhang, J., Wang, Y., Wang, Y., Lan, Y., Qu, S., et al. (2016). Acupuncture decreases blood pressure related to hypothalamus functional connectivity with frontal lobe, cerebellum, and insula: A study of instantaneous and short-term acupuncture treatment in essential hypertension. *Evid. Based Complement Alternat. Med.* 2016:6908710. doi: 10.1155/2016/6908710

Zuo, M., Xu, Y., Zhang, X., Li, M., Jia, X., Niu, J., et al. (2018). Aberrant brain regional homogeneity and functional connectivity of entorhinal cortex in vascular mild cognitive impairment: A resting-state functional MRI study. *Front. Neurol.* 9:1177. doi: 10.3389/fneur.2018.01177

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Acupuncture treatment for post-stroke depression: Intestinal microbiota and its role

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Stroke-induced depression is a common complication and an important risk factor for disability. Besides psychiatric symptoms, depressed patients may also exhibit a variety of gastrointestinal symptoms, and even take gastrointestinal symptoms as the primary reason for medical treatment. It is well documented that stress may disrupt the balance of the gut microbiome in patients suffering from post-stroke depression (PSD), and that disruption of the gut microbiome is closely related to the severity of the condition in depressed patients. Therefore, maintaining the balance of intestinal microbiota can be the focus of research on the mechanism of acupuncture in the treatment of PSD. Furthermore, stroke can be effectively treated with acupuncture at all stages and it may act as a special microecological regulator by regulating intestinal microbiota as well. In this article, we reviewed the studies on changing intestinal microbiota after acupuncture treatment and examined the existing problems and development prospects of acupuncture, microbiome, and poststroke depression, in order to provide new ideas for future acupuncture research.

KEYWORDS

depression, intestinal microbiota, stroke, inflammation, immune response

Abbreviations: 5-HT, 5-hydroxytryptamine; ACTH, adrenocorticotropic hormone; AD, Alzheimer's disease; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; CHR, corticotropin-releasing hormone; CK, cytokine; CNS, central nervous system; CORT, cortisol; CRF, cancer-related fatigue; CUMS, chronic unpredictable mild stress; DA, dopamine; Defα5, defensin alpha 5; DIO, diet-induced obese; DSS, dextran sulfate sodium; FC, functional constipation; GFAP, glial fibrillary acidic protein; HAMD, Hamilton depression; HDL, high density lipoprotein; HFD, high fat diet; HPA, hypothalamic-pituitary-adrenal; IDO, indoleamine 2,3-dioxygenase; KOA, knee osteoarthritis; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NMDAR, N-methyl-D-aspartate receptor; PD, Parkinson's disease; PSD, post-stroke depression; PUD, peptic ulcer disease; PVN, paraventricular nucleus; SCFA, short-chain fatty acid; SN, substantia nigra; SPF, specific pathogen-free; SSRIs, selective serotonin reuptake inhibitors; TcA, tricyclic antide pressive agent; TFF, trilobal factor; TG, triglyceride; Th17, T helper T cell 17; TLRS, toll-like receptors; Treg, Regulatory T cells; UC, ulcerative colitis; VIP, vasoactive intestinal peptide; VPAC2, vasoactive intestinal peptide type 2 receptor; WHO, World Health Organization; β-CaMKII, Calmodulin-dependent protein kinase II-β.

1. Introduction

The most common neuropsychological disorder after stroke is depression, which can occur at any point during the process. It is estimated that more than one-third of stroke survivors experience post-stroke depression (PSD) (Frank et al., 2022), which is a global public health issue that requires urgent attention in national health policy. Depression ranks as one of the leading causes of disability worldwide and contributes significantly to the global burden of disease, according to the World Health Organization (WHO). Additionally, treatment options recommended by the WHO report include, but are not limited to, psychotherapy and/or antidepressants, the most classic of which are tricyclic antide pressive agents (TcAs) and selective serotonin reuptake inhibitors (SSRIs) (Li et al., 2022a). Drugs, however, have inherent side effects, such as the development of drug resistance, and often reported adverse effects including sexual dysfunction and gastrointestinal symptoms, neuropsychiatric symptoms, and other systemic symptoms (Anagha et al., 2021). Throughout the years, medical treatment patterns have gradually changed, patients' vital interests are better served by improving the safety of therapeutic measures while pursuing curative effects. When it comes to treating PSD comprehensively, acupuncture has important advantages as an ideal "green treatment." In recent years, more and more standardized clinical trials have shown acupuncture not only promotes nerve function recovery after stroke, but also significantly benefits in treating patients' depression symptoms as well as improving their quality of life after stroke, when comparing with drugs, acupuncture offers better biosafety and socioeconomic benefits in the treatment of PSD (Hang et al., 2021; Wang Z. et al., 2021).

In addition to the combination of neurological and psychiatric symptoms, it is also common for PSD patients to have abnormal digestive tract function (Jiang, 2022). Researchers have found a significant difference between patients with PSD and those without PSD in terms of gut microbial community and metabolites (Jiang et al., 2021; Zhong et al., 2022). Therefore, it is possible that reasonable gut microbiome composition may play a significant role in maintaining healthy metabolism, and it is increasingly being recognized that depression can be treated with direct changes in intestinal microbiota composition (such as prebiotic intake and fecal microbiota transplantation) (Evrensel and Tarhan, 2021). Studies have shown that mood and behavior are controlled and affected by intestinal flora through neuroimmune mechanisms and nutritional metabolism, whereas unbalanced gut flora can cause mental illness (Liang et al., 2018b; Waclawiková and El Aidy, 2018). Psychoneurotic symptoms in rats can be significantly improved by acupuncture, which may be related to rebalancing the intestinal flora (Li et al., 2021; Xian et al., 2022).

Consequently, maintaining the balance of intestinal microbiota is expected to be a potential target for PSD with acupuncture. Moreover, bibliometric analysis shows that the number of studies focusing on intestinal flora has been increasing over the past 10 years, indicating that acupuncture regulation of intestinal microbiota is a promising research area (Zhang et al., 2022a). In this article, an overview of the relationship between acupuncture and intestinal flora, the relationship between intestinal flora and PSD, the effect and mechanism of acupuncture on the intestinal flora in preventing and treating PSD were provided in an attempt to provide new ideas and targets for studies of traditional Chinese medicine in the treatment of PSD, and we hope to provide some assistance in the decision-making process for acupuncture PSD treatment in the future.

2. The relationship between acupuncture and intestinal microbiota

Human-related microbial communities are mainly found in the large intestine, which mainly composed of prokaryotes (like bacteria), eukaryotes (like fungi and parasites), and viruses (Li et al., 2022b). It is estimated that the total number of bacteria colonized in the human intestinal tract is about 10^{13}–10^{14}, most of which are *Bacteroides* and Firmicutes and the amount of bacteria is about 10 times as much as the total number of cells in the human body and their number of genes is 100 times higher than the human genome (Gill et al., 2006). Although it only weighs 1–1.5 kilograms total, it plays an important role in maintaining the dynamic balance of the internal environment and promoting human health (Bäckhed et al., 2005; Borrel et al., 2020).

Microecological community's succession are intricately intertwined with intestinal various physiological and pathological processes in the body, especially metabolism and immunity (Lynch and Hsiao, 2019). As soon as intestinal microecology homeostasis is disrupted (e.g., reduced richness, dysfunctional microflora, an interference with metabolism or microflora translocation, etc.), it stimulates immune response disorders through different mechanisms and destroys the host immune system as a result and various immune-mediated inflammatory responses will occur, endangering human health (Ruff et al., 2020).

In different disease states, acupuncture can effectively regulate intestinal microbiota, making it a useful microecological regulator. According to existing studies, acupuncture can treat diseases by conducting information exchange between immune-neuro-endocrine-microbial metabolism through brain-intestinal interaction (Xu and Lu, 2020). For example, acupuncture affects the abundance and structure of intestinal bacteria, balancing the number and proportion of probiotics and pathogens in the host body (Xie et al., 2020; Wang J. M. et al., 2021; Wang T. Q. et al., 2021; Li et al., 2022d). In turn, acupuncture reverses a variety of intestinal flora metabolic disorders caused by various diseases by restoring the function and metabolic pathway of key metabolites in human body (Xu et al., 2017; Si et al., 2022). In the restoration of human health, acupuncture plays a crucial role. However, research on acupuncture's regulation of intestinal flora is still limited in the domestic and overseas, facing the problem of limited scope and insufficient depth.

3. The relationship between intestinal flora and PSD

As a secondary to stroke, PSD is characterized by mental and emotional disorders, as well as insomnia, low mood, loss of interest, and loss of appetite and serious people will even exhibit concerning behavioral and psychological characteristics, such as fantasy, delusion, world-weariness, suicide (Wijeratne et al., 2022). Currently, there is no clear pathogenesis for PSD. Current mainstream views is that depression consists of a number of interactions between neurobiology and social psychology and other factors (Li et al., 2022c), and cerebral vascular disease may be a predisposing factor or a motivating factor for depression (Jeon and Kim, 2018).

Depression and digestive problems have strong comorbidities, and many patients with depression go to the hospital for the first attendance usually due to difficult-to-treat gastrointestinal conditions (Liang et al., 2018a; Jiang, 2022). With the deepening of research, researches have gradually illuminated the close connection between psychological factors and gastrointestinal disease over the years. In a meta-analysis, depression was found in 22–38% of patients with irritable bowel syndrome (Hu et al., 2021), and in a cohort study, depression was a comorbid condition for 40.1% of those with inflammatory bowel disease (Lewis et al., 2019). Not only a high prevalence of depression in digestive diseases, but vice versa as well. In another study, researchers found that the rate of gastrointestinal abnormalities among patients with depression was significantly higher than that of those without depression and compared with patients with depression alone, those with depression combined with gastrointestinal symptoms had a more severe depression (Fang and Li, 2022). As a result of these findings, researchers are now paying more attention to gut-brain connection.

According to some available research, changes in the composition of the gut microbiome were strongly associated with the severity of PSD, as shown in **Figure 1** (Jiang et al., 2015; Ye et al., 2021). A neuro-endocrine-immune network exists between the brain and gut flora, called the brain-gut axis, which communicates two-way signals, and the gastrointestinal tract is closely linked to the brain mainly through neural and humoral pathways, allowing this network to circulate and reinforce each other (Begum et al., 2022; Han et al., 2022). Studies have shown that long-term stress responses in depressed patients increase intestinal wall permeability, making it easier for aggressive bacteria or antigens to translocate to the lymphatic system or circulatory system, and then activating immune cells to trigger serum IgA and IgM production, then causing depressive episodes through systemic inflammation (Maes et al., 2008, 2012; O'Malley et al., 2010), or microbial metabolites are more easily to enter the blood circulation through the intestinal wall and pass the blood-brain barrier (BBB), directly affecting the cognitive and behavioral functions of the body (Rao et al., 2021). Notably, due to two-way communication characteristics of brain-gut axis, changes in gut microbiome composition may in turn influence depressive symptoms. Several studies have shown that the gut microbiota of people with depression differs significantly from that of people without depression in terms of diversity and abundance, with abundance being negatively correlated with the severity of depression (Jiang et al., 2015; Hu S. et al., 2019), moreover, some studies have shown that an increase in potentially harmful bacteria or a decrease in beneficial bacteria could reduce short-chain fatty acids (SCFAs) production, leading to intestinal barrier dysfunction and inflammation (Wong et al., 2006; Ramos Meyers et al., 2022). Additionally, a transplantation of patients' gut microbiota caused mice to exhibit depression-like behavior when metabolic processes and inflammatory responses were affected by fecal microbiota transplantation (Liu et al., 2021). Hence, there may be a potential bidirectional interaction between stress and microbiome.

Antibiotics target bacteria, inhibiting their growth and proliferation, and is the most direct, widespread, and important influencing factor for changing intestinal flora composition. Antibiotics have a double-edged effect on depression, on the one hands, antibiotic treatment led to the disappearance of depression-like ethological disorders (Yu et al., 2022), and it has to be pointed out that the first drugs to be used to treat depression was Iproniazid, which was originally developed for tuberculosis (Juli and Juli, 2014; Macedo et al., 2017); on the other hands, antibiotics can damage intestinal flora's homeostasis in the gut, resulting in depression (Hao et al., 2020). Multiple studies have indicated that antibiotic exposure increases depression risk, further, the risk of depression may increase with each additional treatment course and medication, while the declines in risk is characteristic by slow and sustained (Lurie et al., 2015; Köhler et al., 2017; Hu et al., 2022; Pouranayatihosseinabad et al., 2023).

The hypothalamic-pituitary-adrenal (HPA) axis is an important part of the neuroendocrine system. When the human body is exposed to stress, cortisol in the HPA axis is activated, which reduces inflammation and protects against extreme immune responses (Mikulska et al., 2021). However, cortisol elevation caused by chronic stress is also an important factor in the development of depression (Qin et al., 2015). Several studies have demonstrated that neuroendocrine regulation plays an important role in the pathophysiology of neuropsychiatric disorders, and there is an interaction between gut microbiota and HPA axis activity (Ge et al., 2021). Microbial communities can be changed by altering HPA axis activity [such as adrenalectomy, subcutaneous injection of adrenocorticotropic hormone (ACTH) fragments] (Amini-Khoei et al., 2019; Song et al., 2019). In addition to regulating the HPA axis dysfunction caused by stress, probiotics supplementation can also alleviate some depressive behaviors (Liang et al., 2015; Rea et al., 2016).

There are also considerable literatures suggesting a link between the vagus nerve and depression and gastrointestinal disorders (Liu et al., 2020a; Tan et al., 2022). The vagus nerve is one of the most important components of the parasympathetic system, which plays a major role in the regulation of gut-brain axis by acupuncture. Vagus nerve is a hybrid nerve with afferent and efferent fibers that senses gut microbiota metabolites and transmits information about them to the central nervous system (CNS). Additionally, activated efferent vagus nerves can also exert a systemic anti-inflammatory response by directly stimulating the HPA axis and cholinergic pathways, which alleviates damage to intestinal tight junctions and reduces intestinal permeability, thus regulating changes in microbial composition (Borovikova et al., 2000; Hu et al., 2013; Zhou et al., 2013).

4. Acupuncture regulates intestinal microbiota in PSD

Once a patient has a stroke event, due to stroke, drugs, chronic stress, abnormal activation of the HPA axis and vagus nerve, a series of digestive tract symptoms will occur in the

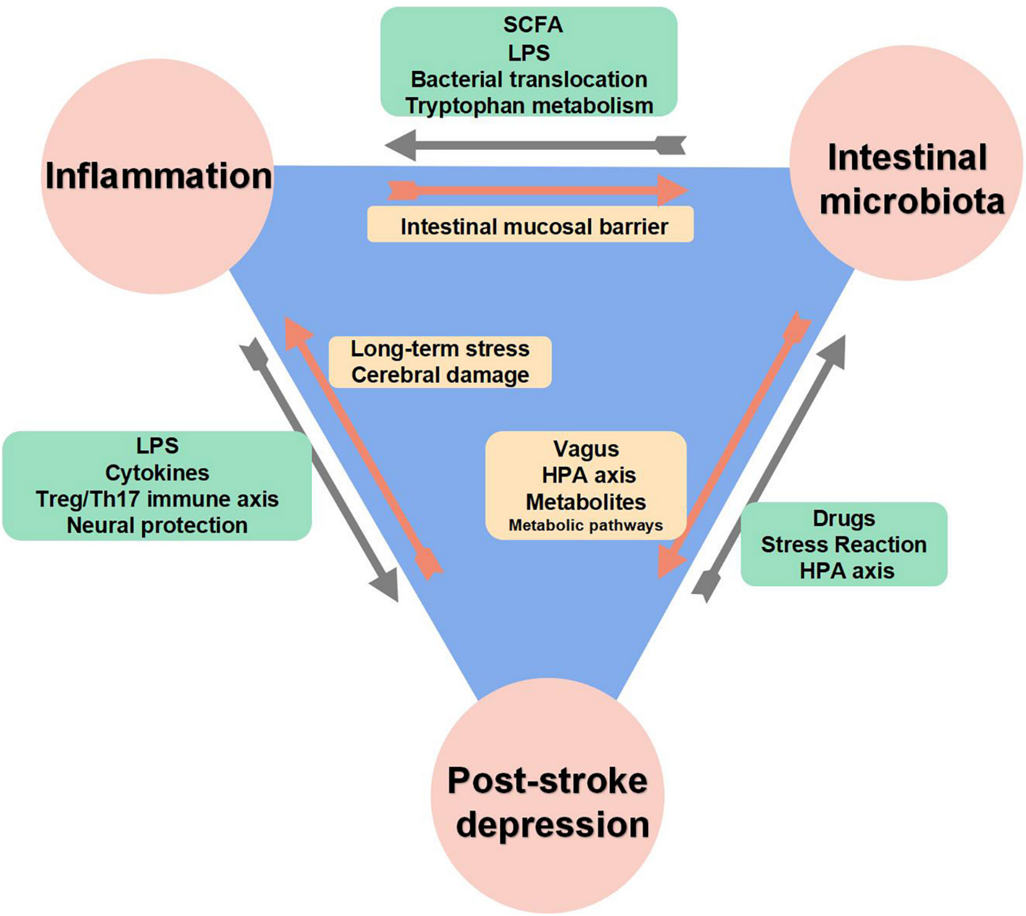

FIGURE 1
The interaction between inflammation, intestinal microbiota and post-stroke depression (PSD).

human body. These processes lead to damage to the intestinal mucosal barrier, resulting in an imbalance of intestinal microbiota due to excessive production of pro-inflammatory substances [lipopolysaccharide (LPS), proinflammatory cytokines (CKs)] and too little production of anti-inflammatory substances (SCFAs, anti-inflammatory cytokines), causing abnormal immune responses (local and systemic inflammatory responses) in the body, ultimately damaging neurons and exacerbating depression. Acupuncture can regulate the structure of intestinal microbiota, inhibit inflammatory storms and improve the symptoms of patients with PSD mainly through the following six ways in **Figure 2**. The original research evidence of acupuncture regulates intestinal microbiota is summarized in **Table 1**.

4.1. Regulation of intestinal microbial structure

Recent studies have gradually shown that acupuncture indirectly alters the microbial composition and communities in various ways, and researchers have found that post-stroke depression-like behavior is strongly associated with intestinal microbial changes after acupuncture treatment (Jiang et al., 2021).

Based on 16S rRNA sequencing, Lv et al. (2022) found that manual acupuncture treatment significantly increased the abundances of Firmicutes, Bacteroidetes, and Patescibacteria and significantly decreased the abundances of Proteobacteria in mice at the phylum level; and the abundance of Candidatus *Arthromitus*, *Lactobacillus*, Muribaculaceae_unclassified, and Clostridia_UCG-014_unclassified were significantly increased and the abundances of Escherichia–Shigella, Burkholderia-Caballeronia-Paraburkholderia, and *Streptococcus* were decreased at the genus level in response to manual acupuncture. In general, the Lv et al. (2022) study demonstrated acupuncture alleviated disease-associated gut microbiome imbalances. Furthermore, there was also a significant correlation observed between the development of depression and the content of Clostridiaceae, Candidatus *Arthromitus*, and *Lactobacillus*. Additionally, Zhang et al. (2022d) observed that manual acupuncture significantly reduced the abundance of Firmicutes, Proteobacteria and Escherichia–Shigella in Alzheimer's disease mice, while significantly increasing the abundance of *Bacteroides*, which led to improvements in intestinal flora. Liu et al. (2020b, 2022) demonstrated that electroacupuncture regulated the overall structure of the intestinal microbiota in the intestinal tract of diseased mice, making the abundance and diversity of Firmicutes is similar to what it is in the healthy mice's intestinal tracts.

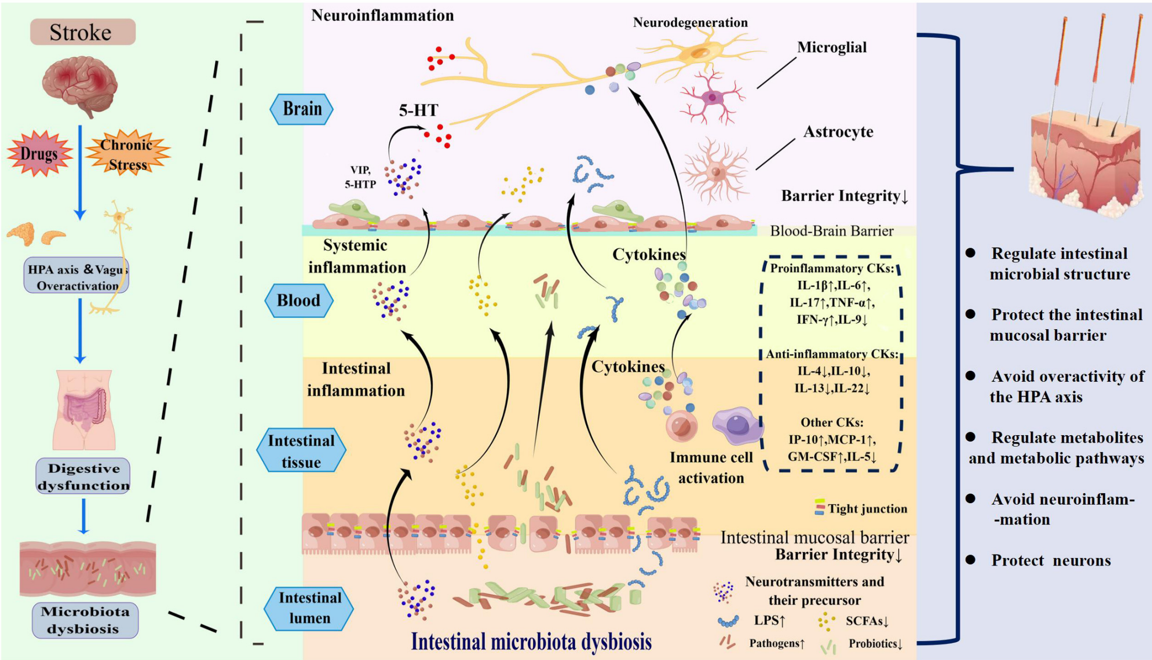

FIGURE 2
The mechanism of intestinal microbiota dysbiosis induced by post-stroke depression (PSD) and how acupuncture regulates the intestinal microbiota to treat PSD.

According to Hao et al. (2022), mice experiencing manual acupuncture showed a significant increase in *Bacteroides* while a decrease in Proteobacteria and Escherichia–Shigella, however, no significant improvement in intestinal microbiota diversity was found, perhaps method of calculating diversity or insufficient samples might limit the result of diversity.

Wang J. M. et al. (2021) observed how electroacupuncture affected patients' flora structures, and observed that part of their flora structures reversed and gradually began to resemble those of healthy individuals, finding the following: at the phylum level, the relative abundance of Firmicutes and the Firmicutes/*Bacteroides* ratio decreased significantly; at the genus level, the relative abundance of *Blautia* increased while the abundance of Escherichia–Shigella decreased. And by the way, Firmicutes and *Bacteroides* are believed to be the predominant bacteria in healthy individuals' intestinal tracts, and using the ratio between them, researchers can assess the degree of intestinal microbial health and establish a landmark parameter for determining the degree of intestinal health (Tang et al., 2019). Furthermore, *Blautia* produces a variety of SCFAs that have anti-inflammatory properties (Koh et al., 2016), and gram-negative bacteria such as Escherichia–Shigella contains LPS, which is a proinflammatory compound found in the cell wall of gram-negative bacterium (Yoo et al., 2022). Consequently, the decreased level of Escherichia–Shigella and the increased relative abundance of *Blautia* in patients treated with electroacupuncture indicate reduced host's inflammation. Xu et al. (2021) found that electroacupuncture rebalances the structure of the intestinal microbiota in mice by reducing Firmicus/*Bacteroides* ratio and the relative abundance of *Roseburia*, *Lachnoclostridium*, and Ruminiclostridium 9 to bring them closer to healthy mice's state.

A manual acupuncture treatment was conducted on rats with depression by Li et al. (2021), and researchers found that manual acupuncture regulation could reduce *Bacteroides*/Firmicutes ratios in the intestinal tract of depressed rats and improve the biodiversity of intestinal flora. Hence, electroacupuncture and manual acupuncture are capable of reversing the proportion of gut bacteria, thus alleviating intestinal ecological disorder in patients (Liu et al., 2020c).

Wang T. Q. et al. (2021) found that electroacupuncture could reverse the increase in the abundance of *Streptococcus* in the disease state, while increasing the abundance of the *Bacteroides* and *Agathobacter* (beneficial bacteria). There was a strong correlation between fecal *Streptococcus* abundance and Hamilton depression scale (HAMD) scores (possibly related to intestinal mucosal barrier and immunity being affected by tryptophan metabolism) (Zhang et al., 2022c), Health-beneficial SCFAs can be produced by *Bacteroides* and *Agathobacter* (beneficial bacteria) in the gut that inhibit opportunistic pathogens and prevent the host from inflammation (Koh et al., 2016; Hua et al., 2020). According to Jang et al. (2020), manual acupuncture restored bacterial abundance and approximately 70% of microbiome composition in the intestinal tract of diseased mice, and increased the number of *Butyricimonas*, which has anti-inflammatory properties by increasing the production of butyrate, a SCFA (Yang et al., 2022).

Xie et al. (2020) carried out 2-week electroacupuncture intervention in mice, and found that electroacupuncture could inhibit proinflammatory shift with promoting the recovery of the relative abundance of *Akkermansia*, *Clostridium*, Lactococcus, and *Butyricimonas* in the intestinal tract, and significantly increase the relative abundance of *Lactobacillus*. A number of bacteria above are capable of inhibiting inflammation, protecting the

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TABLE 1 The original researches on the regulation of intestinal microbiota by acupuncture.

No.	Acupuncture	Model	Mechanism and effect						References
			Intestinal microbial structure	Intestinal mucosal barrier	Hypothalamic-pituitary-adrenal (HPA) axis	Metabolites and metabolic pathways	Inflammatory responses	Central neurons	
1	Manual acupuncture	Female BALB/c mice [cancer-related fatigue (CRF) mice]	The abundance: Candidatus *Arthromitus*↑, *Lactobacillus*↑, Clostridia_UCG-014_unclassified↑; Escherichia–Shigella↓, Burkholderia-Caballeronia-Paraburkholderia↓, *Streptococcus*↓.	Tight junction proteins (ZO-1, Occludin, Claudin-5)↑	Restore adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH) and cortisol (CORT) expression levels	Regulating the differential metabolites N-methylnicotinamide, beta-glycerophosphoric acid, geranyl acetoacetate, serotonin and phenylalanine, tyrosine and tryptophan biosynthesis, taurine and hypotaurine, and beta-alanine metabolic pathways.	In the gut and hippocampus: IL-1β↓, L-6↓, TNF-α↓.		Lv et al., 2022
2	Manual acupuncture	Male APP/PS1 mice [Alzheimer's disease (AD)]	The abundance: *Bacteroides*↑; Firmicutes↓, Proteobacteria↓, Escherichia–Shigella↓.				In serum and brain: LPS↓, TNF-α↓, IL-1β↓.		Zhang et al., 2022d
3	Electroacupuncture	Male C57BL/6 mice [dextran sulfate sodium-(DSS-) induced colitis]	The abundance and diversity: Firmicutes↑, *Akkermansia* muciniphila↑, Bacteroidales↑, Lactobacillales↑, S24-7↑; Enterobacteriaceae↓, Proteobacteria↓, Turicibacterales↓, Erysipelotrichales↓, Turicibacteraceae↓, Clostridiaceae↓, Turicibacter↓, SMB53↓,	Tight junction proteins (Claudin-1, Occludin, ZO-1)↑		Melatonin↑, adiponectin↑, vasoactive intestinal peptide type 2 receptor (VPAC2)↑.	IFN-γ↓, TNF-α↓, IL-6↓, Th2/ILC2 related cytokines (including IL-4, IL-5, IL-9, IL-13, IL-10)↑, ILC3-derived cytokines (IL-22 and GM-CSF)↑.		Liu et al., 2020b, 2022
4	Manual acupuncture	Male APP/PS1 mice (AD)	The abundance: *Bacteroides*↑; Proteobacteria↓, Escherichia–Shigella↓.	The damage of small intestine structures↓, tight junction proteins (Occludin and ZO-1)↑.			Lipopolysaccharide (LPS)↓, TNF-α↓.		Hao et al., 2022
5	Electroacupuncture	Hypertensive patients	Firmicutes/Bacteroidetes ratio↓. The abundance: *Blautia*↑, Escherichia–Shigella↓.						Wang J. M. et al., 2021
6	Electroacupuncture	Female Kunming mice [functional constipation (FC)]	Firmicutes/Bacteroidetes ratio↓. The relative abundance: *Roseburia*↓, *Lachnoclostridium*↓, Ruminiclostridium 9↓.						Xu et al., 2021

(Continued)

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TABLE 1 (Continued)

No.	Acupuncture	Model	Mechanism and effect						References
			Intestinal microbial structure	Intestinal mucosal barrier	Hypothalamic-pituitary-adrenal (HPA) axis	Metabolites and metabolic pathways	Inflammatory responses	Central neurons	
7	Manual acupuncture	Specific pathogen-free male Sprague–Dawley rats [chronic unpredictable mild stress (CUMS)]	*Bacteroides*/Firmicutes ratios↓. The abundance: Firmicutes↑, *Bacteroides*↓.			Dopamine (DA)↑, 5-hydroxytryptamine (5-HT)↑. Affecting the cell growth, the apoptosis pathway, the cofactor and vitamin metabolism pathway, amino acid metabolism pathway, and the carbohydrate metabolism pathway.		Enhancing brain-derived neurotrophic factor (BDNF) signaling. The mRNA and protein expression: BDNF and N-methyl-D-aspartate receptor (NMDAR)↑; Calmodulin-dependent protein kinase II-β (β-CaMKII)↓. Astrocytes↑.	Li et al., 2021
8	Electroacupuncture	Participants with knee osteoarthritis (KOA)	The abundance: *Bacteroides*↑, *Agathobacter*↑; *Streptococcus*↓.						Wang T. Q. et al., 2021
9	Manual acupuncture	Male C57BL/6 mice [Parkinson's disease (PD)]	*Butyricimonas*↑.					Bax↓, NF-κB↓, TNF-α↓, Bcl-2↑. The activation and overexpression of microglia and astrocytes↓, Dopaminergic fibers and neurons↑.	Jang et al., 2020
10	Electroacupuncture	Male Sprague–Dawley rats [diet-induced obese knee osteoarthritis models (DIO-KOA)]	Bacteroidetes/Firmicutes ratio↑. The relative abundance of *Lactobacillus*↑. Recovery of the relative abundance of *Akkermansia*, *Clostridium*, Lactococcus, and *Butyricimonas*.			Total cholesterol↓, triglyceride (TG)↓, low-density lipoprotein (LDL)↓, high density lipoprotein (HDL)↑.	In serum: IP-10↓, IL-1α↓, MCP-1↓. In articular synovial fluid: lipopolysaccharide (LPS)↓.		Xie et al., 2020
11	Electroacupuncture	Male db/db mice (spontaneous T2DM mouse)	Probiotics: *Blautia*↑, *Lactobacillus*↑. Opportunist pathogens: *Alistipes*↓, *Helicobacter*↓, *Prevotella*↓.	Mucosal inflammation, goblet cells, and epithelial damage in the colon.		TG↓, low-density lipoprotein cholesterol (LDL-C) ↓.	SCFAs (acetic acid and butyric acid)↑, LPS↓, IL-6↓.		Zhang et al., 2021

(Continued)

TABLE 1 (Continued)

No.	Acupuncture	Model	Mechanism and effect						References
			Intestinal microbial structure	Intestinal mucosal barrier	Hypothalamic-pituitary-adrenal (HPA) axis	Metabolites and metabolic pathways	Inflammatory responses	Central neurons	
12	Electroacupuncture	Patients with mild to moderate PD	The relative abundance: *Bacteroides*↑; *Parasutterella*↑, the genera *Dialister*↓, *Hungatella*↓, *Barnesiella*↓, *Megasphaera*↓, *Allisonella*↓, *Intestinimon*↓, *Moryella*↓.						Nazarova et al., 2022
13	Acupuncture	Rats with functional dyspepsia				Tryptophan indole metabolites			Zhang et al., 2022b
14	Electroacupuncture	Specific pathogen-free (SPF)-certified male Kunming mice [peptic ulcer disease (PUD) model]	No significant change in the alpha diversity of duodenal microbial.	The arrangement of duodenal mucosal cells was closer to normal, and the villus morphology basically returned to normal.		Trilobal factor (TFF)↑, DA↑			Li et al., 2022d
15	Electroacupuncture	Male C57BL/6J mice [high fat diet (HFD)-induced obese mice]	The abundance of jejunal and cecal microbiota was restored.			Activating Nod-like receptor signaling pathways, Defensin alpha 5 (Defα5)↑, Energy metabolism↑, Lipid metabolism↓.			Xia X. et al., 2022
16	Electroacupuncture	High fat diet (HFD)-induced obese mice	The relative abundances of gut microbiota was restored.			Regulating glycerophospholipid metabolism and primary bile acid biosynthesis.			Si et al., 2022
17	Electroacupuncture	Male Sprague–Dawley rats (HFD-induced obese rats)	Firmicutes/*Bacteroides* ratio↓. The relative abundance: *Prevotella_9*↑.			Regulating lipid metabolism and improving insulin sensitivity and glucose homeostasis.			Wang et al., 2019
18	Electroacupuncture	Ulcerative colitis (UC) mice	The diversity and abundance of gut microbiota↑.				The percentage of Treg cells in CD4+ T lymphocytes↑, the percentage of Th17in CD4+ T lymphocytes↓.		Wei et al., 2019
19	Electroacupuncture	PD mice	The abundance: Erysipelotrichaceae↓.				The mRNA levels: IL-6↓, TNF-α↓.	The loss of dopaminergic neurons↓.	Han Q. Q. et al., 2021

intestinal barrier and preventing depression (Guo et al., 2022; Lai et al., 2022; Ramalho et al., 2022). All of the above bacteria have the potential to inhibit inflammation, protect the intestinal barrier, and prevent depression (Guo et al., 2022; Lai et al., 2022; Ramalho et al., 2022). It can be seen that acupuncture alleviates systemic inflammation in rats overall through the increase in beneficial microorganisms' abundance. Zhang et al. (2021) observed that, at the phylum level, electroacupuncture could modulate the intestinal microbiota structure of T2DM mice to a level similar to that of normal control mice, and researchers found electroacupuncture could increase probiotics (*Blautia* and *Lactobacillus*) and decreased opportunist pathogens (*Alistipes*, *Helicobacter*, *Prevotella*), moreover, a significant correlation was also observed between changes in intestinal flora and changes in LDL-C. After electroacupuncture intervention for 8 weeks, Nazarova et al. (2022) observed changes in intestinal bacteria of participants, and result showed the relative abundance of *Bacteroides* and *Parasutterella* increased significantly at the genus level, whereas the abundances of the genera *Dialister*, *Hungatella*, *Barnesiella*, *Megasphaera*, *Allisonella*, *Intestinimon*, and *Moryella* were significantly lower. Thus, researchers emphasized the role of the gut-brain axis in the process of the treatment in central system diseases.

4.2. Regulating the intestinal mucosal barrier to prevent bacterial translocation

The composition of intestinal microbiota can be indirectly affected by acupuncture. Additionally, protecting the structure and function of the intestinal mucosal barrier system, which indirectly affects the colonization of bacteria and prevents pathogenic antigens penetrating (translocation) the physical barrier, so that human health can be maintained (Macpherson et al., 2002). As soon as the intestinal mucosal barrier is damaged, it increases permeability of the intestinal epithelium (leaky gut), which allows inflammation-related factors and other harmful substances to enter the circulatory system and initiate systemic inflammation (Wasinger et al., 2020; Dou et al., 2022). As part of the intestinal barrier, tight junctions and their proteins protect organisms from pathogens entering from the external environment, which play a significant role in maintaining intestinal barrier integrity (Lin et al., 2022). By establishing cell polarity, tight junctions determine paracellular permeability and serve as a major barrier to the paracellular pathway (Zihni et al., 2016). In intestinal epithelium, tight-junction proteins identify the permeability of paracellular ions at tight junctions, which are located mainly on the lateral sides of the junction tops of adjacent cells (Zeisel et al., 2019). As well as maintaining the integrity of the tight junctions between cells and maintaining the barrier function, it plays a role in the repair of intestinal epithelial damage (Krug and Fromm, 2020).

An experiment conducted by Lv et al. (2022) revealed that acupuncture promoted tight junction proteins (ZO-1, Occludin, Claudin-5) and improved the function of mice's intestinal mucosal immune barriers. Additionally, this study (Lv et al., 2022) showed that intestinal tight-junction protein expression is correlated with changes in intestinal flora abundance after acupuncture intervention.

In two electroacupuncture experiments, Liu et al. (2020b, 2022) found that Claudin-1, Occludin, ZO-1 properties were repaired. Thus, by improving the tight junctions of intestinal epithelial cells, it can stabilize permeability and maintain intestinal homeostasis.

In diseased animals, Hao et al. (2022) observed under an electron microscope that the damage of small intestine structures were significantly reduced after intervention with manual acupuncture. An electron-microscopical examination reveals a mild separation of the epithelium from the lamina propria, an orderly arrangement of intestinal gaps as well as narrower connection gaps. An immunofluorescence experiment reveals the fluorescence structure of tight junction proteins (Occludin and ZO-1) was restored by manual acupuncture intervention, and the fluorescence proteins showed continuity and enhanced intensity, maintaining the intestinal mucosal barrier.

4.3. Regulation of hypothalamic-pituitary-adrenal (HPA) axis disorders

The HPA axis disorder is closely related to the host circadian rhythm disorder and the body's stress response. The HPA axis is regulated by the circadian rhythm cycle, and its abnormal function can trigger sleep disorders and contribute to depression development (Wirz-Justice, 2006; Kim et al., 2015). The result of a cross-sectional study examining the link between insomnia and PSD suggests that insomnia before stroke is an indicator of depression, and stroke is a risk event that can worsen depression (Zheng, 2021). In another clinical cross-sectional study, stroke survivors with poorer subjective sleep were also more likely to suffer from depression (Davis et al., 2019). Patients with PSD often suffer from sleep disorders, so the two frequently require active treatment together (Cai et al., 2021). The composition and function of the gut microbiome also exhibits circadian rhythmicity in relation to the host's activity (Thaiss et al., 2014). This manifests itself in the fact that interference with the sleep pattern of the host can alter the expression of clock genes, ultimately altering the structure and diversity of the gut microbiome (Voigt et al., 2014; Leone et al., 2015), which in turn can drive changes in the circadian rhythm of the host (Thaiss et al., 2016). Moreover, the HPA axis, as one of the key components of stress regulation, can timely perceive pressure and quickly initiate signals in the paraventricular nucleus (PVN) of the hypothalamus, and HPA axis abnormalities may be one of the biological indicators for depression in its early stages (Spalletta et al., 2006; Du and Pang, 2015). Moreover, there is evidence that acute ischemic stroke can act as a stressor to activate the HPA axis (Wexler, 1970; Yoo et al., 2011). There are several basic studies showing that acupuncture can down-regulate the expression of CRH mRNA in hypothalamus, reduce plasma levels of ACTH and CORT (Le et al., 2016; Zheng et al., 2019), which plays an antidepressant role by inhibiting the over-excitation of HPA axis (Han X. et al., 2021). There is also a bidirectional regulatory relationship between the HPA axis and intestinal microecology. Microbiomes in the gut regulate corticosteroid production, including cortisol and glucocorticoids, in turn, the HPA axis can regulate intestinal motility and affect the living environment of intestinal microbiota, and it has been shown that overactivity of the HPA axis can increase intestinal mucosal permeability, activate intestinal immunity, and further alter the composition of microbiome in the intestines, disrupting the gut-microbiome balance (Li et al., 2018; Wu et al., 2018; Młynarska et al., 2022).

According to Lv et al. (2022), manual acupuncture could restore ACTH, CRH and cortisol CORT expression levels, as well as improve dysfunction of the HPA axis. Also, this study found that changes in intestinal flora abundance and hormone expression were correlated after manual acupuncture intervention, suggesting that the regulation of the HPA axis by acupuncture is related to acupuncture's influence on the intestinal flora composition.

4.4. Effect on metabolites and metabolic pathways

There are 100 times more genes in the gut microbiome than in the human genome, and those genes can encode at least 10 times as many unique genes as the host's genes (Ley et al., 2006). It is likely that the products of these genes play an important role in the pathogenesis of depression after entering the circulation and integrating into the host metabolic pathway (Li et al., 2023). Moreover, as a consequence of stroke, the structural integrity of the BBB is affected and under inflammatory conditions, matrix metalloproteinases (MMPs) can degrade basal layer proteins, increasing the BBB's permeability (Zlokovic, 2006; Lakhan et al., 2013). LPS, SCFAs, adiponectin, vasoactive intestinal peptide (VIP), and some neurotransmitter precursors (e.g., 5-HTP) were more readily transported across the BBB to the brain due to increased BBB permeability (Birdsall, 1998; Dogrukol-Ak et al., 2003; Nedorubov et al., 2019; Megur et al., 2020; Formolo et al., 2022; Zhao et al., 2022).

Multiple metabolism pathways and metabolites were altered by manual acupuncture in subjects according to Lv et al. (2022). A serum metabolomics study conducted by Lv et al. (2022) has revealed the following: acupuncture can regulate the differential metabolites, including biosynthesis of N-methylnicotinamide, beta-glycerophosphoric acid, geranyl acetoacetate, serotonin and phenylalanine, tyrosine and tryptophan, as well as metabolic pathways of hypotaurine and beta-alanine taurine and hypotaurine, and beta-alanine. And it should be noted that the metabolic pathways and metabolites described above are closely associated with multiple neurotransmitter precursors of depression-related (Parker and Brotchie, 2011; Strasser et al., 2016; Hüfner et al., 2019). As a result of Lv et al. (2022) correlation analysis of differential microflora and differential metabolites, the authors speculated that the changes of microflora caused by manual acupuncture will affect the changes in serum metabolites, and integrating acupuncture into the process of regulating depression.

It has been reported that the intestinal microbiota affects neurotransmitter production and tryptophan metabolism (O'Mahony et al., 2015), and that tryptophan can produce a variety of indole metabolites under the influence of the microbiome. In the intestinal environment, tryptophan and its indole metabolites are precursors or signaling molecules of many bioactive substances (such as 5-HT, Aryl -Hydrocarbon, Oxindole and Isatin), which have an important role to play in the "gut-brain axis" (Hubbard et al., 2015; De Vadder et al., 2018; Jaglin et al., 2018; Roager and Licht, 2018; Qu et al., 2019; Li et al., 2020; Zhang et al., 2022c). By chemical labeling assisted liquid chromatography-tandem mass spectrometry, Zhang et al. (2022b) successfully determined 15 tryptophan indole metabolites in feces of rats with functional dyspepsia after acupuncture intervention.

Manual acupuncture treatment was administered to depressed rats by Li et al. (2021), and his results showed that the levels of DA and 5-HT in serum and hippocampus increased after treatment. After Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, it was found that manual acupuncture affected the cell growth, the apoptosis pathway, the cofactor and vitamin metabolism pathway, amino acid metabolism pathway, and the carbohydrate metabolism pathway in rats, as well as improving their depression-like behavior through the brain-gut axis.

Li et al. (2022d) detected the diversity and richness of microflora in the stomach and duodenum after electroacupuncture, as well as the content changes of VIP, DA, and trilobal factor (TFF) in serum. Researchers found that electroacupuncture increased TFF and DA levels in the serum, as well as the diversity and richness of stomach microbiota. In addition to being protective to gastrointestinal mucosa (Mashimo et al., 1996; Gyires, 2004; Huang and Wu, 2021), trefoil factor can also reverse depression-like behavior in the same way as dopamine (Shi et al., 2012; Li et al., 2015). Accordingly, electroacupuncture's effectiveness may be related to levels of dopamine and trefoil factor and microbiome structural changes.

Adiponectin, as a adipocyte derived protein, can inhibit the infiltration of macrophages and the increase of pro-inflammatory cytokines, maintain intestinal homeostasis and improve intestinal barrier integrity (Obeid et al., 2017), and is significantly associated with Firmicutes differential OTUs, which may be a key node between intestinal microbiota and depression (Bai et al., 2022). Liu et al. (2020b, 2022) found electroacupuncture restored melatonin and adiponectin levels in plasma to near-normal levels in diseased rats as a result of electroacupuncture, while it could also restore the expression of VIP and its VIP type 1 receptor (VPAC1) and its VIP type 2 receptor (VPAC2). In addition, there is evidence that VIP can improve the immunity of intestinal mucosa (Seillet et al., 2020), and it is also associated with biological depression (Yu et al., 2021; Shukla et al., 2022), thus, acupuncture may be a mediator of gut-brain communication.

According to studies of Xia X. et al. (2022), electroacupuncture activated Nod-like receptor signaling pathways and promoted intestinal defensin production in order to protect the host from intestinal pathogens, thus maintaining intestinal homeostasis. Based on Spearman correlation coefficient analysis, Xia X. et al. (2022) suggested that electroacupuncture of intestinal defensins appeared to be a key mechanism for restoring intestinal microflora homeostasis. Furthermore, electroacupuncture can also upregulated energy metabolism and down-regulate lipid metabolism.

Si et al. (2022) found that electroacupuncture intervention restored 10 significantly altered bacterial genera and 11 metabolites in obese mice to normal levels, as well as that intestinal flora and metabolic levels were strongly correlated. According to researchers' speculation, acupuncture restored gut flora balance primarily by regulating glycerophospholipid metabolism and primary bile acid biosynthesis. Several studies have shown that intestinal microflora is involved in the pathogenesis of depression through glycerophospholipid metabolism and primary bile acid biosynthesis (Gong et al., 2021; MahmoudianDehkordi et al., 2022). Accordingly, it can be speculated that electroacupuncture might be useful in treating depression by regulating intestinal flora's production of glycerophospholipids and bile acids.

The study of Xie et al. (2020) found that electroacupuncture could reduce the level of total cholesterol, TG and LDL in serum, while improving the level of HDL. This result may be related to the increase of the relative abundance of *Lactobacillus*, thus affecting lipid metabolism. And according to relevant study, the aberrant lipid metabolism is one of the predictive biological indicators of PSD (Zhong et al., 2022).

Wang et al. (2019) showed that electroacupuncture could regulate lipid metabolism and improve insulin sensitivity and glucose homeostasis by regulating intestinal flora composition (mainly by reducing Firmicutes/*Bacteroides* ratio and increasing *Prevotella*_9 abundance).

4.5. Effect on inflammatory responses

During times of inflammation or homeostasis disorders, the microbiota can act as a protective force for the body by affecting the immune system's function. Basically, the gut microbiota protects host by controlling the function and number of inflammatory cells, either directly or indirectly, in response to systemic or local infection challenges (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2009; Miller et al., 2009). Certainly, there may also be the overabundance of bacteria in the intestinal tract which have potential to magnify inflammation, leading to local and systemic pathological consequences effects (Belkaid and Hand, 2014). According to the regulation effect of acupuncture, on the one hand, acupuncture can restore the balance of intestinal flora structure, adjust the proportion, abundance and number of pathogenic bacteria and beneficial microorganisms, thus affecting the activation or inhibition of pro-inflammatory and anti-inflammatory cells. On the other hand, acupuncture can protect the structure and function of intestinal mucosal barrier system, prevent the translocation of pathogenic bacteria and inflammation-causing substances, thereby avoiding the occurrence of inflammatory storms in the body.

Basic studies have shown that increased production of proinflammatory cytokines after cerebral ischemia can activate indoleamine 2,3-dioxygenase (IDO) in glial cells and reduce the bioavailability of tryptophan (tryptophan is metabolized mainly *via* two main pathways, the serotonin and kynurenine pathways), as a result, 5-HT is depleted, serotonergic transmission is blocked, as well as neuroactive tryptophan metabolites (such as kynurenine) are produced (O'Connor et al., 2009; Souza et al., 2017; Körtési et al., 2022), which eventually leading to PSD (Spalletta et al., 2006). Despite the lack of a complete understanding of the pathophysiology of depression, inflammation is a key driver of its development, and inflammatory factors is important biological factors that increase the risk of depressive episodes. Several cohort studies have found that the increase of serum levels of proinflammatory factors (such as IL-6, IL-17, TNF-α, and IL-1β) in the acute phase after stroke is independent predictors of depression when using logistics regression analysis (Kim et al., 2017; Hu J. et al., 2019), and reducing the expression of IL-6, TNF-α, and IL-1β in the cortex and hippocampus alleviated depression-like behavior in rats with PSD (Yan et al., 2019).

Post-stroke depression is a brain disease. In addition to stroke itself, which activates glial cell activation and causes CNS inflammation (Lubart et al., 2021; Rayasam et al., 2022; Tariq et al., 2022), peripheral inflammatory factors may establish relationship with the CNS after crossing the BBB as well (Beurel et al., 2020). Furthermore, a significant correlation between CNS inflammation and peripheral inflammation is also supported (Leng et al., 2018; Richards et al., 2018). It is worth noting that inflammation of the CNS is closely associated with microglia and astrocytes, and there has been a lot of evidence that inflammatory microglia and astrocytes play an important role in the development of depression (Rajkowska and Stockmeier, 2013; Peng et al., 2015; Leng et al., 2018; Xia W. et al., 2022; Xie et al., 2022).

As soon as intestinal barrier function is compromised, the bacterial translocation becomes easy, the immune system becomes activated and inflammatory factors increase, resulting in almost all of the changes associated with depression occurring in neural activity (such as neuroendocrine function, neuroplasticity, neurotransmitter signaling, cerebrovascular endothelial cell signaling, circumventricular organ signaling, and peripheral immune cell-to-brain signaling and so on), eventually, neuroinflammation causes behavioral changes and depression (Smith, 1991; Miller et al., 2009; Miller and Raison, 2016; Kronsten et al., 2022).

Lv et al. (2022) found that manual acupuncture inhibited the levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in the gut and hippocampus, and there was a correlation analysis that suggested that acupuncture promoted intestinal microbiota regulation, improved intestinal barrier function, reduced intestinal inflammation, and decreased central inflammation.

Pro-inflammatory cytokines (TNF-α and IL-1β) and LPS may induce depressive symptoms and are the most reliable biomarkers for the presence of inflammation in depressed patients (Spalletta et al., 2006; Maes et al., 2008; Miller et al., 2009). Researchers have proven that intestinal barrier destruction in depression is related to an increase in proinflammatory factors like LPS and TNF-α and IL-1β (Guo et al., 2022). As a consequence of acupuncture, significant reductions in LPS, TNF-α, and IL-1β in serum and brain were observed, and Zhang et al. (2022d) hypothesized that this was the result of manual acupuncture restoring the intestinal barrier and reducing inflammation by regulating the intestinal flora.

Xie et al. (2020) examined the inflammatory cytokines and inflammatory mediators in serum and articular synovial fluid of rats, and the results showed that electroacupuncture could reduce the levels of IP-10, IL-1α, and MCP-1 in serum and LPS in articular synovial fluid, and play an anti-inflammatory role.

Hao et al. (2022) observed that after manual acupuncture intervention, the fluorescence intensity of LPS that could be stained by immunofluorescence decreased, as well as the number of cells that could express glial fibrillary acidic protein (GFAP) in the lamina propria of the intestine and the contents of LPS and TNF-α in serum and intestinal tract. Researchers suggested by reducing the toxic effect of TNF-α on intestinal mucosa and the inflammatory effect of LPS, the structure of tight junction proteins was protected, and inflammatory mediators were reduced into the circulation, thus protecting the CNS.

It has been found that SCFAs, which are metabolized by the gut microbiome, are associated with changes in the gut microbiome in depressed mice (Hao et al., 2022). Electroacupuncture was studied to determine its effect on serum SCFA content, and Ke et al. (2022) found a strong correlation between prognosis of apoplexy rats and intestinal microbiota production of SCFAs (especially acetic acid and propionic acid). Electroacupuncture may improve stroke outcomes by increasing acetic acid and propionic acid levels to restore energy supply to the intestinal epithelium, reduce intestinal inflammation, and stabilize intestinal microbiota. The study of Zhang et al. (2021) showed that the concentration of SCFAs (acetic acid and butyric acid) could be increased in feces as a result of electroacupuncture, which may be related to an increase in *Lactobacillus* and *Blautia*. The study of Zhang et al. (2021) also showed that significantly reduced serum levels of inflammation markers such as LPS and IL-6, and positively correlated with changes in population of *Alistipes*, *Helicobacter* and *Prevotella*, and histopathological analysis revealed that there was significantly less mucosal inflammation, goblet cells, and epithelial damage in the colon.

It is believed that intestinal epithelial cells contain a variety of pattern recognition receptors, including toll-like receptors (TLRS), that are important for the regulation of inflammatory responses by invading pathogens and pathogen-produced toxins (Chassin et al., 2010; Belkaid and Hand, 2014). In their previous experiment, Liu et al. (2020b) discovered that electroacupuncture could inhibit the proinflammatory factors IFN-γ, TNF-α, and IL-6 through TLR4 signaling *via* MyD88-dependent pathway to prevent excessive immune response in the whole body. According to subsequent related experiments, Liu et al. (2022) also found that electroacupuncture could reduce the level of proinflammatory factor IL-6 in plasma, and significantly increase Th2/ILC2 related cytokines (including IL-4, IL-5, IL-9, IL-13, IL-10), as well as increase ILC3-derived cytokines IL-22 and GM-CSF, among which IL-10 is a potent anti-inflammatory cytokine for ILC2s to exert their functions.

Regulatory T cells (Treg) and pro-inflammatory T helper T cell 17 (Th17) cells are a pair of CD4+ T lymphocyte subsets that are functionally opposite, with Th17 promoting tissue inflammation while Treg exhibiting anti-inflammatory properties, and PSD is driven in part by an imbalance between the two cell subsets forming the immune axis (Ju and Wang, 2019; Cui et al., 2021; Westfall et al., 2021). Depressive symptoms can be improved by regulating the gut microbiome's role in regulating the Treg/Th17 immune axis (Westfall et al., 2021). In their study, Wei et al. (2019) found that electroacupuncture regulated the increase in the diversity and abundance of gut microbiota, positively correlated with the improvement in the percentage of Treg cells in CD4+ T lymphocytes, and negatively correlated with the percentage of Th17, indicating that a possible mechanism by which electroacupuncture may regulate gut microbiota structure is through its effects on the internal immune environment.

4.6. Regulation of central neurons

In stroke survivors, depression is associated with survival status of neurons (Yang et al., 2018; Zavvari and Nahavandi, 2020). The findings of Han Q. Q. et al. (2021) suggested that electroacupuncture can reduce the abundance of Erysipelotrichaceae that have pro-inflammatory properties, decrease the mRNA levels of proinflammatory cytokines IL-6, TNF-α and reduce the loss of dopaminergic neurons in the substantia nigra (SN). Researchers speculated that electroacupuncture acted as a neuroprotective role on dopaminergic neurons by inhibiting inflammation in the SN to alleviate behavioral defects in mice, and this effect may be related to the regulation of intestinal microbes. Jang et al. (2020) suggested that the immunomodulatory function of the gut microbiome plays a key role in the process of neuroprotection and anti-inflammation. Manual acupuncture can inhibit the expression of Bax, NF-κB and TNF-α and restore the expression of Bcl-2, and reduce the activation and overexpression of microglia and astrocytes. Neuroprotection occurs through manual acupuncture by blocking neuroinflammation responses and apoptosis, and increasing the level of dopaminergic fibers and neurons in the striatum and SN. Li et al. (2021) conducted manual acupuncture treatment on depressed rats and they found that acupuncture regulates gut microbes and neurotransmitters to alleviate depression-like manifestations in rats. Brain-derived neurotrophic factor (BDNF) signaling was enhanced by manual acupuncture intervention, increasing the mRNA and protein expression of BDNF and N-methyl-D-aspartate receptor (NMDAR), and increasing the number of astrocytes in the hippocampus as a result, at the same time, the mRNA and protein expression of β-CaMKII, which can block BDNF receptor, was decreased in the hippocampus.

5. Conclusion and prospects

Acupuncture can positively promote the prognosis of patients with PSD by maintaining the dynamic balance of intestinal flora structure, which proves that acupuncture is a promising non-drug treatment for reducing depressive symptoms. This paper examines the relationship between intestinal flora and PSD and the role of acupuncture in this relationship to summarize acupuncture will be able to treat PSD through multiple targets (Protect the intestinal mucosal barrier system, avoid overactivity of the HPA axis to activate intestinal immunity, regulate metabolites and metabolic pathways to maintain intestinal homeostasis, control the balance of inflammatory cells and inflammatory factors, avoid neuroinflammation and protect central neurons), and proposes that the common and core link of these mechanism is that intestinal microbiota regulates the local and systemic immune system.

However, the above studies involving acupuncture and intestinal flora structure adjustments are only capable of proving correlation rather than causation. From the current development perspective of acupuncture in the treatment of PSD, the mechanism of acupuncture to maintain the dynamic balance between the type and number of intestinal flora to treat and prevent depression has not been thoroughly studied. And it remains a major challenge to understand the dynamics of microbial ecological adjustment *in vivo*.

The future should involve more researches to explore whether acupuncture can restore ecological balance of intestinal microbes in the immune deficiency model of depression to improve depression, and determine the causal relationship among the three to fill the gaps in current knowledge. It is believed that with more studies, the pathogenesis of depression will be further clarified in the future.

Author contributions

HJ: conceptualization and writing—original draft. SD and JZ: writing—review and editing. BL, WZ, JC, and MZ: investigation. ZM: supervision. CZ: project administration. All authors read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Amini-Khoei, H., Haghani-Samani, E., Beigi, M., Soltani, A., Mobini, G. R., Balali-Dehkordi, S., et al. (2019). On the role of corticosterone in behavioral disorders, microbiota composition alteration and neuroimmune response in adult male mice subjected to maternal separation stress. *Int. Immunopharmacol.* 66, 242–250. doi: 10.1016/j.intimp.2018.11.037

Anagha, K., Shihabudheen, P., and Uvais, N. A. (2021). Side effect profiles of selective serotonin reuptake inhibitors: A cross-sectional study in a naturalistic setting. *Prim. Care Companion CNS Disord.* 23:20m02747. doi: 10.4088/PCC.20m02747

Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., and Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science* 307, 1915–1920.

Bai, S., Bai, H., Li, D., Zhong, Q., Xie, J., and Chen, J. J. (2022). Gut microbiota-related inflammation factors as a potential biomarker for diagnosing major depressive disorder. *Front. Cell. Infect. Microbiol.* 12:831186. doi: 10.3389/fcimb.2022.831186

Begum, N., Mandhare, A., Tryphena, K. P., Srivastava, S., Shaikh, M. F., Singh, S. B., et al. (2022). Epigenetics in depression and gut-brain axis: A molecular crosstalk. *Front. Aging Neurosci.* 14:1048333. doi: 10.3389/fnagi.2022.1048333

Belkaid, Y., and Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell* 157, 121–141. doi: 10.1016/j.cell.2014.03.011

Beurel, E., Toups, M., and Nemeroff, C. B. (2020). The bidirectional relationship of depression and inflammation: Double trouble. *Neuron* 107, 234–256. doi: 10.1016/j.neuron.2020.06.002

Birdsall, T. C. (1998). 5-Hydroxytryptophan: A clinically-effective serotonin precursor. *Altern. Med. Rev.* 3, 271–280.

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., et al. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458–462. doi: 10.1038/35013070

Borrel, G., Brugère, J. F., Gribaldo, S., Schmitz, R. A., and Moissl-Eichinger, C. (2020). The host-associated archaeome. *Nat. Rev. Microbiol.* 18, 622–636. doi: 10.1038/s41579-020-0407-y

Cai, H., Wang, X. P., and Yang, G. Y. (2021). Sleep disorders in stroke: An update on management. *Aging Dis.* 12, 570–585. doi: 10.14336/ad.2020.0707

Chassin, C., Kocur, M., Pott, J., Duerr, C. U., Gütle, D., Lotz, M., et al. (2010). miR-146a mediates protective innate immune tolerance in the neonate intestine. *Cell Host Microbe* 8, 358–368. doi: 10.1016/j.chom.2010.09.005

Cui, M., Dai, W., Kong, J., and Chen, H. (2021). Th17 cells in depression: Are they crucial for the antidepressant effect of ketamine? *Front. Pharmacol.* 12:649144. doi: 10.3389/fphar.2021.649144

Davis, J. C., Falck, R. S., Best, J. R., Chan, P., Doherty, S., and Liu-Ambrose, T. (2019). Examining the inter-relations of depression, physical function, and cognition with subjective sleep parameters among stroke survivors: A cross-sectional analysis. *J. Stroke Cerebrovasc. Dis.* 28, 2115–2123. doi: 10.1016/j.jstrokecerebrovasdis.2019.04.010

De Vadder, F., Grasset, E., Mannerås Holm, L., Karsenty, G., Macpherson, A. J., Olofsson, L. E., et al. (2018). Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6458–6463. doi: 10.1073/pnas.1720017115

Dogrukol-Ak, D., Banks, W. A., Tuncel, N., and Tuncel, M. (2003). Passage of vasoactive intestinal peptide across the blood-brain barrier. *Peptides* 24, 437–444. doi: 10.1016/s0196-9781(03)00059-7

Dou, X., Ma, Z., Yan, D., Gao, N., Li, Z., Li, Y., et al. (2022). Sodium butyrate alleviates intestinal injury and microbial flora disturbance induced by lipopolysaccharides in rats. *Food Funct.* 13, 1360–1369. doi: 10.1039/d1fo03183j

Du, X., and Pang, T. Y. (2015). Is dysregulation of the HPA-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Front. Psychiatry* 6:32. doi: 10.3389/fpsyt.2015.00032

Evrensel, A., and Tarhan, K. N. (2021). Emerging role of Gut-microbiota-brain axis in depression and therapeutic implication. *Progress Neuro Psychopharmacol. Biol. Psychiatry* 106:110138. doi: 10.1016/j.pnpbp.2020.110138

Fang, N., and Li, R. (2022). Analysis of FD, IBS and FD with IBS in depression patients. *Med. Inform.* 35, 124–126.

Formolo, D. A., Cheng, T., Yu, J., Kranz, G. S., and Yau, S. Y. (2022). Central adiponectin signaling - a metabolic regulator in support of brain plasticity. *Brain Plast.* 8, 79–96. doi: 10.3233/bpl-220138

Frank, D., Gruenbaum, B. F., Zlotnik, A., Semyonov, M., Frenkel, A., and Boyko, M. (2022). Pathophysiology and current drug treatments for post-stroke depression: A review. *Int. J. Mol. Sci.* 23:15114. doi: 10.3390/ijms232315114

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., et al. (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 31, 677–689. doi: 10.1016/j.immuni.2009.08.020

Ge, T., Yao, X., Zhao, H., Yang, W., Zou, X., Peng, F., et al. (2021). Gut microbiota and neuropsychiatric disorders: Implications for neuroendocrine-immune regulation. *Pharmacol. Res.* 173:105909. doi: 10.1016/j.phrs.2021.105909

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* 312, 1355–1359.

Gong, X., Huang, C., Yang, X., Chen, J., Pu, J., He, Y., et al. (2021). Altered fecal metabolites and colonic glycerophospholipids were associated with abnormal composition of gut microbiota in a depression model of mice. *Front. Neurosci.* 15:701355. doi: 10.3389/fnins.2021.701355

Guo, D., Park, C., Li, Y., Li, B., Yang, Q., Deng, Y., et al. (2022). Ameliorates depressive disorders in a murine alcohol-LPS (mALPS) model. *Food Funct.* 13, 12766–12776. doi: 10.1039/d2fo01478e

Gyires, K. (2004). Neuropeptides and gastric mucosal homeostasis. *Curr. Top. Med. Chem.* 4, 63–73.

Han, Q. Q., Fu, Y., Le, J. M., Pilot, A., Cheng, S., Chen, P. Q., et al. (2021). Electroacupuncture may alleviate behavioral defects via modulation of gut microbiota in a mouse model of Parkinson's disease. *Acupunct. Med.* 39, 501–511. doi: 10.1177/0964528421990658

Han, W., Wang, N., Han, M., Ban, M., Sun, T., and Xu, J. (2022). Reviewing the role of gut microbiota in the pathogenesis of depression and exploring new therapeutic options. *Front. Neurosci.* 16:1029495. doi: 10.3389/fnins.2022.1029495

Han, X., Gao, Y., Yin, X., Zhang, Z., Lao, L., Chen, Q., et al. (2021). The mechanism of electroacupuncture for depression on basic research: A systematic review. *Chin. Med.* 16:10. doi: 10.1186/s13020-020-00421-y

Hang, X., Li, J., Zhang, Y., Li, Z., Zhang, Y., Ye, X., et al. (2021). Efficacy of frequently-used acupuncture methods for specific parts and conventional pharmaceutical interventions in treating post-stroke depression patients: A network meta-analysis. *Complement. Ther. Clin. Pract.* 45:101471. doi: 10.1016/j.ctcp.2021.101471

Hao, W. Z., Li, X. J., Zhang, P. W., and Chen, J. X. (2020). A review of antibiotics, depression, and the gut microbiome. *Psychiatry Res.* 284:112691. doi: 10.1016/j.psychres.2019.112691

Hao, X., Ding, N., Zhang, Y., Yang, Y., Zhao, Y., Zhao, J., et al. (2022). Benign regulation of the gut microbiota: The possible mechanism through which the beneficial effects of manual acupuncture on cognitive ability and intestinal mucosal barrier function occur in APP/PS1 mice. *Front. Neurosci.* 16:960026. doi: 10.3389/fnins.2022.960026

Hu, J., Zhou, W., Zhou, Z., Yang, Q., Han, J., Yan, Y., et al. (2019). Predictive value of inflammatory indicators for post-stroke depression in patients with ischemic stroke. *Nan Fang Yi Ke Da Xue Xue Bao* 39, 665–671. doi: 10.12122/j.issn.1673-4254.2019.06.06

Hu, K., Smedby, K. E., Sjölander, A., Montgomery, S., Valdimarsdóttir, U., Engstrand, L., et al. (2022). Use of antibiotics and risk of psychiatric disorders in newly diagnosed cancer patients: A population-based cohort study in Sweden. *Cancer Epidemiol. Biomark Prevent.* 31, 528–535. doi: 10.1158/1055-9965.EPI-21-1095

Hu, S., Du, M. H., Luo, H. M., Wang, H., Lv, Y., Ma, L., et al. (2013). Electroacupuncture at zusanli (ST36) prevents intestinal barrier and remote organ dysfunction following gut ischemia through activating the cholinergic anti-inflammatory-dependent mechanism. *Evid. Based Complement. Altern. Med.* 2013:592127. doi: 10.1155/2013/592127

Hu, S., Li, A., Huang, T., Lai, J., Li, J., Sublette, M. E., et al. (2019). Gut microbiota changes in patients with bipolar depression. *Adv. Sci.* 6:1900752. doi: 10.1002/advs.201900752

Hu, Z., Li, M., Yao, L., Wang, Y., Wang, E., Yuan, J., et al. (2021). The level and prevalence of depression and anxiety among patients with different subtypes of irritable bowel syndrome: A network meta-analysis. *BMC Gastroenterol.* 21:23. doi: 10.1186/s12876-020-01593-5

Hua, X., Zhu, J., Yang, T., Guo, M., Li, Q., Chen, J., et al. (2020). The gut microbiota and associated metabolites are altered in sleep disorder of children with autism spectrum disorders. *Front. Psychiatry* 11:855. doi: 10.3389/fpsyt.2020.00855

Huang, F., and Wu, X. (2021). Brain neurotransmitter modulation by gut microbiota in anxiety and depression. *Front. Cell Dev. Biol.* 9:649103. doi: 10.3389/fcell.2021.649103

Hubbard, T. D., Murray, I. A., Bisson, W. H., Lahoti, T. S., Gowda, K., Amin, S. G., et al. (2015). Adaptation of the human aryl hydrocarbon receptor to sense microbiota-derived indoles. *Sci. Rep.* 5:12689. doi: 10.1038/srep12689

Hüfner, K., Fuchs, D., Blauth, M., and Sperner-Unterweger, B. (2019). How acute and chronic physical disease may influence mental health - An Analysis of neurotransmitter precursor amino acid levels. *Psychoneuroendocrinology* 106, 95–101. doi: 10.1016/j.psyneuen.2019.03.028

Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498. doi: 10.1016/j.cell.2009.09.033

Jaglin, M., Rhimi, M., Philippe, C., Pons, N., Bruneau, A., Goustard, B., et al. (2018). Indole, a signaling molecule produced by the gut microbiota, negatively impacts emotional behaviors in rats. *Front. Neurosci.* 12:216. doi: 10.3389/fnins.2018.00216

Jang, J. H., Yeom, M. J., Ahn, S., Oh, J. Y., Ji, S., Kim, T. H., et al. (2020). Acupuncture inhibits neuroinflammation and gut microbial dysbiosis in a mouse model of Parkinson's disease. *Brain Behav. Immun.* 89, 641–655. doi: 10.1016/j.bbi.2020.08.015

Jeon, S. W., and Kim, Y. K. (2018). The role of neuroinflammation and neurovascular dysfunction in major depressive disorder. *J. Inflamm. Res.* 11, 179–192. doi: 10.2147/JIR.S141033

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., et al. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* 48, 186–194. doi: 10.1016/j.bbi.2015.03.016

Jiang, W., Gong, L., Liu, F., Ren, Y., and Mu, J. (2021). Alteration of gut microbiome and correlated lipid metabolism in post-stroke depression. *Front. Cell. Infect. Microbiol.* 11:663967. doi: 10.3389/fcimb.2021.663967

Jiang, Y. (2022). Research progress on the relationship between depression and other systems. *Med. Innov. China* 19, 179–183.

Ju, J., and Wang, L. (2019). Research progress of Th17/Treg cells in the pathogenesis of post-stroke depression. *Stroke Nervous Dis.* 26, 358–361.

Juli, A., and Juli, L. (2014). New antidepressant drugs for new depressions. *Psychiatr. Danubina* 26, 115–116.

Ke, X., Xiang, Q., Jiang, P., Liu, W., Yang, M., Yang, Y., et al. (2022). Effect of electroacupuncture on short-chain fatty acids in peripheral blood after middle cerebral artery occlusion/reperfusion in rats based on gas chromatography-mass spectrometry. *Mediat. Inflamm.* 2022:3997947. doi: 10.1155/2022/3997947

Kim, J. M., Kang, H. J., Kim, J. W., Bae, K. Y., Kim, S. W., Kim, J. T., et al. (2017). Associations of tumor necrosis factor-α and interleukin-1β levels and polymorphisms with post-stroke depression. *Am. J. Geriatr. Psychiatry* 25, 1300–1308. doi: 10.1016/j.jagp.2017.07.012

Kim, T. W., Jeong, J. H., and Hong, S. C. (2015). The impact of sleep and circadian disturbance on hormones and metabolism. *Int. J. Endocrinol.* 2015:591729. doi: 10.1155/2015/591729

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., and Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell* 165, 1332–1345. doi: 10.1016/j.cell.2016.05.041

Köhler, O., Petersen, L., Mors, O., Mortensen, P. B., Yolken, R. H., Gasse, C., et al. (2017). Infections and exposure to anti-infective agents and the risk of severe mental disorders: A nationwide study. *Acta Psychiatr. Scand.* 135, 97–105. doi: 10.1111/acps.12671

Körtési, T., Spekker, E., and Vécsei, L. (2022). Exploring the tryptophan metabolic pathways in migraine-related mechanisms. *Cells* 11:3795. doi: 10.3390/cells11233795

Kronsten, V. T., Tranah, T. H., Pariante, C., and Shawcross, D. L. (2022). Gut-derived systemic inflammation as a driver of depression in chronic liver disease. *J. Hepatol.* 76, 665–680. doi: 10.1016/j.jhep.2021.11.008

Krug, S. M., and Fromm, M. (2020). Special issue on "the tight junction and its proteins: More than just a barrier". *Int. J. Mol. Sci.* 21:4612. doi: 10.3390/ijms21134612

Lai, J., Li, A., Jiang, J., Yuan, X., Zhang, P., Xi, C., et al. (2022). Metagenomic analysis reveals gut bacterial signatures for diagnosis and treatment outcome prediction in bipolar depression. *Psychiatry Res.* 307:114326. doi: 10.1016/j.psychres.2021.114326

Lakhan, S. E., Kirchgessner, A., Tepper, D., and Leonard, A. (2013). Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Front. Neurol.* 4:32. doi: 10.3389/fneur.2013.00032

Le, J. J., Yi, T., Qi, L., Li, J., Shao, L., and Dong, J. C. (2016). Electroacupuncture regulate hypothalamic-pituitary-adrenal axis and enhance hippocampal serotonin system in a rat model of depression. *Neurosci. Lett.* 615, 66–71. doi: 10.1016/j.neulet.2016.01.004

Leng, L., Zhuang, K., Liu, Z., Huang, C., Gao, Y., Chen, G., et al. (2018). Menin deficiency leads to depressive-like behaviors in mice by modulating astrocyte-mediated neuroinflammation. *Neuron* 100, 551–563.e7. doi: 10.1016/j.neuron.2018.08.031

Leone, V., Gibbons, S. M., Martinez, K., Hutchison, A. L., Huang, E. Y., Cham, C. M., et al. (2015). Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 17, 681–689. doi: 10.1016/j.chom.2015.03.006

Lewis, K., Marrie, R. A., Bernstein, C. N., Graff, L. A., Patten, S. B., Sareen, J., et al. (2019). The prevalence and risk factors of undiagnosed depression and anxiety disorders among patients with inflammatory bowel disease. *Inflamm. Bowel Dis.* 25, 1674–1680. doi: 10.1093/ibd/izz045

Ley, R. E., Peterson, D. A., and Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124, 837–848. doi: 10.1016/j.cell.2006.02.017

Li, B., Xu, M., Wang, Y., Feng, L., Xing, H., and Zhang, K. (2023). Gut microbiota: A new target for traditional Chinese medicine in the treatment of depression. *J. Ethnopharmacol.* 303:116038. doi: 10.1016/j.jep.2022.116038

Li, J., Luo, Y., Zhang, R., Shi, H., Zhu, W., and Shi, J. (2015). Neuropeptide trefoil factor 3 reverses depressive-like behaviors by activation of BDNF-ERK-CREB signaling in olfactory bulbectomized rats. *Int. J. Mol. Sci.* 16, 28386–28400. doi: 10.3390/ijms161226105

Li, K., Wang, J., Li, S., Deng, B., and Yu, H. (2022a). Latent characteristics and neural manifold of brain functional network under acupuncture. *IEEE Trans. Neural Syst. Rehabil. Eng.* 30, 758–769. doi: 10.1109/TNSRE.2022.3157380

Li, R., Wang, Y., Hu, H., Tan, Y., and Ma, Y. (2022b). Metagenomic analysis reveals unexplored diversity of archaeal virome in the human gut. *Nat. Commun.* 13:7978. doi: 10.1038/s41467-022-35735-y

Li, X., He, F., Tuo, X., Qiu, Y., Guo, J., Wu, Y., et al. (2022d). Electroacupuncture ameliorates peptic ulcer disease in association with gastroduodenal microbiota modulation in mice. *Front. Cell. Infect. Microbiol.* 12:935681. doi: 10.3389/fcimb.2022.935681

Li, X., Han, G., Zhao, J., Huang, X., Feng, Y., Huang, J., et al. (2022c). Intestinal flora induces depression by mediating the dysregulation of cerebral cortex gene expression and regulating the metabolism of stroke patients. *Front. Mol. Biosci.* 9:865788. doi: 10.3389/fmolb.2022.865788

Li, P., Huang, W., Yan, Y. N., Cheng, W., Liu, S., Huang, Y., et al. (2021). Acupuncture can play an antidepressant role by regulating the intestinal microbes and neurotransmitters in a rat model of depression. *Med. Sci. Monitor* 27:e929027. doi: 10.12659/MSM.929027

Li, Y., Dai, C., Yang, J., Yang, B., and Qu, Y. (2020). Microbial metabolism of indole and its role as a novel signal molecule: A review. *Microbiol. China* 47, 3622–3633. doi: 10.13344/j.microbiol.china.191063

Li, Y., Hao, Y., Fan, F., and Zhang, B. (2018). The role of microbiome in insomnia, circadian disturbance and depression. *Front. Psychiatry* 9:669. doi: 10.3389/fpsyt.2018.00669

Liang, S., Wang, T., Hu, X., Luo, J., Li, W., Wu, X., et al. (2015). Administration of *Lactobacillus helveticus* NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neuroscience* 310, 561–577. doi: 10.1016/j.neuroscience.2015.09.033

Liang, S., Wu, X., Hu, X., Wang, T., and Jin, F. (2018b). Recognizing depression from the microbiota-gut-brain axis. *Int. J. Mol. Sci.* 19:1592. doi: 10.3390/ijms19061592

Liang, S., Wu, X., Hu, X., Niu, Y., and Jin, F. (2018a). The development and tendency of depression researches: Viewed from the microbiota-gut-brain axis. *Chin. Sci. Bull.* 63, 2010–2025.

Lin, P. Y., Stern, A., Peng, H. H., Chen, J. H., and Yang, H. C. (2022). Redox and metabolic regulation of intestinal barrier function and associated disorders. *Int. J. Mol. Sci.* 23:14463. doi: 10.3390/ijms232214463

Liu, C. H., Yang, M. H., Zhang, G. Z., Wang, X. X., Li, B., Li, M., et al. (2020a). Neural networks and the anti-inflammatory effect of transcutaneous auricular vagus nerve stimulation in depression. *J. Neuroinflamm.* 17:54. doi: 10.1186/s12974-020-01732-5

Liu, G. H., Liu, H. M., Chen, Y. S., and Lee, T. Y. (2020b). Effect of electroacupuncture in mice with dextran sulfate sodium-induced colitis and the influence of gut microbiota. *Evid. Based Complement. Altern. Med.* 2020:2087903. doi: 10.1155/2020/2087903

Liu, R. T., Rowan-Nash, A. D., Sheehan, A. E., Walsh, R. F. L., Sanzari, C. M., Korry, B. J., et al. (2020c). Reductions in anti-inflammatory gut bacteria are associated with depression in a sample of young adults. *Brain Behav. Immun.* 88, 308–324. doi: 10.1016/j.bbi.2020.03.026

Liu, G. H., Zhuo, X. C., Huang, Y. H., Liu, H. M., Wu, R. C., Kuo, C. J., et al. (2022). Alterations in gut microbiota and upregulations of VPAC2 and intestinal tight junctions correlate with anti-inflammatory effects of electroacupuncture in colitis mice with sleep fragmentation. *Biology* 11:962. doi: 10.3390/biology11070962

Liu, Y., Wang, H., Gui, S., Zeng, B., Pu, J., Zheng, P., et al. (2021). Proteomics analysis of the gut-brain axis in a gut microbiota-dysbiosis model of depression. *Transl. Psychiatry* 11:568. doi: 10.1038/s41398-021-01689-w

Lubart, A., Benbenishty, A., Har-Gil, H., Laufer, H., Gdalyahu, A., Assaf, Y., et al. (2021). Single cortical microinfarcts lead to widespread microglia/macrophage migration along the white matter. *Cereb. Cortex* 31, 248–266. doi: 10.1093/cercor/bhaa223

Lurie, I., Yang, Y. X., Haynes, K., Mamtani, R., and Boursi, B. (2015). Antibiotic exposure and the risk for depression, anxiety, or psychosis: A nested case-control study. *J. Clin. Psychiatry* 76, 1522–1528. doi: 10.4088/JCP.15m09961

Lv, Z., Liu, R., Su, K., Gu, Y., Fang, L., Fan, Y., et al. (2022). Acupuncture ameliorates breast cancer-related fatigue by regulating the gut microbiota-gut-brain axis. *Front. Endocrinol.* 13:921119. doi: 10.3389/fendo.2022.921119

Lynch, J. B., and Hsiao, E. Y. (2019). Microbiomes as sources of emergent host phenotypes. *Science* 365, 1405–1409. doi: 10.1126/science.aay0240

Macedo, D., Filho, A. J. M. C., Soares de Sousa, C. N., Quevedo, J., Barichello, T., Júnior, H. V. N., et al. (2017). Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. *J. Affect. Disord.* 208, 22–32. doi: 10.1016/j.jad.2016.09.012

Macpherson, A. J., Martinic, M. M., and Harris, N. (2002). The functions of mucosal T cells in containing the indigenous commensal flora of the intestine. *Cell. Mol. Life Sci.* 59, 2088–2096. doi: 10.1007/s000180200009

Maes, M., Kubera, M., and Leunis, J. C. (2008). The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinol. Lett.* 29, 117–124.

Maes, M., Kubera, M., Leunis, J. C., and Berk, M. (2012). Increased IgA and IgM responses against gut commensals in chronic depression: Further evidence for increased bacterial translocation or leaky gut. *J. Affect. Disord.* 141, 55–62. doi: 10.1016/j.jad.2012.02.023

MahmoudianDehkordi, S., Bhattacharyya, S., Brydges, C. R., Jia, W., Fiehn, O., Rush, A. J., et al. (2022). Gut microbiome-linked metabolites in the pathobiology of major depression with or without anxiety-a role for bile acids. *Front. Neurosci.* 16:937906. doi: 10.3389/fnins.2022.937906

Mashimo, H., Wu, D. C., Podolsky, D. K., and Fishman, M. C. (1996). Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science* 274, 262–265. doi: 10.1126/science.274.5285.262

Megur, A., Baltriukienë, D., Bukelskienë, V., and Burokas, A. (2020). The microbiota-gut-brain axis and Alzheimer's disease: Neuroinflammation is to blame? *Nutrients* 13:37. doi: 10.3390/nu13010037

Mikulska, J., Juszczyk, G., Gawrońska-Grzywacz, M., and Herbet, M. (2021). HPA axis in the pathomechanism of depression and schizophrenia: New therapeutic strategies based on its participation. *Brain Sci.* 11:1298. doi: 10.3390/brainsci11101298

Miller, A. H., and Raison, C. L. (2016). The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* 16, 22–34. doi: 10.1038/nri.2015.5

Miller, A. H., Maletic, V., and Raison, C. L. (2009). Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. *Biol. Psychiatry* 65, 732–741. doi: 10.1016/j.biopsych.2008.11.029

Młynarska, E., Gadzinowska, J., Tokarek, J., Forycka, J., Szuman, A., Franczyk, B., et al. (2022). The role of the microbiome-brain-gut axis in the pathogenesis of depressive disorder. *Nutrients* 14:1921. doi: 10.3390/nu14091921

Nazarova, L., Liu, H., Xie, H., Wang, L., Ding, H., An, H., et al. (2022). Targeting gut-brain axis through scalp-abdominal electroacupuncture in Parkinson's disease. *Brain Res.* 1790:147956. doi: 10.1016/j.brainres.2022.147956

Nedorubov, A. A., Pavlov, A. N., Pyatigorskaya, N. V., Brkich, G. E., and Shabalina, M. M. (2019). Pharmacokinetics of nanosomal form of levodopa in intranasal administration. *Open Access Maced J. Med. Sci.* 7, 3509–3513. doi: 10.3889/oamjms.2019.749

Obeid, S., Wankell, M., Charrez, B., Sternberg, J., Kreuter, R., Esmaili, S., et al. (2017). Adiponectin confers protection from acute colitis and restricts a B cell immune response. *J. Biol. Chem.* 292, 6569–6582. doi: 10.1074/jbc.M115.712646

O'Connor, J. C., Lawson, M. A., André, C., Moreau, M., Lestage, J., Castanon, N., et al. (2009). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol. Psychiatry* 14, 511–522. doi: 10.1038/sj.mp.4002148

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., and Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav. Brain Res.* 277, 32–48. doi: 10.1016/j.bbr.2014.07.027

O'Malley, D., Julio-Pieper, M., Gibney, S. M., Dinan, T. G., and Cryan, J. F. (2010). Distinct alterations in colonic morphology and physiology in two rat models of enhanced stress-induced anxiety and depression-like behaviour. *Stress* 13, 114–122. doi: 10.3109/10253890903067418

Parker, G., and Brotchie, H. (2011). Mood effects of the amino acids tryptophan and tyrosine: 'Food for Thought' III. *Acta Psychiatr. Scand.* 124, 417–426. doi: 10.1111/j.1600-0447.2011.01706.x

Peng, L., Verkhratsky, A., Gu, L., and Li, B. (2015). Targeting astrocytes in major depression. *Expert Rev. Neurother.* 15, 1299–1306. doi: 10.1586/14737175.2015.1095094

Pouranayatihosseinabad, M., Bezabih, Y., Hawrelak, J., Peterson, G. M., Veal, F., and Mirkazemi, C. (2023). Antibiotic use and the development of depression: A systematic review. *J. Psychosom. Res.* 164:111113. doi: 10.1016/j.jpsychores.2022.111113

Qin, D., Rizak, J., Chu, X., Li, Z., Yang, S., Lü, L., et al. (2015). A spontaneous depressive pattern in adult female rhesus macaques. *Sci. Rep.* 5:11267. doi: 10.1038/srep11267

Qu, Y., Dai, C., Zhang, X., and Ma, Q. (2019). A new interspecies and interkingdom signaling molecule-Indole. *Sheng Wu Gong Cheng Xue Bao* 35, 2177–2188. doi: 10.13345/j.cjb.190158

Rajkowska, G., and Stockmeier, C. A. (2013). Astrocyte pathology in major depressive disorder: Insights from human postmortem brain tissue. *Curr. Drug Targets* 14, 1225–1236.

Ramalho, J. B., Spiazzi, C. C., Bicca, D. F., Rodrigues, J. F., Sehn, C. P., da Silva, W. P., et al. (2022). Beneficial effects of *Lactococcus lactis* subsp. cremoris LL95 treatment in an LPS-induced depression-like model in mice. *Behav. Brain Res.* 426:113847. doi: 10.1016/j.bbr.2022.113847

Ramos Meyers, G., Samouda, H., and Bohn, T. (2022). Short chain fatty acid metabolism in relation to gut microbiota and genetic variability. *Nutrients* 14:5361. doi: 10.3390/nu14245361

Rao, X., Liu, L., Wang, H., Yu, Y., Li, W., Chai, T., et al. (2021). Regulation of gut microbiota disrupts the glucocorticoid receptor pathway and inflammation-related pathways in the mouse hippocampus. *Exp. Neurobiol.* 30, 59–72. doi: 10.5607/en20055

Rayasam, A., Kijak, J. A., Kissel, L., Choi, Y. H., Kim, T., Hsu, M., et al. (2022). CXCL13 expressed on inflamed cerebral blood vessels recruit IL-21 producing T cells to damage neurons following stroke. *J. Neuroinflamm.* 19:125. doi: 10.1186/s12974-022-02490-2

Rea, K., Dinan, T. G., and Cryan, J. F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiol. Stress* 4, 23–33. doi: 10.1016/j.ynstr.2016.03.001

Richards, E. M., Zanotti-Fregonara, P., Fujita, M., Newman, L., Farmer, C., Ballard, E. D., et al. (2018). PET radioligand binding to translocator protein (TSPO) is increased in unmedicated depressed subjects. *EJNMMI Res.* 8:57. doi: 10.1186/s13550-018-0401-9

Roager, H. M., and Licht, T. R. (2018). Microbial tryptophan catabolites in health and disease. *Nat. Commun.* 9:3294. doi: 10.1038/s41467-018-05470-4

Ruff, W. E., Greiling, T. M., and Kriegel, M. A. (2020). Host-microbiota interactions in immune-mediated diseases. *Nat. Rev. Microbiol.* 18, 521–538. doi: 10.1038/s41579-020-0367-2

Seillet, C., Luong, K., Tellier, J., Jacquelot, N., Shen, R. D., Hickey, P., et al. (2020). The neuropeptide VIP confers anticipatory mucosal immunity by regulating ILC3 activity. *Nat. Immunol.* 21, 168–177. doi: 10.1038/s41590-019-0567-y

Shi, H. S., Zhu, W. L., Liu, J. F., Luo, Y. X., Si, J. J., Wang, S. J., et al. (2012). PI3K/Akt signaling pathway in the basolateral amygdala mediates the rapid antidepressant-like effects of trefoil factor 3. *Neuropsychopharmacology* 37, 2671–2683. doi: 10.1038/npp.2012.131

Shukla, R., Newton, D. F., Sumitomo, A., Zare, H., McCullumsmith, R., Lewis, D. A., et al. (2022). Molecular characterization of depression trait and state. *Mol. Psychiatry* 27, 1083–1094. doi: 10.1038/s41380-021-01347-z

Si, Y. C., Ren, C. C., Zhang, E. W., Kang, Z. X., Mo, X. Y., Li, Q. Q., et al. (2022). Integrative analysis of the gut microbiota and metabolome in obese mice with electroacupuncture by 16S rRNA gene sequencing and HPLC-MS-based metabolic profiling. *Am. J. Chin. Med.* 50, 673–690. doi: 10.1142/S0192415X22500276

Smith, R. S. (1991). The macrophage theory of depression. *Med. Hypothes.* 35, 298–306.

Song, J., Ma, W., Gu, X., Zhao, L., Jiang, J., Xu, Y., et al. (2019). Metabolomic signatures and microbial community profiling of depressive rat model induced by adrenocorticotrophic hormone. *J. Transl. Med.* 17:224. doi: 10.1186/s12967-019-1970-8

Souza, L. C., Jesse, C. R., de Gomes, M. G., Del Fabbro, L., Goes, A. T. R., Donato, F., et al. (2017). Activation of brain indoleamine-2,3-dioxygenase contributes to depressive-like behavior induced by an intracerebroventricular injection of streptozotocin in mice. *Neurochem. Res.* 42, 2982–2995. doi: 10.1007/s11064-017-2329-2

Spalletta, G., Bossù, P., Ciaramella, A., Bria, P., Caltagirone, C., and Robinson, R. G. (2006). The etiology of poststroke depression: A review of the literature and a new hypothesis involving inflammatory cytokines. *Mol. Psychiatry* 11, 984–991. doi: 10.1038/sj.mp.4001879

Strasser, B., Gostner, J. M., and Fuchs, D. (2016). Mood, food, and cognition: Role of tryptophan and serotonin. *Curr. Opin. Clin. Nutr. Metab. Care* 19, 55–61. doi: 10.1097/MCO.0000000000000237

Tan, C., Yan, Q., Ma, Y., Fang, J., and Yang, Y. (2022). Recognizing the role of the vagus nerve in depression from microbiota-gut brain axis. *Front. Neurol.* 13:1015175. doi: 10.3389/fneur.2022.1015175

Tang, W. H. W., Bäckhed, F., Landmesser, U., and Hazen, S. L. (2019). Intestinal microbiota in cardiovascular health and disease: JACC state-of-the-art review. *J. Am. Coll. Cardiol.* 73, 2089–2105. doi: 10.1016/j.jacc.2019.03.024

Tariq, M. B., Lee, J., and McCullough, L. D. (2022). Sex differences in the inflammatory response to stroke. *Semin. Immunopathol.* doi: 10.1007/s00281-022-00969-x [Epub ahead of print].

Thaiss, C. A., Levy, M., Korem, T., Dohnalová, L., Shapiro, H., Jaitin, D. A., et al. (2016). Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* 167, 1495–1510.e12. doi: 10.1016/j.cell.2016.11.003

Thaiss, C. A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, A. C., et al. (2014). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 159, 514–529. doi: 10.1016/j.cell.2014.09.048

Voigt, R. M., Forsyth, C. B., Green, S. J., Mutlu, E., Engen, P., Vitaterna, M. H., et al. (2014). Circadian disorganization alters intestinal microbiota. *PLoS One* 9:e97500. doi: 10.1371/journal.pone.0097500

Waclawiková, B., and El Aidy, S. (2018). Role of microbiota and tryptophan metabolites in the remote effect of intestinal inflammation on brain and depression. *Pharmaceuticals* 11:63. doi: 10.3390/ph11030063

Wang, H., Wang, Q., Liang, C., Su, M., Wang, X., Li, H., et al. (2019). Acupuncture regulating gut microbiota in abdominal obese rats induced by high-fat diet. *Evid. Based Complement. Altern. Med.* 2019:4958294. doi: 10.1155/2019/4958294

Wang, J. M., Yang, M. X., Wu, Q. F., Chen, J., Deng, S. F., Chen, L., et al. (2021). Improvement of intestinal flora: Accompany with the antihypertensive effect of electroacupuncture on stage 1 hypertension. *Chin. Med.* 16:7. doi: 10.1186/s13020-020-00417-8

Wang, T. Q., Li, L. R., Tan, C. X., Yang, J. W., Shi, G. X., Wang, L. Q., et al. (2021). Effect of electroacupuncture on gut microbiota in participants with knee osteoarthritis. *Front. Cell. Infect. Microbiol.* 11:597431. doi: 10.3389/fcimb.2021.597431

Wang, Z., and Zhang, C. (2021). ClinicaI efficacy comparison of acupuncture and western medication in treating PSD. *J. Clin. Acupunct. Moxibust.* 37, 88–93. doi: 10.19917/j.cnki.1005-0779.021105

Wasinger, V. C., Lu, K., Yau, Y. Y., Nash, J., Lee, J., Chang, J., et al. (2020). Spp24 is associated with endocytic signalling, lipid metabolism, and discrimination of tissue integrity for 'leaky-gut' in inflammatory bowel disease. *Sci. Rep.* 10:12932. doi: 10.1038/s41598-020-69746-w

Wei, D., Xie, L., Zhuang, Z., Zhao, N., Huang, B., Tang, Y., et al. (2019). Gut microbiota: A new strategy to study the mechanism of electroacupuncture and moxibustion in treating ulcerative colitis. *Evid. Based Complement. Altern. Med.* 2019:9730176. doi: 10.1155/2019/9730176

Westfall, S., Caracci, F., Zhao, D., Wu, Q. L., Frolinger, T., Simon, J., et al. (2021). Microbiota metabolites modulate the T helper 17 to regulatory T cell (Th17/Treg) imbalance promoting resilience to stress-induced anxiety- and depressive-like behaviors. *Brain Behav. Immun.* 91, 350–368. doi: 10.1016/j.bbi.2020.10.013

Wexler, B. C. (1970). Metabolic changes in response to acute cerebral ischemia following bilateral carotid artery ligation in arteriosclerotic versus nonarteriosclerotic rats. *Stroke* 1, 112–121. doi: 10.1161/01.str.1.2.112

Wijeratne, T., Sales, C., and Wijeratne, C. (2022). A narrative review on the non-pharmacologic interventions in post-stroke depression. *Psychol. Res. Behav. Manag.* 15, 1689–1706. doi: 10.2147/PRBM.S310207

Wirz-Justice, A. (2006). Biological rhythm disturbances in mood disorders. *Int. Clin. Psychopharmacol.* 21, S11–S15. doi: 10.1097/01.yic.0000195660.37267.cf

Wong, J. M. W., de Souza, R., Kendall, C. W. C., Emam, A., and Jenkins, D. J. A. (2006). Colonic health: Fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* 40, 235–243.

Wu, T., Yang, L., Jiang, J., Ni, Y., Zhu, J., Zheng, X., et al. (2018). Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats. *Life Sci.* 192, 173–182. doi: 10.1016/j.lfs.2017.11.049

Xia, W., Xu, Y., Gong, Y., Cheng, X., Yu, T., and Yu, G. (2022). Microglia involves in the immune inflammatory response of poststroke depression: A review of evidence. *Oxid. Med. Cell. Longev.* 2022:2049371. doi: 10.1155/2022/2049371

Xia, X., Xie, Y., Gong, Y., Zhan, M., He, Y., Liang, X., et al. (2022). Electroacupuncture promoted intestinal defensins and rescued the dysbiotic cecal microbiota of high-fat diet-induced obese mice. *Life Sci.* 309:120961. doi: 10.1016/j.lfs.2022.120961

Xian, M., Shen, L., Zhan, S., Chen, S., Lin, H., Cai, J., et al. (2022). Integrated 16S rRNA gene sequencing and LC/MS-based metabolomics ascertained synergistic influences of the combination of acupuncture and NaoMaiTong on ischemic stroke. *J. Ethnopharmacol.* 293:115281. doi: 10.1016/j.jep.2022.115281

Xie, L. L., Zhao, Y. L., Yang, J., Cheng, H., Zhong, Z. D., Liu, Y. R., et al. (2020). Electroacupuncture prevents osteoarthritis of high-fat diet-induced obese rats. *Biomed Res. Int.* 2020:9380965. doi: 10.1155/2020/9380965

Xie, X. H., Lai, W. T., Xu, S. X., Di Forti, M., Zhang, J. Y., Chen, M. M., et al. (2022). Hyper-inflammation of astrocytes in patients of major depressive disorder: Evidence from serum astrocyte-derived extracellular vesicles. *Brain Behav. Immun.* 109, 51–62. doi: 10.1016/j.bbi.2022.12.014

Xu, D., and Lu, W. (2020). Research progress of brain-gut axis related mechanisms regulating diseases. *J. Epileptol. Electroneurophysiol.* 29, 306–310.

Xu, J., Zheng, X., Cheng, K.-K., Chang, X., Shen, G., Liu, M., et al. (2017). NMR-based metabolomics reveals alterations of electro-acupuncture stimulations on chronic atrophic gastritis rats. *Sci. Rep.* 7:45580. doi: 10.1038/srep45580

Xu, M., Wang, L., Guo, Y., Zhang, W., Chen, Y., and Li, Y. (2021). Corrigendum to "positive effect of electro-acupuncture treatment on gut motility in constipated mice is related to rebalancing the gut microbiota". *Evid. Based Complement. Altern. Med.* 2021:9835654. doi: 10.1155/2021/9835654

Yan, Y., Li, T., Wang, D., Zhao, B., and Zhou, Q. (2019). Antidepressant effect of Xingnao Jieyu decoction mediated by alleviating neuroinflammation in a rat model of post-stroke depression. *J. Tradit. Chin. Med.* 39, 658–666.

Yang, N. N., Lin, L. L., Li, Y. J., Li, H. P., Cao, Y., Tan, C. X., et al. (2022). Potential mechanisms and clinical effectiveness of acupuncture in depression. *Curr. Neuropharmacol.* 20, 738–750. doi: 10.2174/1570159X19666210609162809

Yang, Y., Zhang, K., Zhong, J., Wang, J., Yu, Z., Lei, X., et al. (2018). Stably maintained microtubules protect dopamine neurons and alleviate depression-like behavior after intracerebral hemorrhage. *Sci. Rep.* 8:12647. doi: 10.1038/s41598-018-31056-7

Ye, X., Wang, D., Zhu, H., Wang, D., Li, J., Tang, Y., et al. (2021). Gut microbiota changes in patients with major depressive disorder treated with vortioxetine. *Front. Psychiatry* 12:641491. doi: 10.3389/fpsyt.2021.641491

Yoo, J. W., Shin, Y. J., Ma, X., Son, Y. H., Jang, H. M., Lee, C. K., et al. (2022). The alleviation of gut microbiota-induced depression and colitis in mice by anti-inflammatory probiotics NK151, NK173, and NK175. *Nutrients* 14:2080. doi: 10.3390/nu14102080

Yoo, K. Y., Lee, C. H., Choi, J. H., Sohn, Y., Cho, J. H., Hwang, I. K., et al. (2011). Changes in corticosteroid hormone receptors in the ischemic gerbil hippocampal CA1 region following repeated restraint stress. *Neurochem. Res.* 36, 701–712. doi: 10.1007/s11064-010-0384-z

Yu, H. B., Yang, H., Allaire, J. M., Ma, C., Graef, F. A., Mortha, A., et al. (2021). Vasoactive intestinal peptide promotes host defense against enteric pathogens by modulating the recruitment of group 3 innate lymphoid cells. *Proc. Natl. Acad. Sci. U. S. A.* 118:e2106634118. doi: 10.1073/pnas.2106634118

Yu, M., Jia, H. M., Qin, L. L., and Zou, Z. M. (2022). Gut microbiota and gut tissue metabolites involved in development and prevention of depression. *J. Affect. Disord.* 297, 8–17. doi: 10.1016/j.jad.2021.10.016

Zavvari, F., and Nahavandi, A. (2020). Fluoxetine increases hippocampal neural survival by improving axonal transport in stress-induced model of depression male rats. *Physiol. Behav.* 227:113140. doi: 10.1016/j.physbeh.2020.113140

Zeisel, M. B., Dhawan, P., and Baumert, T. F. (2019). Tight junction proteins in gastrointestinal and liver disease. *Gut* 68, 547–561. doi: 10.1136/gutjnl-2018-316906

Zhang, L., Chen, X., Wang, H., Huang, H., Li, M., Yao, L., et al. (2021). Adjusting internal organs and dredging channel" electroacupuncture ameliorates insulin resistance in type 2 diabetes mellitus by regulating the intestinal flora and inhibiting inflammation. *Diabet. Metab. Syndr. Obesity* 14, 2595–2607. doi: 10.2147/DMSO.S306861

Zhang, L., Zhang, H., Xie, Q., Xiong, S., Jin, F., Zhou, F., et al. (2022a). A bibliometric study of global trends in diabetes and gut flora research from 2011 to 2021. *Front. Endocrinol.* 13:990133. doi: 10.3389/fendo.2022.990133

Zhang, Q. F., Xiao, H. M., Zhan, J. T., Yuan, B. F., and Feng, Y. Q. (2022b). Simultaneous determination of indole metabolites of tryptophan in rat feces by chemical labeling assisted liquid chromatography-tandem mass spectrometry. *J. Chin. Chem. Lett.* 33, 4746–4749.

Zhang, X., Hou, Y., Li, Y., Wei, W., Cai, X., Shao, H., et al. (2022c). Taxonomic and metabolic signatures of gut microbiota for assessing the severity of depression and anxiety in major depressive disorder patients. *Neuroscience* 496, 179–189. doi: 10.1016/j.neuroscience.2022.06.024

Zhang, Y., Ding, N., Hao, X., Zhao, J., Zhao, Y., Li, Y., et al. (2022d). Manual acupuncture benignly regulates blood-brain barrier disruption and reduces lipopolysaccharide loading and systemic inflammation, possibly by adjusting the gut microbiota. *Front. Aging Neurosci.* 14:1018371. doi: 10.3389/fnagi.2022.1018371

Zhao, Y., Jaber, V. R., Pogue, A. I., Sharfman, N. M., Taylor, C., and Lukiw, W. J. (2022). Lipopolysaccharides (LPSs) as potent neurotoxic glycolipids in Alzheimer's disease (AD). *Int. J. Mol. Sci.* 23:12671. doi: 10.3390/ijms232012671

Zheng, S. (2021). Analysis of post-stroke depression related factors in ischemic stroke patients. *Chin. J. Modern Drug Appl.* 15, 105–107. doi: 10.14164/j.cnki.cn11-5581/r.2021.12.037

Zheng, Y., He, J., Guo, L., Yao, L., Zheng, X., Yang, Z., et al. (2019). Transcriptome analysis on maternal separation rats with depression-related manifestations ameliorated by electroacupuncture. *Front. Neurosci.* 13:314. doi: 10.3389/fnins.2019.00314

Zhong, J., Chen, J., Cao, M., Fang, L., Wang, Z., Liao, J., et al. (2022). Elevated plasma intestinal fatty acid binding protein and aberrant lipid metabolism predict post-stroke depression. *Heliyon* 8:e11848. doi: 10.1016/j.heliyon.2022.e11848

Zhou, H., Liang, H., Li, Z. F., Xiang, H., Liu, W., and Li, J. G. (2013). Vagus nerve stimulation attenuates intestinal epithelial tight junctions disruption in endotoxemic mice through α7 nicotinic acetylcholine receptors. *Shock* 40, 144–151. doi: 10.1097/SHK.0b013e318299e9c0

Zihni, C., Mills, C., Matter, K., and Balda, M. S. (2016). Tight junctions: From simple barriers to multifunctional molecular gates. *Nat. Rev. Mol. Cell Biol.* 17, 564–580. doi: 10.1038/nrm.2016.80

Zlokovic, B. V. (2006). Remodeling after stroke. *Nat. Med.* 12, 390–391. doi: 10.1038/nm0406-390

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Activation of POMC neurons to adiponectin participating in EA-mediated improvement of high-fat diet IR mice

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Background: Insulin resistance (IR) is one of the common pathological manifestations of metabolic-related diseases, and the prevalence of relevant diseases is high. Acupuncture is beneficial to IR patients, but the central mechanism underlying this treatment remains unclear. This study provides mechanistic insights into how electroacupuncture (EA) improves IR through the response of Pro-opiomelanocortin (POMC) neurons to adiponectin (Adipo).

Methods: Glucose tolerance tests (GTT), Insulin tolerance tests (ITT) and fasting blood glucose (FBG) were detected by glucometer. Serum insulin, Adipo and skeletal muscle adiponectin receptor 1 (AdipoR1) protein levels were examined by ELISA. Homeostasis model assessment estimated insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting insulin (FINS) (mU/L) × FBG (mmol/L)/22.5. The expression levels of AdipoR1 and Adipo mRNA in skeletal muscle were detected by real-time PCR quantification. The co-marking of c-Fos/AdipoR1 and POMC neurons were investigated using immunofluorescence. Spontaneous excitatory postsynaptic currents (sEPSCs) of POMC neurons and the response of POMC neurons to Adipo were detected via electrophysiology.

Results: EA significantly ameliorated HFD-induced impairment of GTT, ITT, FBG, and HOMA-IR which was correlated with recovery of the expression level of AdipoR1 and Adipo in skeletal muscle. The improved response of POMC neurons to Adipo in the hypothalamus may be a key factor in correcting abnormal glucose tolerance and improving IR.

Conclusion: This study demonstrates that EA can ameliorate HFD-induced impaired glucose tolerance through improved response of POMC neurons to Adipo in the hypothalamus, providing insight into the central mechanism of improving IR through EA.

KEYWORDS

electroacupuncture, ZuSanLi, insulin resistance, adiponectin, POMC

Introduction

Insulin resistance (IR) is defined as the reduced sensitivity of target cells expressing insulin receptors to insulin (Petersen and Shulman, 2018). Diseases closely related to insulin resistance include diabetes, obesity, non-alcoholic fatty liver disease, polycystic ovary syndrome, and cardiovascular diseases, especially type 2 diabetes (Rice et al., 2005; Mojiminiyi et al., 2007; Unlühizarci et al., 2007; Diamanti-Kandarakis and Dunaif, 2012; Nakata et al., 2016; Wu et al., 2016; Dahan and Reaven, 2019; Lee et al., 2020; Stener-Victorin et al., 2020). Globally, the prevalence of diabetes is expected to increase from 382 million in 2013 to 592 million in 2035, with type 2 diabetes accounting for approximately 90-95% of all cases (Laakso and Kuusisto, 2014). Metformin induces energy stress by inhibition of complex I of the mitochondrial respiratory chain, leading to changes in the ratio of ATP to AMP and activation of classically related protein kinases, which are key molecules in the regulation of metabolism and thus impact IR (Scherer et al., 1995). Direct activation of relevant protein kinases can recover IR in a variety of animal models (Fullerton et al., 2013; Cokorinos et al., 2017). However, inactivation of related protein kinases in the liver has no effect on the efficacy of drugs, while muscle-specific loss of related protein kinases rescues the effect of drugs. Therefore, muscle-associated protein kinases have been established as the main therapeutic target of IR (Cokorinos et al., 2017), suggesting that muscles may play an important role in improving insulin resistance.

Adipo is a hormone mainly secreted by adipocytes. By acting on key organs, including muscle tissue, pancreas, liver, adipose tissue, and brain, Adipo regulates glucose metabolism and insulin action. It can increase insulin sensitivity and reduce plasma concentration associated with insulin resistance (Fang and Judd, 2018). In IR, the level of Adipo in the blood is generally impaired (Hung et al., 2008; Sull et al., 2009). Regarded as insulin sensitizers, thiazolidinedione drugs (TZDs) can increase the ability of peripheral tissues to clear glucose, thus improving glucose metabolism (Miles et al., 2003; Zinman et al., 2009). The decrease of Adipo in rodents is related to decreased insulin response and high glucose levels, while systemic Adipo treatment or transgenic Adipo overexpression can reduce blood glucose (Qi et al., 2004). Additionally, TZD called pioglitazone can significantly improve IR in diabetic *ob/ob* mice, but not in Adipo-deficient *ob/ob* mice (Kubota et al., 2006). Adipo expression and number of mitochondria were decreased in the adipose tissue of obese mice, and these changes were reversed by rosiglitazone (Koh et al., 2007). Taken together, these indicate that Adipo has a therapeutic effect on insulin resistance.

Alongside its aforementioned role, Adipo also stimulates the activity of hypothalamic-related protein kinases (Yamauchi and Kadowaki, 2008). POMC neurons are key regulatory factors of metabolism and reproduction in the arcuate nucleus (ARC) of the hypothalamus and play a fundamental role in food and metabolism regulation. AdipoR1 is present in POMC neurons (Guillod-Maximin et al., 2009). A low molecular form of Adipo can cross the brain-blood barrier (BBB) and is also found in human cerebrospinal fluid (Kubota et al., 2007). Adipo enters the cerebrospinal fluid from the circulation, increases the activity of related protein kinases in the hypothalamus, and increases food intake through AdipoR1 (Thundyil et al., 2012). Under starvation conditions, Adipo triggers related protein kinases in the hypothalamus to promote food uptake (Yamauchi and Kadowaki, 2008). After intracerebroventricular injection of Adipo in mice, energy consumption is increased (Guillod-Maximin et al., 2009), food intake (Coope et al., 2008), and body weight are decreased. Adipo level correlates with the excitatory or inhibitory effect of Adipo on POMC neurons activity and feeding in ARC (Suyama et al., 2016). Thus, the peripheral and central roles of Adipo are closely related to energy metabolism and food intake. However, current studies have focused on peripheral target organs and the level of blood factors, while the central system mainly focuses on the influence of Adipo on food intake through POMC neurons and the participation of other pathway factors. Whether Adipo signal impacts the activity of peripheral skeletal muscle protein kinase and glucose absorption through the regulation of POMC neurons, and whether the regulation of Adipo signal by POMC neurons plays a key role in improving insulin resistance remain unclear.

Experimental studies have shown that acupuncture can correct various metabolic disorders such as hyperglycemia, overweight, hyperlipidemia, inflammation, altered sympathetic nervous system activity, and defective insulin signaling (Benrick et al., 2014; Lin et al., 2014; Li et al., 2018; Huang et al., 2020), all of which contribute to the improvement of IR. Lin (Lin et al., 2014) hypothesized that ST36 EA treatment stimulates the secretion of insulin-sensitive substances by releasing acetylcholine, and improves glucose tolerance and insulin activity by impacting insulin-sensitive target organs (chiefly muscles). Our previous study (Huang et al., 2016) demonstrated that acupuncture is capable of improving fasting blood glucose (FBG), plasma fasting insulin (FINS), insulin resistance index (HOMA-IR), and insulin signal molecules in OLETF rats. In this study, molecular biology, electrophysiology, and other experimental methods were used to observe the changes of peripheral molecular biology and the effects of central Adipo on POMC neurons activity in IR mice by EA, and to explore the mechanism of Adipo participating in EA to improve IR.

Materials and methods

Animals

Male C57BL/6J mice (10–14g; aged 4 weeks) were supplied by the Beijing Hua Fukang Biotechnology Co., LTD., and managed by the South China Research Center for Acupuncture and Moxibustion (Guangzhou, China). Mice were kept in a temperature-controlled (22–24°C, 50-70% humidity range) holding room with 12h light/dark cycle. Mice were given free access to water and food. The protocols were approved by the Ethics Committee of Guangzhou University of Chinese Medicine (No. 20210604003).

Abbreviations: Adipo, adiponectin; AdipoR1, adiponectin receptor 1; AMPK, AMP-activated protein kinase; ARC, arcuate nucleus; AUC, area under curve; BCA, bicinchoninic acid; BMI, body mass index; EA, electroacupuncture; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; IPGTT, intraperitoneal glucose tolerance tests; IPITT, intraperitoneal insulin tolerance tests; IR, insulin resistance; PBS, phosphate buffered saline; POMC, pro-opiomelanocortin; sEPSCs, spontaneous excitatory postsynaptic currents; TZDs, thiazolidinediones.

Establishment of IR model in mice

After 3 days of adaptive feeding, mice were randomly divided into 2 groups, CD and HFD groups. Mice in CD and HFD groups were fed a control standard diet [20% kcal protein, 70% kcal carbohydrate and 10% kcal fat, H10010, Beijing HFK BIOSCIENCE CO.,LTD.) or high-fat diet (20% kcal protein, 20% kcal carbohydrate and 60% kcal fat, H10060, Beijing HFK BIOSCIENCE CO.,LTD.)], respectively. Body weights were measured once a week. After 8 weeks of feeding, mice were randomly selected from the CD group and HDF group for examination via glucose tolerance test (GTT), insulin tolerance test (ITT), fasting blood glucose (FBG), HOMA-IR, and serum insulin levels to establish the model.

Electroacupuncture (EA) treatment

After the establishment of an IR model, the HFD group was randomly divided into an HFD group and an HFD + EA group. The mice were anesthetized by isoflurane and kept under anesthesia during EA treatment. The HFD group only received anesthesia without electroacupuncture. Disposable sterile acupuncture needles (0.16 mm × 7 mm; Beijing Zhongyan Taihe Medical InstrumentCp. Ltd., Tianjin, China) were parallelly inserted at a depth of 4-5 mm into the murine equivalent of the human ZuSanLi (ST36, about 3mm below the knee joint in the tibialis anterior muscle) acupoints bilaterally. The needle handles were connected to Huatuo electroacupuncture instrument. The EA stimulation was applied for 20 min with a dense-dispersed wave of 2Hz. Two groups were treated 6 days every week for 4 weeks.

Glucose tolerance tests (GTT)

Glucose tolerance tests were performed on 12h-fasted animals. After determination of fasting blood glucose (FBG) levels at the end of mouse tails at 9 a.m., each animal received an intraperitoneal injection of 50% glucose (10 mL/kg) (G7201, Sigma). Blood glucose levels were measured after 30, 60, 90, and 120 min.

Insulin tolerance tests (ITT)

Glucose tolerance tests were performed on 12h-fasted animals. After determination of fasting blood glucose (FBG) levels at the end of mouse tails at 9 a.m., each animal received an intraperitoneal injection of 1U/mL Insulin (10 mL/kg) (25R, Lilly France). Blood glucose levels were measured after 30, 60, 90, and 120 min.

Serum analyses

All mice fasted for 12 h after prior acupuncture. On the second morning, fasting blood glucose (FBG) levels were tested from mouse tails. Mice were then anesthetized with tribromoethanol and blood samples were collected by eye enucleation. Samples were measured by ELISA to detect fasting insulin (FINS) (E-EL-M1382c; Elabscience, Wuhan, China) and Adipo (E-EL-M0002c; Elabscience, Wuhan, China). ELISA was performed according to the manufacturer's instructions. Additionally, the following formula was used to calculate the homeostasis model assessment estimated insulin resistance (HOMA-IR) = FINS (mU/L) × FBG (mmol/L)/22.5.

Real-time PCR quantification of Adipo and AdipoR1

Total skeletal muscle RNA was extracted using TRIzol Reagent (Invitrogen, 15596-026) as described by the manufacturer. cDNA was obtained by reverse transcription of 1 μg of RNA with random primers using PrimeScript RT (Takara, RR055A). qPCR was performed on all samples using an Applied Biosystems 7,300 System and TB Green Premix Ex Taq II (Takara, FY18936). The following primers were used to detect Adipo: (forward: 5′- CCAATGTACCCATTCGCTTTAC-3′; reverse: 5′-GAAGTAGTAGAGTCCCGGAATG-3′), AdipoR1: (forward: GAAAGACAACGACTACCTGCTA; reverse: ATAG CACAAAACCAAGCAGATG), reference gene GAPDH binding protein (forward: 5′-GCACCACCAACTGCTTAG-3′; reverse: 5′-CAGTGATGGCATGGACTG-3′). The $\Delta\Delta$CT method was used to analyze relative expression levels and statistics were performed using $\Delta\Delta$CT values.

Skeletal muscle AdipoR1 protein levels measured by ELISA

Extraction of skeletal muscle tissue was performed as described above. Tissues were then homogenized and the total protein concentration was determined using bicinchoninic acid (BCA), with the concentration not exceeding 0.3 mg. ELISA was performed according to the manufacturer's instructions (EM0308; Wuhan Fine Biotech Co., Ltd., Wuhan, China).

Immunofluorescence

For EA treatment, mice were anesthetized and perfused transcardially with 4% paraformaldehyde, and brains were removed and stored in 4% paraformaldehyde overnight. Sagittal brain slices (30 μm) containing the arcuate hypothalamic nucleus (ARC) were prepared on a freezing microtome. After antigen retrieval, the sections were subsequently blocked in phosphate buffered saline (PBS) with 10% normal goat serum and 0.5% Triton X-100 for 1 h at 37°C. POMC neurons and c-Fos were detected using a rabbit anti-POMC antibody (ab254257; abcam; 1:500) and a guinea pig anti-c-Fos antibody (226004; Synaptic System; 1:800) overnight at 4°C. Tissue was then washed in PBS 3 times and incubated with Alexa Fluor 555-conjugated goat anti-rabbit secondary antibody (ab150078; abcam; 1:800) and Alexa Fluor 488-conjugated goat anti-guinea pig (A11073; Invitrogen; 1:500). AdipoR1 was detected using a rabbit anti-AdipoR1 antibody (ab126611; abcam; 1:200)

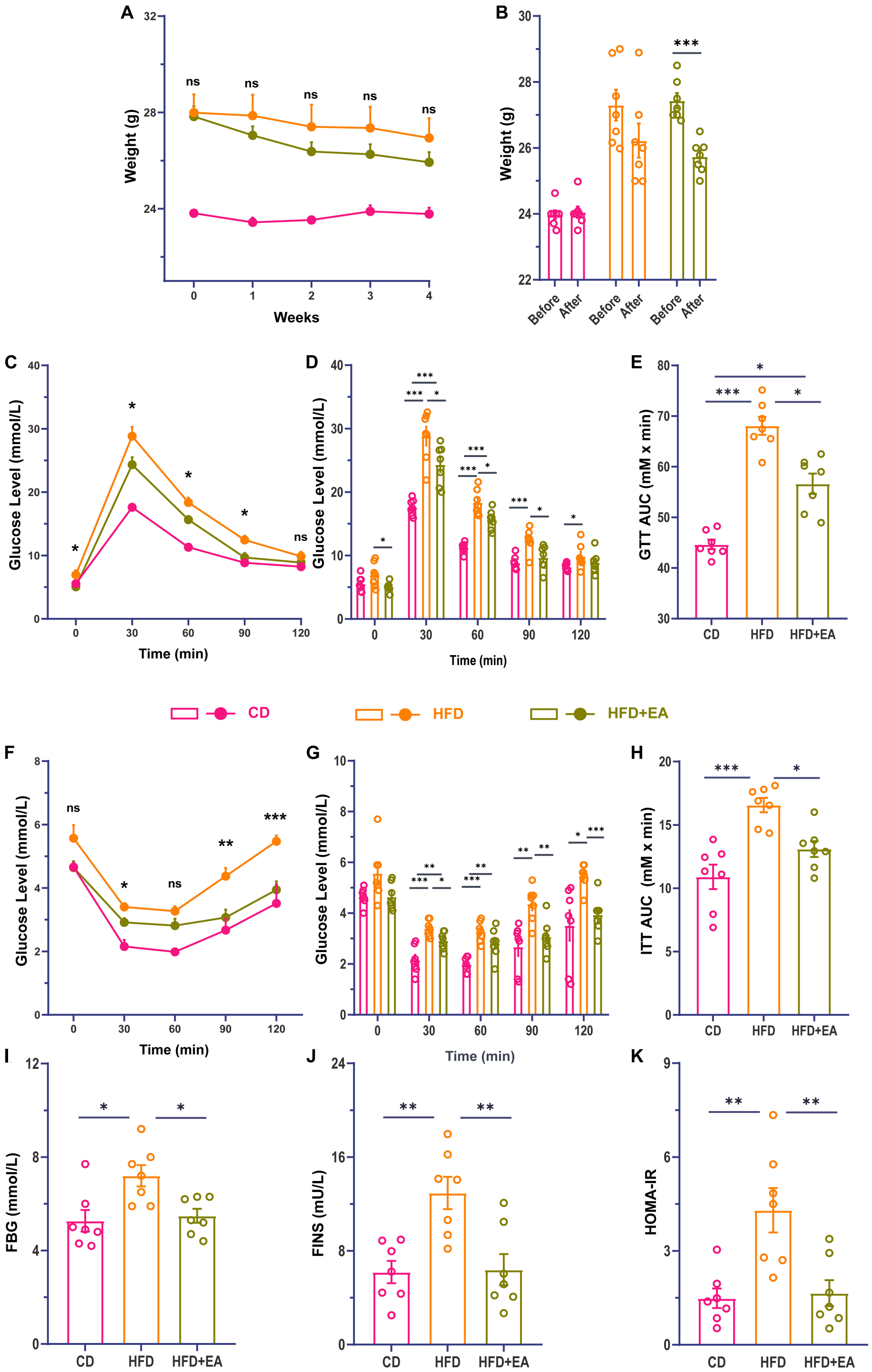

FIGURE 1
EA ameliorate high-fat diet-induced insulin resistance in mice. **(A)** Comparison of body weight changes during EA. **(B)** Comparison of body weight changes before and after EA. **(C)** Line chart of blood glucose change during glucose tolerance test in mice 4 weeks after EA. **(D)** Histogram of blood glucose change during glucose tolerance test in mice 4 weeks after EA. **(E)** Area under the glucose tolerance curve. **(F)** Line chart of blood glucose change during insulin tolerance test in mice 4 weeks after EA. **(G)** Histogram of blood glucose changes during insulin tolerance test in mice 4 weeks after EA. **(H)** Area under the insulin tolerance curve. **(I)** Fasting blood glucose of mice after EA. **(J)** Fasting serum insulin concentration of mice after EA. **(K)** Insulin resistance index of mice after EA. CD: control group. HFD: high-fat diet group. HFD + EA: model + electroacupuncture group. $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (one-way analysis of variance with *post hoc* Bonferroni correction or Tamhane correction).

and then incubated with Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (ab150181; abcam; 1:800). Subsequently, rabbit anti-POMC antibody and Alexa Fluor 555-conjugated goat anti-rabbit secondary antibody were used. Fluorescence imaging was conducted by confocal laser microscopy. Colocalization of POMC neuronal immunoreactivity and c-Fos expression was observed and documented after counting of labeled cells from a total of 16 representative brain slices and every 4 slices were included for each mice.

Stereotactic surgery

Mice were anesthetized with tribromoethanol (125 mg/kg) injection (i.p.) and fixed on a stereotaxic apparatus. The skull was exposed and holes were drilled in the target area. rAAV-EF1α-DIO-mCherry-WPRE-pA was injected at a rate of 30 nL/min according to the following coordinates: ARC (AP:-1.45; ML: ± 0.22; DV:-5.60). After 5 min, the needle was retracted and the incision was closed with sterile suture. Coordinate positioning and injection were performed by RWD Biotechnology, Shenzhen, China.

Electrophysiology

Whole cell patch clamp recording and data analysis were performed on POMC neurons prepared from hypothalamus sections. 16-week-old POMC-cre mice were anaesthetized and infused with cold artificial cerebrospinal fluid (ACSF: 25 mM D-glucose, 25 mM $NaHCO_3$, 1.25 mM $NaH_2PO_4 \cdot 2H_2O$, 2.5 mM KCl, 0.1 mM $MgCl_2$, 127 mM NaCl, 2 mM $CaCl_2 \cdot 2H_2O$). Immediately after the brain was removed, it was placed in cold ACSF containing 95% O_2 and 5% CO_2. Coronal sections (280 uM) were cut on Leica VT1200S vibrating microtome and incubated for 30 min in oxygen-containing ACSF at 36°C and incubated at room temperature for at least 1 h before recording. Slices were transferred to recording tanks and soaked in oxygen-containing ACSF (34°C) at a flow rate of 2 mL/min. Electrophysiological signals were recorded using a Multi-Clamp 700 B amplifier (Molecular Devices). The resistance of the recording electrode was 5-8 MΩ when filling the solution with sodium k-gluconate. Insulin (2.5 um, HY-P0035, MCE) and Adipo (600 nM, 315-26-100, PeproTech) were added to the recording tank for specific experiments.

Statistical analyses

Data and statistical analyses were performed using IBM SPSS (version 26.0) and GraphPad Prism (version 8.0.1). All data are presented as mean ± standard error of the mean (SEM). The differences between groups were analyzed by the unpaired *t*-test or one-way analysis of variance (ANOVA) with *post hoc* Bonferroni correction or Tamhane correction, statistical significance was defined as $P < 0.05$ *, $P < 0.01$ ** and $P < 0.001$ ***.

Results and discussion

EA ameliorate high-fat diet-induced insulin resistance in mice

After 8 weeks of feeding, HFD-fed mice showed significant weight gain (**Supplementary Figure 1A**), impaired glucose tolerance (**Supplementary Figures 1B, C**), as well as impaired insulin tolerance (**Supplementary Figures 1D, E**). Meanwhile, fasting blood glucose (FBG), serum insulin (FINS), and HOMA-IR in the HFD group were considerably increased compared with the CD group (**Supplementary Figures 1F–H**). These results suggest that HFD-fed induced insulin resistance was successfully established.

After the establishment of our IR model, the HFD group was randomly divided into an HFD group and an HFD + EA group. Following EA intervention for 4 weeks, the HFD + EA group showed a marked trend in weight loss (**Figures 1A, B**). Compared to the HFD group, the HFD + EA group presented improved glucose and insulin tolerance (**Figures 1C–H**). Compared with HFD group, the area under the curve (AUC) of ITT in the HFD + EA group decreased significantly, and there was no significant difference between the HFD + EA and CD groups (**Figure 1H**). FBG, FINS, and HOMA-IR levels were returned to normal levels after EA acupuncture treatment (**Figures 1I–K**).

Adipo and AdipoR1 participate in EA-mediated improvement of HFD-IR mice

Studies have found that Adipo can increase insulin sensitivity and improve insulin resistance (Fang and Judd, 2018). Adipo receptors include receptor 1 and receptor 2, with receptor 1 mainly distributed in skeletal muscle and receptor 2 in the liver (Borst, 2004; Yadav et al., 2013; James et al., 2021). Although both receptor 1 and receptor 2 may influence glucose metabolism, findings imply that receptor 1 may be more involved in glucose metabolism (Tan et al., 2005). Kadowaki et al. proved that pioglitazone, a commonly used drug for the treatment of type 2 diabetes is likely to play an important role through Adipo (Kubota et al., 2006; Quaresma et al., 2016). Therefore, we investigated whether Adipo was also involved in the improvement of IR by EA. We examined Adipo and AdipoR1 levels in mice, observing that HFD-fed mice were associated with a significant decrease in serum Adipo and skeletal muscle AdipoR1 levels (**Figures 2A, C, E**). These results suggest that HFD-fed mice develop insulin resistance with decreased Adipo and AdipoR1 levels. After EA treatment, the serum Adipo level of the HFD + EA group was significantly improved with no significant difference compared to CD group (**Figure 2B**). We then examined the expression levels of AdipoR1 mRNA and protein in skeletal muscle to determine whether AdipoR1 participated in the improvement of IR in the skeletal muscle of mice through EA intervention. Our results showed that AdipoR1 mRNA expression in skeletal muscle of HFD mice had a noteworthy change after EA (**Figure 2D**). Correspondingly, AdipoR1 protein expression in skeletal muscle was significantly increased in the HFD + EA group and returned to normal levels (**Figure 2F**). We also detected Adipo mRNA in skeletal muscle. Interestingly, the HFD group showed a significant increase in Adipo mRNA (**Figure 2G**), which was restored by EA. This result may be related to the compensatory increase of skeletal muscle Adipo in IR conditions. These results suggest that EA treatment can improve insulin resistance in HFD mice, and Adipo and AdipoR1 might contribute to this process.

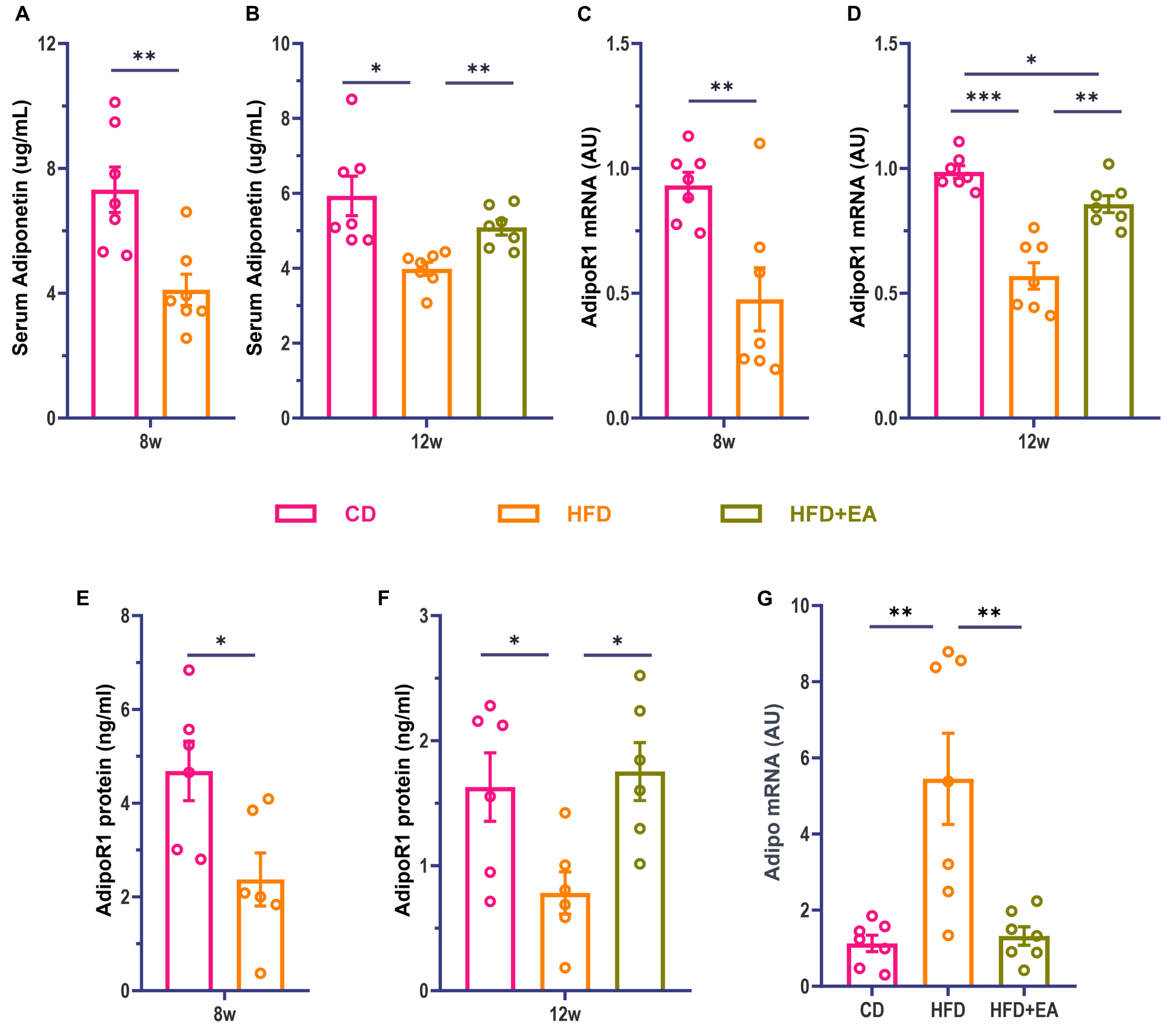

FIGURE 2
HFD + EA mice exhibited improved levels of Adipo and AdipoR1. **(A)** Serum Adipo concentration of mice after HFD modeling. **(B)** Serum Adipo concentration of mice after EA. **(C)** Expression of AdipoR1 mRNA in skeletal muscle of mice after HFD modeling. **(D)** Expression of AdipoR1 mRNA in skeletal muscle of mice after EA. **(E)** AdipoR1 protein concentration in skeletal muscle of mice after HFD modeling. **(F)** AdipoR1 protein concentration in skeletal muscle of mice after EA. **(G)** Expression of Adipo mRNA in skeletal muscle of mice after EA. CD: control group. HFD: high-fat diet group. HFD + EA: model + electroacupuncture group. $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ [unpaired *t*-test in panels **(A,C,E)**; one-way analysis of variance with *post hoc* Bonferroni correction or Tamhane correction in panels **(B,D,F,G)**].

EA treatments activate hypothalamic POMC neurons

As observed above, EA treatment of ST36 improved HFD-induced insulin resistance, leading to questions about whether this was related to central regulation. Previous studies have shown that POMC neurons in the arcuate nucleus of the hypothalamus play an important role in regulating metabolism (Schwartz et al., 1992; Sipols et al., 1995; Hill et al., 2010; Zeltser et al., 2012). We wanted to know whether POMC neurons could be activated by EA treatment. Mice were anesthetized 1h after EA treatment and their brains were harvested for immunofluorescence staining. Anti-c-Fos and anti-POMC were jointly labeled (Figure 3A). There was no significant difference in the number of POMC neurons among the three groups (Figure 3B). The activated POMC neurons were significantly enriched in the HFD + EA group compared with the HFD group (Figure 3C) and returned to CD group level. We then examined the electrophysiological properties of POMC neurons using a patch clamp. Cells were clamped at –60mV and sEPSCs were recorded (Figure 3D). In the HFD group, the frequency of sEPSCs decreased meaningfully and the HFD + EA group had rescued this phenomenon and returned to normal level (Figure 3E). Amplitude of sEPSCs showed the same tendency (Figure 3F).

EA treatments activate hypothalamic POMC neuron responses to Adipo

Although POMC neurons can respond to insulin, mice with an insulin receptor deletion in POMC neurons show normal blood glucose parameters (Hill et al., 2010), suggesting that the effect of insulin on POMC neurons is not the most important factor in

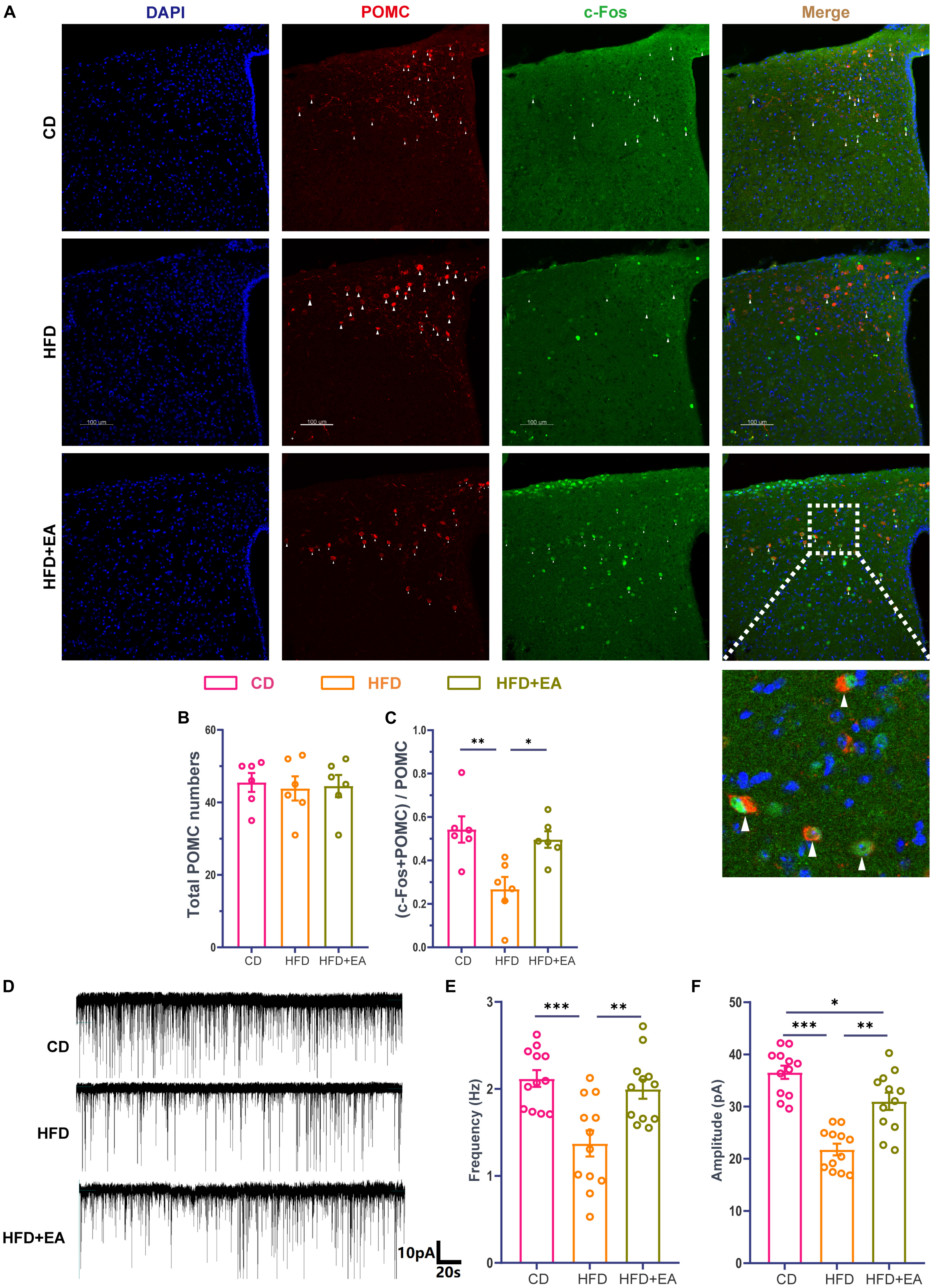

FIGURE 3

EA treatments activate hypothalamic POMC neurons. **(A)** Co-labeled immunofluorescence representation of POMC neurons and c-Fos. **(B)** Total POMC neurons number. **(C)** Ratio of common standard count of POMC neurons and c-Fos in total POMC neurons. **(D)** Representation of sEPSCs in POMC neurons. **(E)** sEPSCs frequency of POMC neurons. **(F)** sEPSCs amplitude of POMC neurons. "n" in the immunofluorescence graph represents the number of slices, with 3 mice in each group. "n" in the patch clamp graph represents the number of cells (the number of mice in each group ≥ 3). CD: control group. HFD: high-fat diet group. HFD + EA: model + electroacupuncture group. $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (one-way analysis of variance with *post hoc* Bonferroni correction or Tamhane correction). Scale bars: 100 μm.

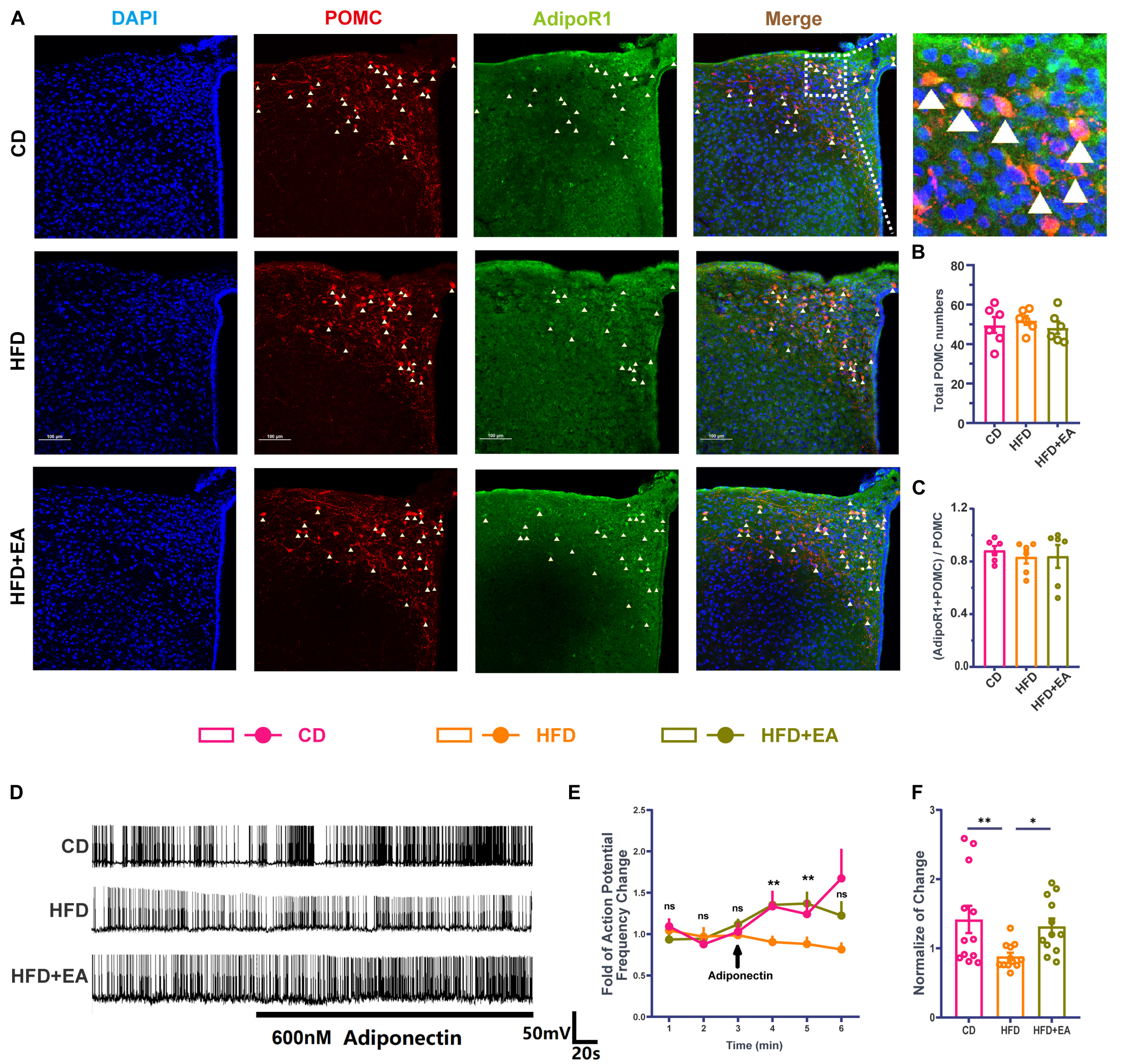

FIGURE 4
EA treatments activate hypothalamic POMC neurons' response to Adipo. **(A)** Co-labeled immunofluorescence representation of POMC neurons and AdipoR1 in the arcuate nucleus of the hypothalamus. **(B)** Total POMC numbers. **(C)** Ratio of common standard count of POMC neurons and AdipoR1 in the total POMC number. **(D)** Frequency representation of spontaneous action potential of POMC neurons before and after Adipo perfusion. **(E)** Spontaneous action potential frequency line diagram of POMC neurons before and after Adipo perfusion. **(F)** Normalization of action potential frequency change. "n" in the immunofluorescence graph represents the number of slices, with 3 mice in each group. "n" in the patch clamp graph represents the number of cells (the number of mice in each group ≥ 3). Panel **(E)** shows the statistical results of HFD group and HFD + EA group. CD: control group. HFD: high-fat diet group. HFD + EA: model + electroacupuncture group. $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$ (one-way analysis of variance with *post hoc* Bonferroni correction or Tamhane correction). Scale bars: 100 μm.

improving IR. At the same time, Adipo has been demonstrated to interact directly with POMC neurons under high concentrations of glucose (Suyama et al., 2016), suggesting that the direct effect of Adipo on POMC neurons may be the reason for improving IR. We therefore performed immunofluorescence co-staining in the ARC region of AdipoR1 and POMC neurons (Figure 4A). The result showed no significant difference in the ratio of the co-stained number of AdipoR1 and POMC neurons to the total number of POMC neurons in the CD group, HFD group, and HFD + EA group (Figures 4B, C), suggesting that EA may not improve IR by changing the number of AdipoR1 receptors on POMC neurons.

Pioglitazone (PIO), a diabetes drug, improves the effect of insulin/leptin in the hypothalamus, partly by activating the Adipo/AdipoR1/AMPK axis in the hypothalamus (Quaresma et al., 2016), indicating that Adipo and AdipoR1 may play an important role in improving the effect of hypothalamus IR. Therefore, this research further detected the change of spontaneous action potential frequency of POMC neurons after Adipo perfusion (Figure 4D). In the CD group, Adipo can increase the action potential frequency of POMC neurons. However, POMC neurons cannot respond to Adipo in the HFD group, while EA can restore the response degree of POMC neurons to Adipo (Figure 4E). The normalization of action potential frequency change also indicates that EA can improve the response of POMC neurons to Adipo (**Figure 4F**). Based on previous studies and the above experiments, we propose a hypothesis that EA ST36 may improve IR through activating POMC neurons responses to Adipo.

Discussion

In this study, EA at ST36 improved IR induced by high-fat diet while increasing Adipo and AdipoR1 levels. In addition, the activity of POMC neurons and their response to Adipo were weakened in IR and improved after EA. Our results suggest that the enhanced response of POMC neurons to Adipo plays an important role in EA-mediated improvement of IR.

Obesity is closely related to IR, and most obese experimental animals showed impaired insulin response (Al-Massadi et al., 2019; Rodrigues et al., 2019; Zhang F. et al., 2020), while some experimental results demonstrated that FINS concentration in mice fed with a high-fat diet did not change significantly (Hanning et al., 2019). In this study, FBG, FINS, and HOMA-IR increased in model mice, similar to clinical IR. The differences between experimental results and prior research results may be related to the following: (1) dietary nutrient ratio of the mice; (2) Daily caloric intake of the mice; (3) Modeling time; (4) Sampling time. In the streptozotocin-(STZ-) induced insulin-dependent diabetes (IDDM) rat model, Man (Man et al., 2021) found that EA at ST36 can be an effective strategy to reduce plasma glucose and the level of free fatty acids which is one of the major lipids in human body. Ji et al. (2013) concluded that EA ST36 produces an up-regulation of anorexigenic factor POMC production in the NTS/HN, which inhibited food intake and reduced body weight. Meanwhile, Zhang Y. et al. (2020) and Han et al. (2021) also found the similarly result, which suggest that single point stimulation at ST36 can reduce body weight. In this experiment, it was observed that the body weight of the HFD + EA group only a decreased compared with the HFD group during EA, suggesting that a single EA treatment could not restore the body weight of model mice to a normal level. In intragroup comparison, it was observed that the body weight of the HFD + EA group decreased significantly compared to before EA, and there was no significant difference in body weight between the HFD group and the CD group. This may be related to the small size of the mice themselves and the insignificant weight change. Although weight gain is inextricably linked to IR, weight is just one of the manifestations of IR, and IR may also can occur without high body mass index (BMI) (Brown and Walker, 2016). In clinical practice, weight control is often combined with exercise and drugs to reduce weight (Bray et al., 2016; Lundgren et al., 2021). Therefore, we propose that acupuncture at a single point may not be the ideal method to improve body weight of IR individuals. In this study, EA could improve IPGTT, IPITT, FBG, FINS levels, and HOMA-IR in IR mice, which was consistent with the results of others (Yi et al., 2007; Chen et al., 2017; Huang et al., 2019).

The improvement of IR by acupuncture may be related to the improvement of glucose transport capacity in the hypothalamus (Hong et al., 2017). POMC neurons located in the ARC region of the hypothalamus are key neurons in regulating metabolism and energy. Some studies suggest that POMC neurons show different responses to different concentrations of glucose (Suyama et al., 2016; Fioramonti et al., 2017), indicating that insulin may also have similar effects on POMC neurons. At the same time, POMC neurons are innervated by other neurons (Newton et al., 2013; Trotta et al., 2020). In this research, EA was observed to improve the expression of c-Fos in POMC neurons, which may be related to the speed of releasing the transmitter from the presynaptic membrane of POMC neurons and the changes in the number of receptors in the postsynaptic membrane.

Skeletal muscle is the main target of Adipo and a major location of AdipoR1. Although skeletal muscle is considered to be one of the targets of pharmacological treatment for IR or diabetes (Dong et al., 2021; Wei et al., 2021; Yu et al., 2022), some studies have shown that acupuncture ST36 may stimulate skeletal muscle and improve the recovery of behavioral activity in rats subjected to cerebral ischemia or reperfusion injury (Yang et al., 2022). Sun demonstrated that acupuncture at ST36 increased motor cortical excitation (Sun et al., 2019). The deep layer of ST36 is skeletal muscle, and ST36 is the only acupuncture point selected in this experiment. Therefore, skeletal muscle probably will be one of the targets of acupuncture treatment for IR or diabetes. Based on previous studies (da Silva Rosa et al., 2021), we know that there is a degradation of Adipo during insulin resistance, which can also lead to a decrease in Adipo levels and thus an inability to maintain normal insulin sensitivity. Meanwhile, our research indicates that the expression levels of AdipoR1 mRNA and protein in skeletal muscle of the HFD group were significantly decreased, suggesting that the ability of skeletal muscle to process Adipo in IR was impaired, which may be one of the reasons for decreased insulin sensitivity in model mice. EA at ST36 heightened the expression of AdipoR1 mRNA and protein in skeletal muscle of HFD group mice and rescued Adipo levels in both serum and skeletal muscle.

Pioglitazone (PIO), a diabetes drug, improves the effect of insulin/leptin in the hypothalamus, partly by activating the Adipo/AdipoR1/AMPK axis in the hypothalamus (Quaresma et al., 2016), suggesting that Adipo and AdipoR1 may play an important role in improving the effect of IR in hypothalamus. Therefore, this experiment further demonstrated the change of spontaneous action potential frequency of POMC neurons after Adipo perfusion. Under physiological conditions, Adipo can increase the discharge frequency of POMC neurons, and this function is reduced in the IR pathological state, while EA can restore the response degree of POMC neurons to Adipo. According to previous research, the peripheral injections (Kandasamy et al., 2012) or intraportal injection (Shklyaev et al., 2003) of lipocalin encoding vectors can drive expression of lipocalin cDNA, which would suggest that muscle-derived lipocalin would be secreted into the bloodstream thereby increasing serum lipocalin levels, while acting to reduce body weight and food intake (Tang et al., 2021). At the same time, there are some viewpoints that peripheral adiponectin can be transferred to the brain through the blood-brain barrier (Koch et al., 2014) and the expression of adiponectin in hypothalamic mHypoPOMC and SH-SY5Y neurons and their regulation at the neuronal level (Abgrall et al., 2022). Based on previous studies and the above experiments, we propose a hypothesis that EA at ST36 may regulate Adipo through POMC neurons, thereby improving IR. However, there are still other neurons in this location that may impact IR in addition to POMC neurons.

Studies have shown that Adipo can independently enhance the inhibitory postsynaptic current of NPY neurons at the physiological level (Suyama et al., 2017), and glucose levels may influence the excitatory or inhibitory effect of Adipo on the activity and feeding of arcuate POMC neurons. In subsequent experiments, we will specifically regulate AdipoR1 on POMC neurons to observe the role of POMC neurons in regulating Adipo in IR.

Conclusion

This study investigated the effect of EA at ST36 on IR in peripheral and central functions. Our study illustrates the possibility that EA regulates metabolism and energy through the response of POMC neurons to Adipo. Future research should explore the underlying mechanisms of intrinsic excitatory changes and how central signals are transmitted to the periphery. In addition, further research is needed on central neurons that regulate metabolism and energy.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

This animal study was reviewed and approved by the Ethics Committee of Guangzhou University of Chinese Medicine (No. 20210604003).

Author contributions

WX: study design, performance of the experiments, data analysis, statistical analysis, and writing and revision of the manuscript. JL: performance of the animal feeding work, assistance with the experiments, and writing and revision of the manuscript. CJ: study design and assistance with the molecular experiments. DF: assistance with the animal feeding work and molecular experiments. LY and NX: study design and revision of the manuscript. WY: study design and writing and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1145079/full#supplementary-material

References

Abgrall, A., Poizat, G., Prevost, M., Riffault, L., De La Barrera, L., Hanine, R., et al. (2022). Evidence for the neuronal expression and secretion of adiponectin. *Cells* 11:2725. doi: 10.3390/cells11172725

Al-Massadi, O., Quiñones, M., Clasadonte, J., Hernandez-Bautista, R., Romero-Picó, A., Folgueira, C., et al. (2019). MCH regulates SIRT1/FoxO1 and reduces POMC neuronal activity to induce hyperphagia, adiposity, and glucose intolerance. *Diabetes* 68, 2210–2222. doi: 10.2337/db19-0029

Benrick, A., Maliqueo, M., Johansson, J., Sun, M., Wu, X., Mannerås-Holm, L., et al. (2014). Enhanced insulin sensitivity and acute regulation of metabolic genes and signaling pathways after a single electrical or manual acupuncture session in female insulin-resistant rats. *Acta Diabetol.* 51, 963–972. doi: 10.1007/s00592-014-0645-4

Borst, S. E. (2004). The role of TNF-alpha in insulin resistance. *Endocrine* 23, 177–182. doi: 10.1385/endo:23:2-3:177

Bray, G. A., Frühbeck, G., Ryan, D. H., and Wilding, J. P. (2016). Management of obesity. *Lancet* 387, 1947–1956. doi: 10.1016/s0140-6736(16)00271-3

Brown, A. E., and Walker, M. (2016). Genetics of insulin resistance and the metabolic syndrome. *Curr. Cardiol. Rep.* 18:75. doi: 10.1007/s11886-016-0755-4

Chen, H., Zhang, Z. L., Wang, X., and Yang, Y. Q. (2017). Effect of "spleen-stomach harmonizing" needling on insulin resistance and expression of insulin receptor substrate-1, −2, and glucose transporter-4 in insulin resistance type 2 diabetes rats. *Zhen Ci Yan Jiu* 42, 197–201.

Cokorinos, E. C., Delmore, J., Reyes, A. R., Albuquerque, B., Kjøbsted, R., Jørgensen, N. O., et al. (2017). Activation of skeletal muscle AMPK promotes glucose disposal and glucose lowering in non-human primates and mice. *Cell Metab.* 25, 1147–1159.e10. doi: 10.1016/j.cmet.2017.04.010

Coope, A., Milanski, M., Araújo, E. P., Tambascia, M., Saad, M. J., Geloneze, B., et al. (2008). AdipoR1 mediates the anorexigenic and insulin/leptin-like actions of adiponectin in the hypothalamus. *FEBS Lett.* 582, 1471–1476. doi: 10.1016/j.febslet.2008.03.037

da Silva Rosa, S. C., Liu, M., and Sweeney, G. (2021). Adiponectin synthesis, secretion and extravasation from circulation to interstitial space. *Physiology* 36, 134–149. doi: 10.1152/physiol.00031.2020

Dahan, M. H., and Reaven, G. (2019). Relationship among obesity, insulin resistance, and hyperinsulinemia in the polycystic ovary syndrome. *Endocrine* 64, 685–689. doi: 10.1007/s12020-019-01899-9

Diamanti-Kandarakis, E., and Dunaif, A. (2012). Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications. *Endocr. Rev.* 33, 981–1030. doi: 10.1210/er.2011-1034

Dong, Z. H., Lin, H. Y., Chen, F. L., Che, X. Q., Bi, W. K., Shi, S. L., et al. (2021). Berberine improves intralipid-induced insulin resistance in murine. *Acta Pharmacol. Sin.* 42, 735–743. doi: 10.1038/s41401-020-0493-4

Fang, H., and Judd, R. L. (2018). Adiponectin regulation and function. *Compr. Physiol.* 8, 1031–1063. doi: 10.1002/cphy.c170046

Fioramonti, X., Chrétien, C., Leloup, C., and Pénicaud, L. (2017). Recent advances in the cellular and molecular mechanisms of hypothalamic neuronal glucose detection. *Front. Physiol.* 8:875. doi: 10.3389/fphys.2017.00875

Fullerton, M. D., Galic, S., Marcinko, K., Sikkema, S., Pulinilkunnil, T., Chen, Z. P., et al. (2013). Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat. Med.* 19, 1649–1654. doi: 10.1038/nm.3372

Guillod-Maximin, E., Roy, A. F., Vacher, C. M., Aubourg, A., Bailleux, V., Lorsignol, A., et al. (2009). Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. *J. Endocrinol.* 200, 93–105. doi: 10.1677/joe-08-0348

Han, X., Chen, X., Wang, X., Gong, M., Lu, M., Yu, Z., et al. (2021). Electroacupuncture at ST36 improve the gastric motility by affecting neurotransmitters in the enteric nervous system in type 2 diabetic rats. *Evid. Based Complement. Alternat. Med.* 2021:6666323. doi: 10.1155/2021/6666323

Hanning, A. R., Wang, X., Hashemi, Z., Wan, S., England, A., Jacobs, R. L., et al. (2019). Both low- and regular-fat cheeses mediate improved insulin sensitivity and modulate serum phospholipid profiles in insulin-resistant rats. *J. Nutr. Biochem.* 64, 144–151. doi: 10.1016/j.jnutbio.2018.10.018

Hill, J. W., Elias, C. F., Fukuda, M., Williams, K. W., Berglund, E. D., Holland, W. L., et al. (2010). Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. *Cell. Metab.* 11, 286–297. doi: 10.1016/j.cmet.2010.03.002

Hong, H., Cui, Z. Z., Zhu, L., Fu, S. P., Rossi, M., Cui, Y. H., et al. (2017). Central IGF1 improves glucose tolerance and insulin sensitivity in mice. *Nutr. Diabetes.* 7:2. doi: 10.1038/s41387-017-0002-0

Huang, Q., Song, Y. J., Yu, Z. M., Ren, J. F., Liang, F. X., Chen, R., et al. (2019). Electroacupuncture improves inflammatory reaction and insulin sensitivity in insulin-resistant obese rats. *Zhen Ci Yan Jiu* 44, 898–905. doi: 10.13702/j.1000-0607.190209

Huang, X. Y., Zhang, L., Sun, J., Xu, N. G., and Yi, W. (2016). Acupuncture alters expression of insulin signaling related molecules and improves insulin resistance in OLETF rats. *Evid. Based Complement. Alternat. Med.* 2016:9651592. doi: 10.1155/2016/9651592

Huang, X., Huang, K., Li, Z., Bai, D., Hao, Y., Wu, Q., et al. (2020). Electroacupuncture improves cognitive deficits and insulin resistance in an OLETF rat model of Al/D-gal induced aging model via the PI3K/Akt signaling pathway. *Brain Res.* 1740:146834. doi: 10.1016/j.brainres.2020.146834

Hung, J., McQuillan, B. M., Thompson, P. L., and Beilby, J. P. (2008). Circulating adiponectin levels associate with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity. *Int. J. Obes.* 32, 772–779. doi: 10.1038/sj.ijo.0803793

James, D. E., Stöckli, J., and Birnbaum, M. J. (2021). The aetiology and molecular landscape of insulin resistance. *Nat. Rev. Mol. Cell Biol.* 22, 751–771. doi: 10.1038/s41580-021-00390-6

Ji, B., Hu, J., and Ma, S. (2013). Effects of electroacupuncture Zusanli (ST36) on food intake and expression of POMC and TRPV1 through afferents-medulla pathway in obese prone rats. *Peptides* 40, 188–194. doi: 10.1016/j.peptides.2012.10.009

Kandasamy, A. D., Sung, M. M., Boisvenue, J. J., Barr, A. J., and Dyck, J. R. (2012). Adiponectin gene therapy ameliorates high-fat, high-sucrose diet-induced metabolic perturbations in mice. *Nutr. Diabetes* 2:e45. doi: 10.1038/nutd.2012.18

Koch, C. E., Lowe, C., Legler, K., Benzler, J., Boucsein, A., Böttiger, G., et al. (2014). Central adiponectin acutely improves glucose tolerance in male mice. *Endocrinology* 155, 1806–1816. doi: 10.1210/en.2013-1734

Koh, E. H., Park, J. Y., Park, H. S., Jeon, M. J., Ryu, J. W., Kim, M., et al. (2007). Essential role of mitochondrial function in adiponectin synthesis in adipocytes. *Diabetes* 56, 2973–2981. doi: 10.2337/db07-0510

Kubota, N., Terauchi, Y., Kubota, T., Kumagai, H., Itoh, S., Satoh, H., et al. (2006). Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin-dependent and -independent pathways. *J. Biol. Chem.* 281, 8748–8755. doi: 10.1074/jbc.M505649200

Kubota, N., Yano, W., Kubota, T., Yamauchi, T., Itoh, S., Kumagai, H., et al. (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab.* 6, 55–68. doi: 10.1016/j.cmet.2007.06.003

Laakso, M., and Kuusisto, J. (2014). Insulin resistance and hyperglycaemia in cardiovascular disease development. *Nat. Rev. Endocrinol.* 10, 293–302. doi: 10.1038/nrendo.2014.29

Lee, E. S., Kwon, M. H., Kim, H. M., Woo, H. B., Ahn, C. M., and Chung, C. H. (2020). Curcumin analog CUR5-8 ameliorates nonalcoholic fatty liver disease in mice with high-fat diet-induced obesity. *Metabolism* 103:154015. doi: 10.1016/j.metabol.2019.154015

Li, Z., Lan, D., Zhang, H., Zhang, H., Chen, X., and Sun, J. (2018). Electroacupuncture mitigates skeletal muscular lipid metabolism disorder related to high-fat-diet induced insulin resistance through the AMPK/ACC signaling pathway. *Evid. Based Complement. Alternat. Med.* 2018:7925842. doi: 10.1155/2018/7925842

Lin, R. T., Tzeng, C. Y., Lee, Y. C., Chen, Y. I., Hsu, T. H., Lin, J. G., et al. (2014). Acupoint-specific, frequency-dependent, and improved insulin sensitivity hypoglycemic effect of electroacupuncture applied to drug-combined therapy studied by a randomized control clinical trial. *Evid. Based Complement. Alternat. Med.* 2014:371475. doi: 10.1155/2014/371475

Lundgren, J. R., Janus, C., Jensen, S. B. K., Juhl, C. R., Olsen, L. M., Christensen, R. M., et al. (2021). Healthy weight loss maintenance with exercise liraglutide, or both combined. *N. Engl. J. Med.* 384, 1719–1730. doi: 10.1056/NEJMoa2028198

Man, K. M., Lee, Y. C., Chen, Y. I., Chen, Y. H., Chang, S. L., and Huang, C. C. (2021). Electroacupuncture at bilateral ST36 acupoints: Inducing the hypoglycemic effect through enhancing insulin signal proteins in a streptozotocin-induced rat model during isoflurane anesthesia. *Evid. Based Complement. Alternat. Med.* 2021:5852599. doi: 10.1155/2021/5852599

Miles, P. D., Barak, Y., Evans, R. M., and Olefsky, J. M. (2003). Effect of heterozygous PPARgamma deficiency and TZD treatment on insulin resistance associated with age and high-fat feeding. *Am. J. Physiol. Endocrinol. Metab.* 284, E618–E626. doi: 10.1152/ajpendo.00312.2002

Mojiminiyi, O. A., Abdella, N. A., Al Arouj, M., and Ben Nakhi, A. (2007). Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. *Int. J. Obes.* 31, 213–220. doi: 10.1038/sj.ijo.0803355

Nakata, M., Yamamoto, S., Okada, T., Gantulga, D., Okano, H., Ozawa, K., et al. (2016). IL-10 gene transfer upregulates arcuate POMC and ameliorates hyperphagia, obesity and diabetes by substituting for leptin. *Int. J. Obes.* 40, 425–433. doi: 10.1038/ijo.2015.201

Newton, A. J., Hess, S., Paeger, L., Vogt, M. C., Fleming Lascano, J., Nillni, E. A., et al. (2013). AgRP innervation onto POMC neurons increases with age and is accelerated with chronic high-fat feeding in male mice. *Endocrinology* 154, 172–183. doi: 10.1210/en.2012-1643

Petersen, M. C., and Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiol. Rev.* 98, 2133–2223. doi: 10.1152/physrev.00063.2017

Qi, Y., Takahashi, N., Hileman, S. M., Patel, H. R., Berg, A. H., Pajvani, U. B., et al. (2004). Adiponectin acts in the brain to decrease body weight. *Nat. Med.* 10, 524–529. doi: 10.1038/nm1029

Quaresma, P. G., Reencober, N., Zanotto, T. M., Santos, A. C., Weissmann, L., de Matos, A. H., et al. (2016). Pioglitazone treatment increases food intake and decreases energy expenditure partially via hypothalamic adiponectin/adipoR1/AMPK pathway. *Int. J. Obes.* 40, 138–146. doi: 10.1038/ijo.2015.134

Rice, S., Christoforidis, N., Gadd, C., Nikolaou, D., Seyani, L., Donaldson, A., et al. (2005). Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. *Hum. Reprod.* 20, 373–381. doi: 10.1093/humrep/deh609

Rodrigues, A. R., Salazar, M. J., Rocha-Rodrigues, S., Gonçalves, I. O., Cruz, C., Neves, D., et al. (2019). Peripherally administered melanocortins induce mice fat browning and prevent obesity. *Int. J. Obes.* 43, 1058–1069. doi: 10.1038/s41366-018-0155-5

Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H. F. (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *J. Biol. Chem.* 270, 26746–26749. doi: 10.1074/jbc.270.45.26746

Schwartz, M. W., Sipols, A. J., Marks, J. L., Sanacora, G., White, J. D., Scheurink, A., et al. (1992). Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* 130, 3608–3616. doi: 10.1210/endo.130.6.1597158

Shklyaev, S., Aslanidi, G., Tennant, M., Prima, V., Kohlbrenner, E., Kroutov, V., et al. (2003). Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14217–14222. doi: 10.1073/pnas.2333912100

Sipols, A. J., Baskin, D. G., and Schwartz, M. W. (1995). Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* 44, 147–151. doi: 10.2337/diab.44.2.147

Stener-Victorin, E., Padmanabhan, V., Walters, K. A., Campbell, R. E., Benrick, A., Giacobini, P., et al. (2020). Animal models to understand the etiology and pathophysiology of polycystic ovary syndrome. *Endocr. Rev.* 41:bnaa010.

Sull, J. W., Kim, H. J., Yun, J. E., Kim, G., Park, E. J., Kim, S., et al. (2009). Serum adiponectin is associated with family history of diabetes independently of obesity and insulin resistance in healthy Korean men and women. *Eur. J. Endocrinol.* 160, 39–43. doi: 10.1530/eje-08-0603

Sun, Z. G., Pi, Y. L., Zhang, J., Wang, M., Zou, J., and Wu, W. (2019). Effect of acupuncture at ST36 on motor cortical excitation and inhibition. *Brain Behav.* 9:e01370. doi: 10.1002/brb3.1370

Suyama, S., Lei, W., Kubota, N., Kadowaki, T., and Yada, T. (2017). Adiponectin at physiological level glucose-independently enhances inhibitory postsynaptic current onto NPY neurons in the hypothalamic arcuate nucleus. *Neuropeptides* 65, 1–9. doi: 10.1016/j.npep.2017.03.003

Suyama, S., Maekawa, F., Maejima, Y., Kubota, N., Kadowaki, T., and Yada, T. (2016). Glucose level determines excitatory or inhibitory effects of adiponectin on arcuate POMC neuron activity and feeding. *Sci. Rep.* 6:30796. doi: 10.1038/srep30796

Tan, G. D., Debard, C., Funahashi, T., Humphreys, S. M., Matsuzawa, Y., Frayn, K. N., et al. (2005). Changes in adiponectin receptor expression in muscle and adipose tissue of type 2 diabetic patients during rosiglitazone therapy. *Diabetologia* 48, 1585–1589. doi: 10.1007/s00125-005-1835-y

Tang, N., Zhang, X., Chen, D., and Li, Z. (2021). The controversial role of adiponectin in appetite regulation of animals. *Nutrients* 13:3387. doi: 10.3390/nu13103387

Thundyil, J., Pavlovski, D., Sobey, C. G., and Arumugam, T. V. (2012). Adiponectin receptor signalling in the brain. *Br. J. Pharmacol.* 165, 313–327. doi: 10.1111/j.1476-5381.2011.01560.x

Trotta, M., Bello, E. P., Alsina, R., Tavella, M. B., Ferrán, J. L., Rubinstein, M., et al. (2020). Hypothalamic Pomc expression restricted to GABAergic neurons suppresses Npy overexpression and restores food intake in obese mice. *Mol. Metab.* 37:100985. doi: 10.1016/j.molmet.2020.100985

Unlühizarci, K., Ozocak, M., Tanriverdi, F., Atmaca, H., and Keleştimur, F. (2007). Investigation of hypothalamo-pituitary-gonadal axis and glucose intolerance among the first-degree female relatives of women with polycystic ovary syndrome. *Fertil. Steril.* 87, 1377–1382. doi: 10.1016/j.fertnstert.2006.11.075

Wei, X., Yang, B., Chen, X., Wen, L., and Kan, J. (2021). Zanthoxylum alkylamides ameliorate protein metabolism in type 2 diabetes mellitus rats by regulating multiple signaling pathways. *Food Funct.* 12, 3740–3753. doi: 10.1039/d0fo02695f

Wu, H. T., Ou, H. Y., Hung, H. C., Su, Y. C., Lu, F. H., Wu, J. S., et al. (2016). A novel hepatokine, HFREP1, plays a crucial role in the development of insulin resistance and type 2 diabetes. *Diabetologia* 59, 1732–1742. doi: 10.1007/s00125-016-3991-7

Yadav, A., Kataria, M. A., Saini, V., and Yadav, A. (2013). Role of leptin and adiponectin in insulin resistance. *Clin. Chim. Acta* 417, 80–84. doi: 10.1016/j.cca.2012.12.007

Yamauchi, T., and Kadowaki, T. (2008). Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *Int. J. Obes.* 32, S13–S18. doi: 10.1038/ijo.2008.233

Yang, Y., Deng, P., Si, Y., Xu, H., Zhang, J., and Sun, H. (2022). Acupuncture at GV20 and ST36 improves the recovery of behavioral activity in rats subjected to cerebral ischemia/reperfusion injury. *Front. Behav. Neurosci.* 16:909512. doi: 10.3389/fnbeh.2022.909512

Yi, W., Xu, N. G., Sun, J., and Jia, Z. (2007). Effects of acupuncture on serum insulin antibody and tumor necrosis factor alpha in the experimental rat with insulin resistance. *Zhongguo Zhen Jiu* 27, 525–527.

Yu, M., Wu, S., Gong, C., and Chen, L. (2022). Neuregulin-1β increases glucose uptake and promotes GLUT4 translocation in palmitate-treated C2C12 myotubes by activating PI3K/AKT signaling pathway. *Front. Pharmacol.* 13:1066279. doi: 10.3389/fphar.2022.1066279

Zeltser, L. M., Seeley, R. J., and Tschöp, M. H. (2012). Synaptic plasticity in neuronal circuits regulating energy balance. *Nat. Neurosci.* 15, 1336–1342. doi: 10.1038/nn.3219

Zhang, F., Ma, D., Zhao, W., Wang, D., Liu, T., Liu, Y., et al. (2020). Obesity-induced overexpression of miR-802 impairs insulin transcription and secretion. *Nat. Commun.* 11:1822. doi: 10.1038/s41467-020-15529-w

Zhang, Y., Li, J., Wei, D., Mo, G., Yu, C., Wang, L., et al. (2020). Genome-wide regulation of electroacupuncture and treadmill exercise on diet-induced obese rats. *Evid. Based Complement. Alternat. Med.* 2020:8764507. doi: 10.1155/2020/8764507

Zinman, B., Gerich, J., Buse, J. B., Lewin, A., Schwartz, S., Raskin, P., et al. (2009). Efficacy and safety of the human glucagon-like peptide-1 analog liraglutide in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD-4 Met+TZD). *Diabetes Care* 32, 1224–1230. doi: 10.2337/dc08-2124

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New insights into acupuncture techniques for poststroke spasticity

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With the trend of aging population getting more obvious, stroke has already been a major public health problem worldwide. As a main disabling motor impairment after stroke, spasticity has unexpected negative impacts on the quality of life and social participation in patients. Moreover, it brings heavy economic burden to the family and society. Previous researches indicated that abnormality of neural modulation and muscle property corelates with the pathogenesis of poststroke spasticity (PSS). So far, there still lacks golden standardized treatment regimen for PSS; furthermore, certain potential adverse-events of the mainstream therapy, for example, drug-induced generalized muscle weakness or high risk related surgery somehow decrease patient preference and compliance, which brings challenges to disease treatment and follow-up care. As an essential non-pharmacological therapy, acupuncture has long been used for PSS in China and shows favorable effects on improvements of spastic hypertonia and motor function. Notably, previous studies focused mainly on the research of antispastic acupoints. In comparison, few studies lay special stress on the other significant factor impacting on acupuncture efficacy, that is acupuncture technique. Based on current evidences from the clinic and laboratory, we will discuss certain new insights into acupuncture technique, in particular the antispastic needling technique, for PSS management in light of its potential effects on central modulations as well as peripheral adjustments, and attempt to provide some suggestions for future studies with respect to the intervention timing and course, application of acupuncture techniques, acupoint selection, predictive and aggravating factors of PSS, aiming at optimization of antispastic acupuncture regimen and improvement of quality of life in stroke patients. More innovations including rigorous study design, valid objective assessments for spasticity, and related experimental studies are worthy to be expected in the years ahead.

KEYWORDS

stroke, spasticity, motor dysfunction, acupuncture technique, rehabilitation, non-pharmacological intervention, review, clinical and experimental evidences

Introduction

Stroke is a critical public health problem worldwide and is the leading cause of death in China (1). As the most common complication poststroke, spasticity is reconsidered as velocity- and muscle length- dependent increase in resistance to externally imposed muscle stretch, which can be explained by hyperexcitable descending excitatory brainstem pathways and secondary exaggerated stretch reflex responses (2). The prevalence of poststroke spasticity (PSS) ranges from 17% to 43% during the first year, and approximately two-thirds of patients with spasticity have both upper and lower extremities involvement (3, 4). After lesions to the cerebral cortex and its descending pathway (cortico-spinal tract, CST), weakness occurs immediately, while spasticity emerges and develops later (2). Spasticity interacts with weakness, resulting in disabling motor impairments and complex complications like muscle contracture, motor dysfunction and spastic pain, which negatively impacts on patients' quality of life (5, 6). The direct costs of PSS patients are four times to those without spasticity, imposing heavy financial burdens to the family and society (7). Nowadays, a wide range of treatment options are available, including oral drugs, physical techniques, botulinum toxin injection and surgery to target central and/or peripheral factors (8). However, certain unpredictable adverse-events such as drug-induced hepatorenal toxicity and generalized muscle weakness, and high risks related surgery still remain problematic (9–11). Therefore, a safer, effective and a more economical treatment modality is desired by both doctors and patients.

Acupuncture has long been used for treating PSS in China. The incidence of adverse-effects in this ancient art of healing technique is much lower than that of western medicine (12). As we know, there are two major factors determining the efficacy of acupuncture, one is acupoint selection, the other acupuncture technique. Apparently, majority of previous studies focused mainly on acupoint research, committed to finding out the most effective antispastic acupoint or acupoint combinations. For example, in views of traditional Chinese medicine (TCM) theory, acupoints either target to regulate disordered brain functions, such as scalp acupoints/lines (e.g., MS6-parietal anterior temporal oblique line, and MS7-parietal posterior temporal oblique line), governor vessel acupoints (e.g., GV20-*Baihui*, GV16-*Fengfu*, GV24-*Shenting*) and *Jiaji* acupoints (Ex-B2), or act directly on the spastic muscles (13–17). A very new systematic review that involves 88 RCTs with 6,431 patients in total has further confirmed that acupuncture possesses a reliable antispastic effect for stroke patients and summarized the most frequently used acupoints: LI4-*Hegu*, LI15-*Jianyu*, LI11-*Quchi*, SJ5-*Waiguan*, LI10-*Shousanli*, LU5-*Chize* and PC6-*Neiguan* for the upper limb, and GB34-*Yanglingquan*, ST36-*Zusanli*, SP6-*Sanyinjiao*, LR3-*Taichong*, SP10-*Xuehai*, SP9-*Yinlingquan* and ST41-*Jiexi* for the lower limb, which all locate at the muscle surrounding the affected joints (18). In comparison, research on antispastic acupuncture techniques is insufficient. In fact, specific acupuncture techniques applied on the acupoints are crucial for treatment outcome, which could be traced back to The Yellow Emperor's Inner Classic (19). Nowadays, the perception of applying suitable acupuncture technique for relevant diseases is widely accepted in the clinical settings by more acupuncturists than ever worldwide (20–22).

As a major motor impairment following stroke, spasticity usually leads to structural and functional changes in skeletal muscles such as shortening and stiffness of muscle fibers, and discordant relationship between the flexor and extensor muscles, which in turn exacerbates motor dysfunction (23). Thus, the aim of PSS treatment should take into consideration not only alleviation of the disordered muscle itself but its impact on motor function. Motion-style acupuncture (MSA) is a distinctive branch of multiple acupuncture techniques. It belongs to the compound acupuncture technique, which is characterized by active or passive body movement (motor training) during needle retention (24, 25). Thus, MSA is not equal to conventional acupuncture plus motor training. It has been recommended for treating muscle and tendon disorders, such as spasticity, weakness, muscle soreness and restricted joint movement (26). Although an increasing number of clinical studies have indicated that the overall therapeutic effects of commonly used MSA techniques including waggle needling, *Dongqi* needling (DN), Fu's subcutaneous needling (FSN) and motion-style scalp acupuncture (MSSA) are superior to conventional acupuncture/motor training alone, or their simple combination (27–35), few studies tried to unmask the possible mechanisms behind them. Based on the pathogenesis of PSS, combined with the characteristic of MSA techniques, certain new insights into acupuncture techniques for PSS in light of central and peripheral aspects will be discussed, with the purpose of optimizing antispastic acupuncture regimen and thereby helping with the improvement of patient's quality of life.

Understanding the plausible pathophysiology of PSS

In human, three important descending pathways are closely related to motor system, including CST originated from the cerebral cortex, and reticulospinal tract (RST) and vestibulospinal tract (VST) from the brain stem (36). Selective lesions to the CST only produce negative upper motor neuron (UMN) signs, such as weakness, hypotonia, and hyporeflexia, whereas the other two would cause positive consequences (e.g., spasticity) (6, 37). Although the pathogenesis of PSS has not been fully understood, mounting evidences indicate that spasticity is a result of changes in supraspinal origin, intraspinal network and peripheral muscle, leading to hyperexcitable stretch reflex (6).

First of all, supraspinal origin provides a balanced descending regulation for spinal stretch reflex circuit, which is predominantly mediated by RST and VST (38, 39). Specifically, the dorsal RST, receiving facilitation from the premotor cortex (PMC) and supplementary motor area (SMA) *via* corticoreticular pathway (CRP), descends paralleling with CST in the dorsolateral funiculus and wields a powerful inhibitory effect on stretch reflex (40). Conversely, the medial RST and VST, being independent from the contralateral motor cortex, in particular the anterior limb of the internal capsule with fibers from PMC, descend in the ventromedial cord and provide an excitatory effect (5, 41). Nevertheless, the contribution degree of VST to spasticity leaves a question open. It was shown that cutting off VST only caused transient alleviation of extensor tone of lower extremity in the spastic cat, however, spasticity markedly eased when the medial RST was broken (42). In stroke with cortical lesions, the medullary reticular inhibitory center, to a great degree, loses facilitatory inputs from the cortex and presents as weakened descending inhibitory effect. The medial RST, at this time, is in predominant state resulting in hyperexcitability of spinal stretch arc (5, 43).

Second, α-motoneuron (α-MN) hyperexcitability is deemed the abnormal intraspinal change in PSS (44). The reason is probably as follows: augmented sensitivity spindles increase peripheral afferent input to the spinal MN, setting the condition for oversensitivity of the stretch arc (39). Moreover, changes in intrinsic properties of the spinal MN lead to weakened presynaptic and reciprocal inhibition that enable the MN to spontaneously discharge, and ultimately decrease reflex threshold (39, 45). It is likely that resultant α-MN hyperexcitability is a plastic reorganization following lopsided supraspinal descending inputs to the spinal network (6). Bernice et al. has found that within limited temporal window poststroke, both brain and spinal cord initiate enhanced structural reorganization depending on the degree of cortical insult, which further promotes functional recovery (46).

Likewise, levels or expressions of certain neurotransmitters in the central nervous system (CNS) have long been elucidated crucial in the pathophysiology of spasticity as well. These neurotransmitters could be basically divided into two categories: one is inhibitory neurotransmitters including γ-aminobutyric acid (GABA) and glycine (Gly), the other excitatory ones like glutamate (Glu) and aspartate (Asp) (47). Normally, neurotransmitters bond to their receptors to modulate monosynaptic and poly synaptic reflexes in spinal level and mutually maintain the balance between excitation and inhibition of neurons (48, 49). However, once the balance is disrupted, i.e., either increased release of excitatory neurotransmitters or lessened activity of inhibitory inputs, spasticity occurs or aggravates (47, 48, 50). In addition, few studies reported that the inflammatory response is also involved in the pathogenesis behind PSS that we will discuss later (51, 52).

Last but not least, it must be stressed that PSS is a complex clinical phenomenon, which is not only considered as a neurologic problem but an indication of muscle property alteration. The alteration, for example, includes increased proportion of connective tissues, decreased amount and shortened length of sarcomeres (41). When a paralyzed muscle is held in an abnormally shortened position, it would lose sarcomeres to adjust its length and thus potentiate contracture, which in turn aggravates spasticity (5). Besides, researchers found that there is a strong connection between increased proportion of type I muscle fibers and muscle hypertonia in spasticity patients (53). To sum up, hyperexcitability of the medial RST tends to be the key mechanism, while changes in intraspinal network processing and peripheral muscle property are secondary factors that contribute to the development of PSS (5, 6, 43; Figure 1A).

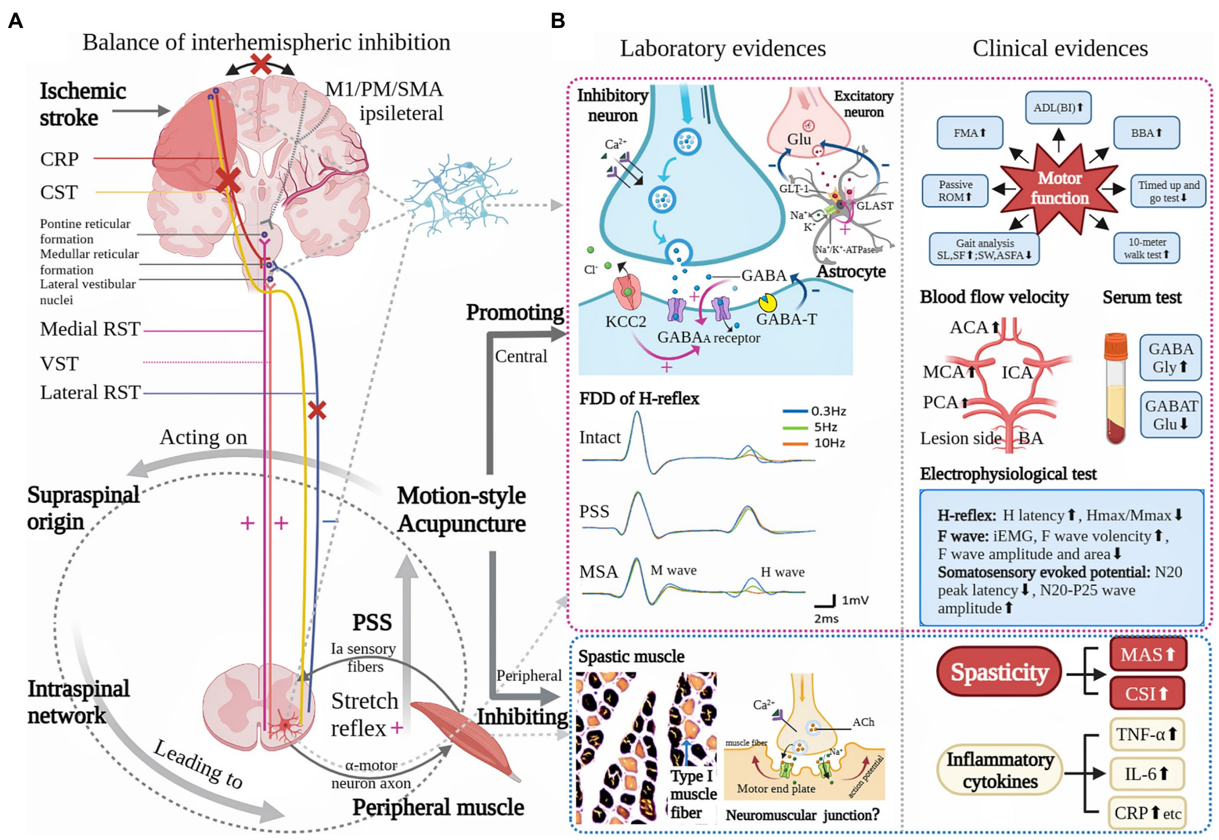

FIGURE 1
Underlying pathophysiology of poststroke spasticity (PSS) and role of motion-style acupuncture (MSA) for PSS. **(A)** is modified from Li et al. (2) showing that the possible primary mechanism for a loss of inhibitory control after a stroke is an UMN lesion while the secondary factors are altered intraspinal network processing and peripheral muscular changes. **(B)** Based on current clinical and laboratory evidences, the possible role of MSA for PSS is mainly reflected in two aspects: one is its modulation on the CNS, the other peripheral muscle. Central modulation presents relative neuroprotective effect (improvement of blood perfusion and inhibition of inflammatory response), regulation of balance between excitatory and inhibitory neurotransmitters, functional improvement of sensorimotor pathway, and inhibition of spinal stretch reflex. Peripheral muscle adjustment includes decreased proportion of type 1 muscle fiber. ACA: anterior cerebral artery, Ach: acetylcholine, ADL: activity of daily life, ASFA: affected side foot angle, BA: basilar artery, BBA: Brunel balance assessment, BI: Barthel Index, CRP: C-reactive protein, CSI: clinical spasticity index, CST: cerebral-spinal tract, FDD: frequency-dependent depression, FMA: Fugl-Meyer assessment, GABA: γ-aminobutyric acid, GABAA: γ-aminobutyric acid subtype A, GABAT: γ-aminobutyric acid transaminase, GLAST: glutamate aspartate transporter, GLT-1: Glutamate transporter-1, Glu: glutamate, Gly: glycine, ICA: internal cerebral artery, iEMG: integrated electromyography, IL-6: interleukin-6, KCC2: K(+)-Cl(–) co-transporter, M1: primary motor cortex, MAS: modified Ashworth scale, MCA: middle cerebral artery, PCA: posterior cerebral artery, PM: premotor cortex, ROM: range of motion, RST: reticulospinal tract, SF: slide frequency, SL: slide length, SMA: supplementary motor area, SW: slide width, TNF-α: Tumor necrosis factor-α, VST: vestibular spinal tract. (+): excitatory, (–): inhibitory, (x): impaired, (⬆): increased, (⬇): decreased.

Characteristics of commonly used MSA techniques

As mentioned before, waggle needling, DN, FSN as well as MSSA all highlight effective integration of acupuncture technique and rehabilitation, that is cooperating with motor training during needle retention. Because of this, MSA treatment not only promotes positive interaction between acupuncturists and patients, but creates conditions for those with neuromuscular retraining, which helps to improve patient compliance and treatment outcomes (24, 26). Nevertheless, each of MSA techniques has its own characteristic. For example, as for needling sensation, waggle needling, DN and MSSA are supposed to evoke relatively strong yet tolerable needling sensation; however, not in FSN. The principle of acupoint selection varies among them as well, which could be preliminarily classified as local and remote point selection. Waggle needling and FSN are always applied on the points in the affected areas, e.g., some classic acupoints closely related to tendons (like GB34, SP9, ST41, LI11 and LI15) (28) and the most significant tender point (positive reaction point) (54–56). However, the exact point of needle insertion is quite different. In waggle needling, the tender point should be needled at its exact location, while in FSN, 2–3 *cun* (1 cun = 3.33 cm) superior or inferior towards it. Given that different needling directions towards the tender point would not influence therapeutic effects of FSN (57), thus, the point selection of FSN is more flexible and adjustable than the waggle needling. In contrast, the stimulating points of DN and MSSA are far away from the affected muscles and tendons, named remote point selection. The primary distal acupoints (*Tung's* extraordinary points) on the healthy side (58) and specific areas or lines (MS6, MS7) on the scalp (59) are the main needling target for DN and MSSA respectively, which differs considerably from the traditional acupuncture theory. It has to point out that long-durational needle retention can be realized in both MSSA and FSN, particularly the latter, for up to 24–48 h, attributing to superficial needling depth that has no impact on deep muscle layers and limb exercise, which may increase the total amount of stimulation and contribute to long-term therapeutic effect (32, 57). Detailed characteristics among these MSA techniques are summarized in Table 1.

Evidences from clinical studies

MSA techniques alleviate muscle tone and improve motor functions in PSS patients

In the real clinical setting, except for spasticity, stroke patients may suffer from additional complications, for instance, motor dysfunctions manifesting as low task-oriented executive ability, joint immobilization, abnormal posture and spastic muscle pain. Therefore, employing combined approaches rather than conventional monotherapy to counteract those complex conditions has aroused wide attention among doctors and researchers. Loads of clinical studies have shown that the antispastic effect of MSA is superior to routine needling or rehabilitation alone. In one RCT, 121 patients with PSS were randomly sorted into control group by routine needling and treatment group by waggle needling. All these patients were given similar usual care, including neurotrophic supplement, anti-infection, blood pressure control, microcirculation improvement, and so on. Although both groups showed positive effects in spastic hemiplegia after 2-weeks of intervention, shown as decreased modified Ashworth scale (MAS, the most commonly used scale for spasticity assessment) score but increased Fugl-Meyer assessment (FMA, to evaluate overall motor function) and Barthel Index (BI, to evaluate activities of daily living) score, inter group comparison displayed that the overall effect of waggle needling is better than routine needling ($p < 0.05$) (28). Another study (34) conducted a RCT on 140 PSS patients, comparing MSSA treatment ($n = 70$) with intelligent upper-limb feedback training alone ($n = 70$). The results showed that after 8-weeks treatment, the spasticity degree of the elbow (69.07 ± 9.39) and wrist (33.04 ± 7.33) joints in the MSSA group were significantly lower than that in the rehabilitation training group (80.65 ± 7.98 and 35.91 ± 7.50, respectively). Besides, the overall treatment outcomes (MAS, FMA, BI) and long-term efficacy of MSSA is much superior to rehabilitation training alone after 1-month follow-up. Ge et al. (58) observed similar outcomes. Sixty PSS patients were randomly assigned into DN group ($n = 30$) and routine needling group ($n = 30$). The MAS, FMA, BI, balance function (Brunel balance assessment, BBA), walking ability including timed up and go test (TUG) and 10-meters walking speed test, and stroke specific quality of life scale before and 4-weeks after intervention were measured. The results showed that while the above outcome measures significantly improved in both groups ($p < 0.05$), the improvements of motor function of lower limbs in DN group (BBA: 40.19 ± 5.21, TUG: 21.77 ± 2.65, 10-meters walking speed: 48.22 ± 5.37) are better than those in the routine needling group (27.56 ± 2.73, 28.91 ± 3.50, 39.05 ± 4.53, respectively) (all $p < 0.01$). On top of its remarkable effects on meaningful reduction in the degree of spasticity with improvements in motor function and quality of life in patients, FSN owns superiority in immediate pain relieving in comparison with other three MSA techniques, possibly thanks to its effect on directly loosening tissue adhesion by swaying manipulation (32, 55, 61).

Recently, a meta-analysis involving 31 RCTs with total 2,488 PSS patients indicated that a range of outcome measures including marked efficiency, MAS classification, MAS score, and clinical spasticity index (CSI) score, in electroacupuncture plus rehabilitation training group were all statistically better than those in electroacupuncture or rehabilitation training alone groups (67), inspiring us that combination of different therapies (e.g., MSA plus professional rehabilitation training) may maximize spasticity management. Song et al. (62) observed the effect of combination therapy versus monotherapy. One hundred and twenty PSS patients were randomly distributed to rehabilitation group ($n = 40$), FSN group ($n = 40$) and FSN plus rehabilitation group ($n = 40$). The final outcomes showed that the muscle tone (MAS), hand motor function (FMA) and range of motion (ROM) of wrist joint were all significantly improved among the three groups ($p < 0.05$), however, the overall effects of combination group are far better than those of FSN or rehabilitation alone group ($p < 0.05$). No statistical differences existed between the FSN and rehabilitation group ($p > 0.05$). Similar outcomes were also shown in other two studies that antispastic effect of combination group (DN + rehabilitation) is superior to DN treatment or rehabilitation alone (30, 31). In addition, spasticity is reported to become worse in cold (6), which is consistent with the TCM theory that cold pathogen could contract muscles, obstruct meridians and thereby aggravate symptoms. Wang et al. observed therapeutic effects of combination therapy (waggle needling with moxibustion) for PSS. The results

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TABLE 1 Characteristics among the commonly used MSA techniques.

References	Similarities				Differences			
	Style of acupuncture	Clinical indications	Instrument	Area of needle stimulation	Principle of acupoint-selection: the main points	Needling sensation	Long needle-retention	Manipulation characteristics
Waggle Needling (28, 29, 60)	Motion-style acupuncture is unique in that effective and synchronous combination of acupuncture with body movement. Simultaneous activation of sensory and motor conduction pathways favors reconstruction of the right neural feedback mechanism and improvement of motor ability	Neurological and muscular diseases (e.g., spastic hypertonia, contracture, joint immobilization, muscle stiffness and pain)	Filiform needle	A greatest area with muscle tissues at multiple layers in a three - dimensional area around the stimulated point	Local point-selection: Points around the joint (e.g., LI15, LI11, SJ5, LI14 for the upper limb; GB34, SP9, SP10, ST36, ST41, SP6 for low limb) or the tender point, mainly on the affected side	Strong yet tolerable	No (For about 30 min)	Penetrating from the skin to the muscle layers followed by lifting-thrusting methods in multi-directional angles
Fu's subcutaneous needling (61–63)			Fu's needle	A greater area with subcutaneous tissues in a fan-shaped area outwards from the inserting point	Local point-selection: 2–3 *cun* superior or inferior to the most significant tender point, mainly on the affected side	None-requirement	Yes (Up to 24–48 h)	Puncturing subcutaneously followed by swaying method, with a long-period of soft tube retention
Dongqi needling (30, 31, 58)			Filiform needle	A limited area with the muscle tissues in the longitudinal area at the stimulated points and its deep tissues	Remote point-selection: *Tung's* extraordinary acupoints [e.g., T 77.18 (bilaterally) and T 22.05, T 22.04, T 77.07 on heathy side]	Strong yet tolerable	No (For about 30 min)	Penetrating from the skin to the muscle layers with twirling or lifting-thrusting methods
Motion-style scalp acupuncture (64–66)			Filiform needle	A limited area with the tissue in the transverse area at the stimulated points and its relatively shallow tissues	Remote point-selection: the anterior (MS6) and posterior (MS7) oblique lines of the vertex-temporal on the affected side	Strong yet tolerable	No (For about 30 min)	Penetrating from the skin to the epicranial aponeurosis followed by quick twirling method for about 200 rpm/min

showed that the combination group presented higher integrated electromyography (iEMG) values and F wave velocity in both biceps and triceps, but lower F wave amplitude and area than the waggle needling group, indicating alleviation of spasticity and increasement of muscle strength (56). This study enlightens us that keeping warm during MSA intervention may help relax muscles of patients easily affected by cold temperature and achieve better outcomes.

To date, mounting studies have indicated that the order of treatment or timing of combination of different therapies, such as synchronous or asynchronous application of acupuncture and rehabilitation, may bring different prognostic outcomes to patients (35, 64, 65). For instance, a RCT by Qi et al. (68) has shown that after 6-months treatment, muscle hypertonia and motor functions were all substantially improved among three treatment groups (rehabilitation alone, rehabilitation after scalp acupuncture and MSSA groups) in comparison with before treatment. And compared to the other two groups, MSSA possessed preferable therapeutic effects ($p < 0.05$). Zhang et al. (33) found that the therapeutic effect of conventional scalp acupuncture plus rehabilitation is better than scalp acupuncture alone, but inferior to synchronous combination of scalp acupuncture and rehabilitation (MSSA). The efficacy is reflected in ameliorating patients' gait parameters, including increased stride frequency and stride length and lowered foot angle of the affected side, and increased passive ROM of joints (the hip, knee and ankle). Some researchers held the opinion that in the process of neuroplasticity, active and proper rehabilitation inducement is particularly essential to avoid establishment of abnormal movement pattern and unexpected motor compensation. In MSA, the sensory and motor conduction pathways can be activated simultaneously, owning to the dual of acupuncture manipulation and active/passive body movement, which conduces to restoration of right neural feedback mechanism and reconstruction of motor ability (34, 66, 68). Taken together, results from these clinical trials (although still limited) imply that MSA is a promising and effective therapy for patients with PSS.

Evidences from animal studies

MSA techniques impact on behavioral performances in rats with PSS

In contrast to the wealth of clinical studies, comparatively fewer experiments exist exploring the effect of and underlying mechanism of MSA techniques against PSS in rodent animals. Currently, the most popular PSS model of rats is established through middle cerebral artery occlusion (MCAO) surgery, featuring by high reliability and good reproducibility (69). Our previous studies employed Zea Longa score and MAS to evaluate neurological deficit and muscle tone in the model rats, separately (70–73). At the very beginning, all rats scored 0 in Zea Longa and MAS before MCAO, indicating there is no neurological deficit and no spastic hypertonia. Three days after modeling, Zea Longa score was still 0 in the normal and sham-operated groups. Meanwhile, the score in other modeled groups (model, waggle needling, routine needling, and Baclofen groups) were all statistically higher than the sham-operated group (all $p < 0.01$), indicating the neurological deficit was successfully induced. After 7-consecutive-days intervention, Zea Longa score has obviously decreased in the three intervention groups ($p < 0.01$ or $p < 0.05$) in comparison with the model group. And waggle needling showed similar therapeutic effect on alleviation of neurological deficit as Baclofen does ($p > 0.05$). The MAS score normally increases 3 days after MCAO, maintains at a high level within 9 days, and decreases gradually later, which has been proved by previous animal models (69). In order to exclude the influence of animal self-recovery on the experimental outcomes, we started the intervention at day three after modeling and terminated the experiment at day nine. The results showed that acupuncture, in particular waggle needling, significantly decreased MAS score when compared to the model group ($p < 0.05$). These data confirmed that waggle needling wields preferable effects on neurological deficit and spastic hypertonia. As for the underlying mechanisms, they are possibly related to microcirculation improvement of peri-infarct areas, inhibition of inflammatory responses, and alterations of neurotransmitters, spinal reflex as well as muscle property, which will be discussed in the following part.

In addition, efforts have been made to quantify the behavioral deficits in animals with some indirect assessments like screen test and gait analysis. We found that rats suffered from motor dysfunctions immediately after MCAO and manifested with weakness and imbalance, which might be caused by damage to the cerebral cortex and CST. The screen test scored five in the sham-operated group but only about two in the modeled groups 1 day following surgery. With acupuncture intervention, motor function was obviously elevated at day seven and day nine in the waggle needling group, compared with the model group (all $p < 0.01$). No statistical differences existed between routine needling and model groups ($p > 0.05$) (71). An experiment conducted by Mu et al. (74) observed hindlimb balance and state of gait movement in rats with PSS. The PH-200 ft. balance tester showed that rats in the sham-operated group showed no changes in the static weight ratio throughout the experiment, however, the ratio in model, waggle needling and placebo needling groups obviously decreased on the day three after MCAO. Following 7-treatment-days, the ratio was effectively augmented by waggle needling compared to the model group ($p < 0.001$). Likewise, before treatment, significant enlargements of swing, stance, step angle and stance width but reductions of peak paw area and stride length were shown in the model, waggle needling and placebo needling groups when compared to the sham-operated (all $p < 0.01$). Interestingly, this situation was significantly reversed by waggle needling after treatment, indicating that waggle needling possesses the ability to facilitate motor function recovery. Recently, a study has proved that spastic behavior of rat was consistent and reproducible during swimming test (75), such a behavioral test is desired to bring more convinced evidences to the antispastic effect of MSA techniques.

Modern insights of MSA techniques for PSS

Inspiration for MSA techniques in light of facilitating central neuroplasticity and neuromodulation

Neuroplasticity is defined as the ability of neurons and circuits to modify their functional activity and the synaptic reconstruction in accordance with variations in activity (16). Enhancement of neuroplastic activity generally exerts a positive effect on motor recovery. After a stroke, neuroplasticity initiates immediately in bilateral cortices, which could be explained by structural change and regeneration, cortical reorganization (neural excitability, CST integrity and intracortical excitability) and molecular biology changes (76–79).

Impaired nerve cells in the penumbra surrounding the ischemic core could be saved only if the blood perfusion is timely restored (80). Ischemic insult triggers angiogenesis to support penumbra, and induces neurogenesis and synaptogenesis in the perilesional cortex (81, 82). Restoration of blood perfusion to the ischemic areas was therefore considered as a vital therapeutic target for rehabilitation outcomes (16). Both clinical and experimental studies have proved the satisfying effects of MSA techniques (DN, waggle needling) on microcirculation improvements, including promoting mean blood flow velocity of posterior cerebral artery (PCA), middle cerebral artery (MCA) as well as anterior cerebral artery (ACA) in patients with PSS (58), and reducing cerebral infarct volumes in the model rats (70–72, 74). Additionally, there is a study indicating that inflammatory response during ischemic process can induce a cascade of negative consequences, for instance, promotion of leukocyte infiltration and obstruction of capillary blood flow, which in turn aggravates the extent of ischemic areas and cell necrosis (52). Early acupuncture intervention, in particular MSA (waggle needling), could effectively inhibit the expression of inflammatory markers (e.g., Tumor necrosis factor-α, interleukin-6 and C-reactive protein) in the serum in patients with PSS, which favors the improvement of regeneration and functional recovery of CNS (52, 60). These outcomes can mean that MSA possesses relative neuroprotective effects on ischemic stroke. Relevant studies have declared that certain neuroprotective factors such as brain-derived neurotrophic factor, postsynaptic density protein 95, synaptophysin favor neurogenesis, synaptic plasticity (83); and the active neural plasticity in the perilesional cortex may create opportunity for new synaptic connection (e.g., CRP), which contributes to regaining the facilitation from the contralateral PMC to the medullar inhibitory center and thereby restores downward inhibition to the stretch reflex.

It was reported that activation of contralateral hemisphere is greater than that of the ipsilateral in the subacute stage so as to compensate for severely damaged motor control; while the activation will shift back to the ipsilateral that facilitates recovery of voluntary control on the paretic side in the chronic stage (79, 84, 85). For treating stroke patients suffering from motor deficits, scalp acupuncture could markedly activate motor cortex, strengthen the activities of the brain regions related to sensory integration and motor coordination, enhance bilateral frontal lobe motor control and thereby conduce to the cooperation of bilateral sensorimotor networks and the balance between inter-hemisphere (86–89). After stroke, mountains of neurons and nerve fibers are damaged, directly causing sensory and motor deficits with abnormal somatosensory evoked potential (SEP) responses that mainly presents prolonged relative peak latency and lowered wave amplitude. Interestingly, recent studies have indicated that the N20 peak latency of the median and tibial nerves were obviously shortened, while the N20-P25 wave amplitude of these two nerves were increased in MSA group (motion-style scalp acupuncture) as compared to conventional scalp acupuncture plus rehabilitation group or scalp acupuncture group (64, 66).

Disequilibrium of neurotransmitter adjustment such as overexpression of excitatory neurotransmitter (like Glu) and/or insufficiency of inhibitory neurotransmitters (GABA, Gly) was found in the biomolecular mechanisms underlying PSS in both human and animal studies. Clinical results showed that in comparison with routine needling alone, spastic hypertonia and motor dysfunction were significantly alleviated in MSA (DN, MSSA or waggle needling) group, accompanying by suppression of Glu, and elevations of GABA and Gly in serum in patients with PSS (58, 60, 66). Animal studies also confirmed that the antispastic effect of waggle needling was superior to routine needling and equivalent to Baclofen (70–73). Underlying mechanisms could be explained as follows: In the perilesional cortex, the expression/activity of GABA was enhanced but GABAT (a key metabolism enzyme of GABA) was inhibited. In addition, expressions of $GABA_A\gamma 2$ receptor and KCC2 (a chloride extruder that maintains the inhibitory effect of GABA) were all elevated among the cerebral cortex, brain stem and lumber spine of PSS rats. Furthermore, downregulations of Na^+/K^+-ATPase and Glu transporters (EAATs) such as EAAT1 (GLAST) and EAAT2 (GLT-1), and upregulation of Glu in the hippocampus were effectively reversed by waggle needling. Such experiments are needed to help us understand the biochemical mechanisms behind MSA techniques for PSS.

MSA techniques contribute to the improvement of peripheral muscular conditions

The possible primary mechanism for a loss of inhibitory control after a stroke is an UMN lesion while the secondary factors are altered intraspinal network processing and peripheral muscular changes, which can both result in PSS (6). These imbalanced inputs from the supraspinal levels partly lead to α-MN hyperexcitability, subthreshold or spontaneous discharging of motor units, associated with spastic hypertonia and muscle contraction (6). Moreover, peripheral muscular conditions have changed as well, including muscle fiber shortening and stiffness, loss of sarcomeres in series, connective tissue adhesion, and increased proportion of type I muscle fibers, which additionally exacerbate joint immobilization and motor dysfunction in patients (53, 90–92). However, those conditions could be soothed with prolonged slow passive stretching, to prevent excessive muscle contracture and lessen spasticity-induced pain (10, 93).

According to the classical acupuncture theory, therapeutic effects of acupuncture are determined by acquired needling sensation (*deqi* in Chinese) (94, 95), and different types of stimulation normally bring about diverse clinical outcomes (96). It was shown that stroke patients suffering from spastic hemiplegia react especially well to a type of acupuncture with strong needle-stimulation (97). Effective needling manipulation, particularly like multi-directional lifting-thrusting method in waggle needling, or swaying method in FSN, enables increased changes in local blood perfusion and soft-tissue displacement, delivering mechanical signals into the subcutaneous tissue, inducing the release of pain-related substances and consequently triggering the nerve-immune-secretion network to relieve spastic muscle pain (94, 98). Modern research shows that immediate alleviation of spasticity and increase of active ROM in PSS patients are positively influenced by quick lifting-thrusting needling method at trigger point, which correlated with decreased frequency of motor unit spontaneous discharging (99). H-reflex is a monosynaptic reflex triggered by activation of Ia afferents, MN and muscle fibers, and is extensively used as a valid tool to quantify the excitability of the MN (100). Certain parameters of H-reflex changed in patients with PSS, such as shortened H-reflex latency, amplified Hmax/Mmax ratio. Animal research also demonstrated that motor threshold, frequency-dependent depression (FDD) of H-reflex were all increased, indicating that occurrences of hyperreflexia and spastic hypertonia correlated with hyperexcitability of MN. While those conditions could be effectively reversed by MSA techniques (63, 74). Besides, the proportion of type I muscle fibers in the spastic gastrocnemius muscle is markedly declined after waggle needling intervention in comparison with that of routine needling group ($p < 0.05$) (72). As reported, the direct action of lifting-thrusting needling method on peripheral muscular tissues (e.g., neuromuscular junction and muscle fibers) possibly correlates with breaking down of muscle fibers and temporary depletion of acetylcholine neurotransmitters (101). Whether those mechanisms are involved in the antispastic effect of MSA (especially waggle needling and FSN that directly act on spastic muscles) remains to be further investigated. To sum up, to improve the intrinsic structure of spastic muscles and to attenuate over-excitation of the spinal reflex circuit may serve as meaningful therapeutic targets for MSA against PSS. Such electrophysiological studies will help to provide more objective evidences in showing the effects of different MSA techniques on peripheral muscular conditions. Relevant evidences are presented in Figure 1B.

Discussion and expectation

Given that both clinical and experimental evidences (although still limited) has confirmed the preferable antispastic effect of MSA techniques, also seeing that at present there still lacks standardized regimen for PSS management, MSA deserves to be considered as a part of comprehensive therapeutic protocol for stroke patients who suffer from spasticity, with its merits of preferable effect, few acupoint-selection as well as affordable price, and importantly, attributable to its potentially regulatory effects on the CNS and peripheral muscles. However, it has to be mentioned that MSA techniques involved in previous studies are normally based on TCM theory or personal experiences. In views of TCM, obstruction of meridians, *qi* and blood stagnation, or poor nourishment of muscles and tendons after stroke consequently led to edema, pain, numbness, stiffness and disadvantageous movement of distal limbs. Intensive amount of needle stimulation (e.g., relatively strong but tolerable stimulation intensity, large stimulation area or relatively long-durational needle retention) integrated with tailored motor training determines that MSA plays a meaningful role in accelerating the flow of *qi* and blood, unblocking meridians and easing muscles in PSS patients (50). However, the intervention timing and course, acupoint-selection, and exercise mode are different among them, limiting the curative efficacy and credibility of MSA studies to some extent. To further optimize treatment protocol of MSA for PSS, certain insights based on the practice implications are provided from the following aspects.

Intervention timing and course

Proper intervention within optimal time window could facilitate neuroplasticity, and to a great extent, avoid maladaptive neuroplasticity (spasticity). Neuroplasticity begins within hours after ischemia, peaks at 7–14 days poststroke in rodent animals (102), while it peaks within 3 months poststroke in human beings (79). It has been found that in animals, a very early exercise within 24 h poststroke results in overexpressed inflammatory responses (103). Conversely, rehabilitation commencing later (24–72 h poststroke), is favorable to suppressions of ischemic volumes (82), inflammations (104) and apoptosis (105), yet promotion of neurogenesis (83). Similar outcomes are observed in human beings that exercise less than 24 h after stroke is related to infarct expansion (106), but within 72 h is considered beneficial (107). Considering that early acupuncture intervention could induce significant ischemia tolerance (108, 109), and the intensity of motor training (active/passive body movement-based) in MSA is relatively moderate, on the basis of internal medicine care that helps to open the obstructed blood vessels timely (e.g., application of recombinant tissue-type plasminogen activator, t-PA) (110), it might be a feasible choice to apply MSA as early as possible (after hyperacute phase, ≥24 h), and to insist on it at least 2 to 3 months as most of previous MSA studies did (33, 34, 54, 65, 66), for stroke patients to actively combat ischemic injury and facilitate neuroplasticity.

Selection and application of MSA techniques

Previous research has demonstrated that pathogenesis of spasticity in the early subacute phase (> 7 days–< 3 months) after stroke could be explained by neural changes; whereas, as time goes by, during the late subacute to chronic stages (≥3 months-2 years), it might link with intrinsic muscle alterations (4). A factorial study supported the view that both "needling stimulation" and "acupoint selection" contribute to the acupuncture efficacy (97). Although there is insufficient scientific evidence of high-quality suggesting that needling technique wields more impacts on the clinical result, some experts have argued that it is more important to perform suitable acupuncture techniques than to select exactly the right acupoint with routine needling technique (111). Therefore, the choice of needling technique should also be flexible for spasticity treatment at different poststroke phases. In light of previous evidences from the clinic to the laboratory, also taking needling features of MSA techniques into consideration, an auxiliary MSA treatment protocol for PSS is designed as follows.

Early after a stroke (in the hyperacute and acute phases), before spasticity forms, treatment should focus mainly on promoting neuroplasticity and avoiding maladaptive neural reorganization. Given that scalp acupuncture tends to enhance cerebral blood reperfusion and facilitate integration and cooperation of sensorimotor cortex network (86, 112, 113), and also considering that the contralateral needling (in healthy side) in DN technique might activate the uncrossed CST to compensate for contralateral CST, which is based on symmetry constraint (114), MSSA and DN techniques are therefore recommended in the early poststroke stage, in light of facilitating neuroplasticity and neuromodulation. Over time, stroke patients, without effective intervention, would undergo a series of motor impairments, such as synergic movement pattern and different degrees of spasticity. At this time, not only the CNS lesions should be considered, but the changes in the intrinsic muscle properties should be paid attention to. As connective tissue adhesion and increased proportion of type I muscle fibers potentiate the degree of spastic hypertonia (53, 92), applying waggle needling at disordered muscles (the tender point or tendon-related acupoints) with multi-directional lifting-thrusting method can be regarded as a good way to sooth spastic muscle and improve its structure (28, 29). Besides, except for directly acting on local areas as waggle needling does, FSN possesses the ability in immediate pain-relieving by loosening connective tissue adhesions with swaying method (54, 57, 61), which ultimately helps with restoration of mechanical alignment and improvement of motor performance.

Moreover, it is noteworthy that both hemodynamics and electrophysiology studies support the view that stroke patients who have received bilateral needling showed preferable effects as compared to unilateral needling (115–117). After treatment, the blood velocity of ACA, MCA, PCA or basilar artery was all strengthened among the unilateral needling, bilateral needling and conventional medicine groups ($p < 0.01$ or $p < 0.05$). Most importantly, the blood velocity of ACA, MCA and basilar artery (contralateral side) in the bilateral needling group was superior to unilateral needling group ($p < 0.05$). Likewise, bilateral needling group had more advantages in the improvement of SEP responses (e.g., reduction of latency, increasement of amplitude, reproduction of missing amplitude), hinting that neurons and fibers recruited during bioelectrical activity were increased, and so was bilateral cortical excitability to some extent. In addition, rehabilitation research demonstrated that bilateral hands rehabilitation led to decreased intracortical inhibition yet increased intracortical facilitation in stroke patients, while these alterations only occurred in the contralateral hemisphere in patients with unilateral hand training (118, 119). In line with these mechanisms of improvements after stroke, applying MSA bilaterally might be a noteworthy insight due to its better effects on enhancing neuroprotection and rebalancing of interhemispheric inhibition.

Learning from and integrating with modern rehabilitation strategy

In view of the fact that the exercise mode in most of MSA techniques is active/passive movement of the disordered limb, lacking of standardized rehabilitation strategy, to some extent, limits curative efficacy of MSA in real clinic settings. Because of the complexity and variability of PSS, apart from classic pharmacological modulations on CNS, advanced rehabilitation strategies targeting to improve peripheral muscle conditions (11, 120–122), for instance, biomechanical restoration *via* muscle stretching, improvement of motor control by body weight–supported treadmill training or robot-assisted training, enhancement of muscle strength by resistance training program or aquatic therapy, and improvement of endurance *via* treadmill exercise and circuit training, are worthy to be learned and implemented in the portion of motor training in MSA practice. Of course, the intensity of motor training should also depend on the patient's condition. For patients with mild to moderate motor impairments, active motor trainings such as weight–supported treadmill training or task-oriented training are particularly recommended during MSSA and FSN treatments, because transverse needling could ensure the range of movement as large as possible. On the contrary, passive motor trainings, e.g., muscle stretching, robot-assisted training, proprioceptive neuromuscular facilitation, are more suitable for those with severe neurological impairments that cannot perform tasks by themselves, and can also be combined with MSA techniques flexibly. Notably, stroke patients tend to manifest less endurance with easy fatigue (2). Thus, the frequency and intensity of needling manipulation and motor training should not be in a state of excess, that is, avoidance of a single excessive stimulation to insure against hyperalgesia (26, 123). Instead, with the suggestion that repeated needling manipulation and motor training at proper intervals during needle retention is taken into consideration along with achieving proper stimulation intensity and tolerance of patients. For safety reason, the patient's body position should be adjustable and comfortable throughout the whole treatment process to avoid acupuncture-related adverse-events such as bending or breaking of the needle, or fainting during treatment.

Predictive and aggravating factors of spasticity

The aim of PSS treatment should take into consideration not only alleviation of spastic hypertonia but its underlying impact on motor rehabilitation. Early positive treatment may not terminate PSS progress, but may greatly reduce the incidence of severe spasticity and prevent intractable complications (8, 124). Certain factors are reckoned as important and independent predictors of PSS: lesion location (especially in the brain stem), degree of neurological impairments (severe paresis and hemihypesthesia, National Institutes of Health Stroke Scale >2, Mini-Mental State Examination <27), functional limitations (Modified Rankin Scale >2, low BI), large infarct volumes, increased MAS score, younger age and stroke-related pain (8, 125). Thus, to know these possible PSS predictors and to solve these problems pointedly with certain neuroprotectants, e.g., calcium channel blockers, Glu antagonist, free radical scavenger, or anti-inflammation drugs are advantageous to reduce the stress response under brain pathological conditions, inhibit inflammatory responses, and promote neural regeneration and neural repair in acute ischemic stroke, which may create positive prerequisites for MSA techniques to achieve the best therapeutic outcomes (126, 127).

Besides, clinical observations have also indicated that spasticity changes with posture, temperature weather (worse in cold), pain and emotion (anxiety, anger) (6). Tailored and systematic TCM nursing protocol, for example, normal limb placement plus exercise nursing, body warm-keeping with moxibustion or hot compress therapy, pain care as well as emotional nursing care (128), are especially recommended to deal with up-mentioned aggravating factors. Meanwhile, given that poor nutritional status is common among stroke survivors that not only leads to muscle loss but negatively impacts on poststroke recovery (129), positive solution to malnutrition is therefore considered as a meaningful supplement for the conventional treatment.

Limitations and future research

Although acupuncture, in particular MSA techniques, is supported by a vast amount of clinical and experimental results in dealing with various motor impairments following stroke, however, there still exists certain limitations. Quite a few MSA studies are limited to relatively small sample sizes. In addition, other factors including methodological deficits in randomization, inconsistent assessment of spasticity (e.g., using different scales), subjective evaluations (clinical scale-based evaluations like MAS and FMA), incompatible inclusion/exclusion criteria (e.g., ischemic or hemorrhagic stroke types), differences in the time between stroke onset and assessment, differences in the intervention timing and course, acupoint-selection, comparatively simple exercise mode used in MSA techniques, absence of long-term follow-up, partly reduced the quality of the current MSA studies. Few studies were able to determine whether the observed effectiveness of MSA was due to placebo effects, the intensity of practitioner contact, or the physiologic effect of needling. In brief, although positive outcomes were found that the antispastic effects of MSA outdo conventional acupuncture alone, rigorous evidence-based meta-analysis is highly expected in the future, to provide acupuncturists with more powerful guidance.

For better verification of the efficacy of MSA techniques on PSS, we propose that controls such as routine needling and sham needling, should be designed in future clinical studies, including patient's self-report of needle sensation as well as objective measurements which may optimize clinical outcomes and improve patient adherence (130–132). Use of valid and reliable assessment tools, such as iEMG and shear wave elastography (133), could produce safe and timely evaluation on peripheral muscular changes. Functional magnetic resonance imaging (fMRI) (6, 86) and repetitive transcranial magnetic stimulation (rTMS) (134), could evaluate the excitability of individual brainstem nuclei and cortex in real-time respectively, and thereby provide direct evidences for neuromodulation mechanisms of MSA against PSS. Diffusion tensor imaging (DTI) can help us to observe the microstructural changes of fibers like CRP (40). In addition, the acoustic startle reflex (ASR), a brainstem-mediated reflex *via* RST, is also a good way to examine RST excitability non-invasively (135). Certain objective methods for PSS assessment and their prospective applications in MSA studies are exhibited in Figure 2.

Conclusion

With the trend of aging population worldwide getting more obvious, the incidence of stroke is increasingly high. Spasticity, as a major medical problem following stroke, usually leads to abnormal posture and movement patterns, and greatly reduces the quality of life in patients. Unfortunately, the pathogenesis of PSS has not been fully understood till now, and its ideal and specific treatment regimen is deficient. Previous studies have demonstrated that occurrence of spasticity correlates with abnormalities in the central modulation and muscular adjustment. Facing the complicated situation, multidisciplinary approaches that could reconcile the central with the peripheral is considered as a noteworthy insight for doctors and researchers in the management of spasticity. As a portion of comprehensive remedy of PSS, MSA techniques, which are unique in that effective and synchronous combination of acupuncture and rehabilitation, have shown promising practical values in the clinical settings, with meaningful reduction in spastic hypertonia and improvement in motor performance, which may correlate with improvements of neuroplasticity and muscle condition. In order to ensure the effectiveness of treatment, it is necessary to consider not only the intervention timing and course, choice of needling technique, stimulation intensity,

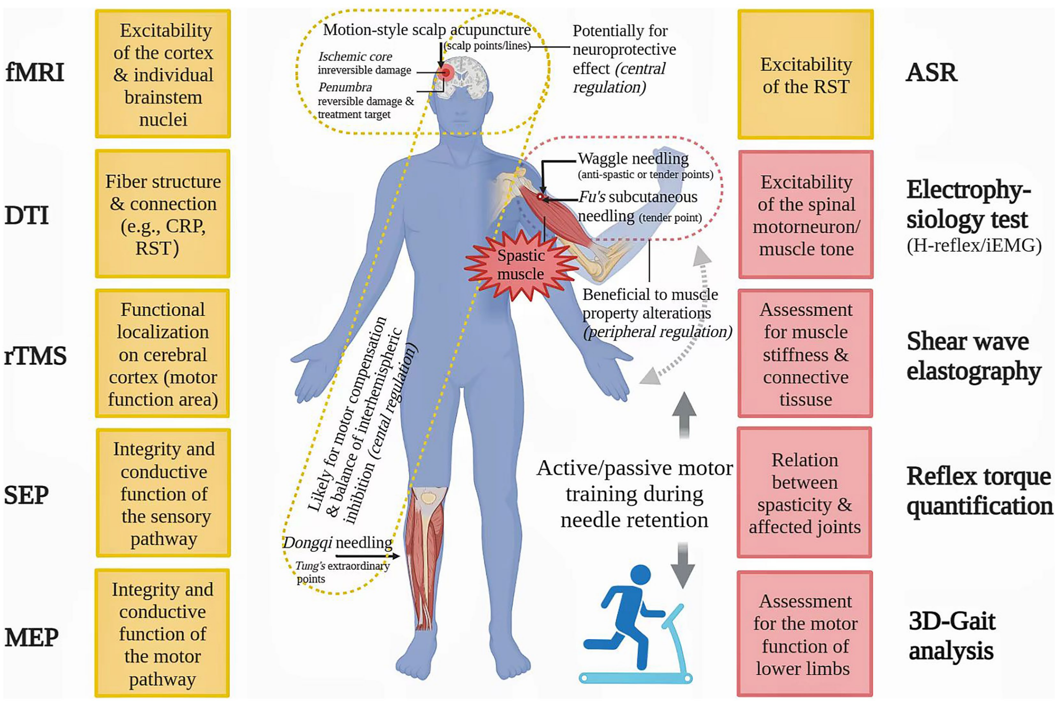

FIGURE 2
Advanced methods for spasticity assessment and their potential application in future motion-style acupuncture research. ASP: acoustic startle reflex, CRP: corticoreticular pathway, DTI: diffusion tensor imaging, fMRI: functional magnetic resonance imaging, iEMG: integrated electromyography, MEP: motor evoked potential, rTMS: repetitive transcranial magnetic stimulation, RST: reticulospinal tract, SEP: somatosensory evoked potential.

acupoint-selection (local/remote, bilateral/unilateral), exercise mode, predictive/aggravating factors of PSS, but the specific conditions (e.g., poststroke phases, extent of neurological deficit) and tolerance of patients (e.g., fatigue tolerance, pain tolerance). To avoid decreased efficacy and tolerance of acupuncture induced by frequent application of traditional acupoints and the same needling technique, using MSA techniques alternatively can be regarded as a feasible choice, especially suitable for those with long and complex disease progression. We hope this review can provide certain new insights for future research on acupuncture against PSS. Given that the quality of the current MSA studies is not optimal, rigorous study design, valid assessment tools for spasticity and related animal studies are expected to provide more substantial scientific evidences for MSA studies.

Author contributions

J-XW wrote and revised the manuscript, also drew the figures and table. L-XM revised the manuscript. J-XW and OF checked the language together. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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References

1. Feigin VL, Vos T, Nichols E, Owolabi MO, Carroll WM, Dichgans M, et al. The global burden of neurological disorders: translating evidence into policy. *Lancet Neurol*. (2020) 19:255–65. doi: 10.1016/S1474-4422(19)30411-9

2. Li S, Francisco GE, Rymer WZ. A new definition of Poststroke spasticity and the interference of spasticity with motor recovery from acute to chronic stages. *Neurorehabil Neural Repair*. (2021) 35:601–10. doi: 10.1177/15459683211011214

3. Zeng H, Chen J, Guo Y, Tan S. Prevalence and risk factors for spasticity after stroke: a systematic review and meta-analysis. *Front Neurol*. (2021) 11:616097. doi: 10.3389/fneur.2020.616097

4. Wissel J, Manack A, Brainin M. Toward an epidemiology of poststroke spasticity. *Neurology*. (2013) 80:S13–9. doi: 10.1212/WNL.0b013e3182762448

5. Li S. Spasticity, motor recovery, and neural plasticity after stroke. *Front Neurol*. (2017) 8:120. doi: 10.3389/fneur.2017.00120

6. Li S, Francisco GE. New insights into the pathophysiology of post-stroke spasticity. *Front Hum Neurosci*. (2015) 9:192. doi: 10.3389/fnhum.2015.00192

7. Lundström E, Smits A, Borg J, Terént A. Four-fold increase in direct costs of stroke survivors with spasticity compared with stroke survivors without spasticity: the first year after the event. *Stroke*. (2010) 41:319–24. doi: 10.1161/STROKEAHA.109.558619

8. Francisco GE, McGuire JR. Poststroke spasticity management. *Stroke*. (2012) 43:3132–6. doi: 10.1161/STROKEAHA.111.639831

9. Winstein CJ, Stein J, Arena R, Bates B, Cherney LR, Cramer SC, et al. Guidelines for adult stroke rehabilitation and recovery: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2016) 47:e98–e169. doi: 10.1161/STR.0000000000000098

10. Thibaut A, Chatelle C, Ziegler E, Bruno MA, Laureys S, Gosseries O. Spasticity after stroke: physiology, assessment and treatment. *Brain Inj*. (2013) 27:1093–105. doi: 10.3109/02699052.2013.804202

11. Katalinic OM, Harvey LA, Herbert RD. Effectiveness of stretch for the treatment and prevention of contractures in people with neurological conditions: a systematic review. *Phys Ther*. (2011) 91:11–24. doi: 10.2522/ptj.20100265

12. MacPherson H, Thomas K, Walters S, Fitter M. A prospective survey of adverse events and treatment reactions following 34,000 consultations with professional acupuncturists. *Acupunct Med*. (2001) 19:93–102. doi: 10.1136/aim.19.2.93

13. Wang HQ, Hou M, Bao CL, Min L, Li H. Effects of acupuncture treatment on lower limb spasticity in patients following hemorrhagic stroke: a pilot study. *Eur Neurol*. (2019) 81:5–12. doi: 10.1159/000499133

14. Li H, Liu H, Liu C, Shi G, Zhou W, Zhao C, et al. Effect of "Deqi" during the study of needling "Wang's Jiaji" acupoints treating spasticity after stroke. *Evid Based Complement Alternat Med*. (2014) 2014:715351. doi: 10.1155/2014/715351

15. Wang BH, Lin CL, Li TM, Lin SD, Lin JG, Chou LW. Selection of acupoints for managing upper-extremity spasticity in chronic stroke patients. *Clin Interv Aging*. (2014) 9:147–56. doi: 10.2147/CIA.S53814

16. Zhu W, Ye Y, Liu Y, Wang XR, Shi GX, Zhang S, et al. Mechanisms of acupuncture therapy for cerebral ischemia: an evidence-based review of clinical and animal studies on cerebral ischemia. *J Neuroimmune Pharmacol*. (2017) 12:575–92. doi: 10.1007/s11481-017-9747-4

17. Qian X, Ma LX, Sun TY, Mu JD, Zhang Z, Yu WY, et al. Practical value and thought on "co-regulation of body and mind" in treatment of post-stroke spasticity with acupuncture. *Zhongguo Zhen Jiu*. (2022) 42:803–6. doi: 10.13703/j.0255-2930.20210718-k0003

18. Xue C, Jiang C, Zhu Y, Liu X, Zhong D, Li Y, et al. Effectiveness and safety of acupuncture for post-stroke spasticity: a systematic review and meta-analysis. *Front Neurol*. (2022) 13:942597. doi: 10.3389/fneur.2022.942597

19. Neal E. Introduction to *Neijing* classical acupuncture part III: clinical therapeutics. *J Chin Med*. (2014) 104:5–23.

20. Wang JX, Ma LX, Mohammad Reza AF, Mohammadi A. Use of specific acupuncture techniques in lingering nummular eczema: a case report. *J Tradit Chin Med Sci*. (2021) 8:166–70. doi: 10.1016/j.jtcms.2021.03.001

21. Qiu X, Gao Y, Zhang Z, Cheng S, Zhang S. Fire acupuncture versus conventional acupuncture to treat spasticity after stroke: a systematic review and meta-analysis. *PLoS One*. (2021) 16:e0249313. doi: 10.1371/journal.pone.0249313

22. Zou DH, Liu T, Wang HB, Chang H, Li JN, Li Q, et al. Discussion on clinical application of "touching-bone" acupuncture technique. *Zhongguo Zhen Jiu*. (2020) 40:54–7. doi: 10.13703/j.0255-2930.20181210-0005

23. SCHILLEBEECKX F, de GROEF A, de BEUKELAER N, DESLOOVERE K, VERHEYDEN G, PEERS K. Muscle and tendon properties of the spastic lower leg after stroke defined by ultrasonography: a systematic review. *Eur J Phys Rehabil Med*. (2021) 57:495–510. doi: 10.23736/S1973-9087.20.06462-X

24. Shi GX, Liu BZ, Wang J, Fu QN, Sun SF, Liang RL, et al. Motion style acupuncture therapy for shoulder pain: a randomized controlled trial. *J Pain Res*. (2018) Volume 11:2039–50. doi: 10.2147/JPR.S161951

25. Kim D, Park KS, Lee JH, Ryu WH, Moon H, Park J, et al. Intensive motion style acupuncture treatment (MSAT) is effective for patients with acute whiplash injury: a randomized controlled trial. *J Clin Med*. (2020) 9:2079. doi: 10.3390/jcm9072079

26. Shin JS, Ha IH, Lee J, Choi Y, Kim MR, Park BY, et al. Effects of motion style acupuncture treatment in acute low back pain patients with severe disability: a multicenter, randomized, controlled, comparative effectiveness trial. *Pain*. (2013) 154:1030–7. doi: 10.1016/j.pain.2013.03.013

27. Zhang Y, Ma TM, Bai ZH, Sun BW, Zhao HY. Meta-analysis on the therapeutic effect of acupuncture at Meridian sinew for spastic paralysis after stroke. *Zhen Ci Yan Jiu*. (2017) 42:178–82.

28. Yan RJ, Cheng B, Chen LS, Shen XY, Zong L. Waggle needling plus joint needling for post-stroke spastic hemiplegia: a randomized controlled trail. *Shanghai J Acupunct Moxib*. (2016) 35:930–4. doi: 10.13460/j.issn.1005-0957.2016.08.0930

29. Li XH, Gan JX. Therapeutic effects of waggle needling at meridian-muscle nodes for spastic hemiplegia after stroke: a randomized controlled trail. *Chin J Integr Med Cardio-Cerebrovasc Dis*. (2017) 15:858–60.

30. Chen JA, Yu KC, Zhong Z, Zheng Y, Qu SS, Huang Y. Effect of Tung's acupuncture plus rehabilitation on stroke patients with upper limb spastic hemiplegia. *Chin J Rehabil Theory Pract*. (2015) 31:330–3.

31. Chen JA, Zhen Y, Yu KC, Zhong Z, Qu SS, Huang Y. Impacts of Dongqi needling at Tung's extraordinary points integrated with motor training on the spastic upper limb paralysis in stroke patients: a randomized controlled trail. *Chin J Rehabil Med*. (2015) 30:715–7.

32. Wang XY, Wen X, Zeng KX, Liu T. Fu's subcutaneous needling at starting and ending points of spastic muscle for elbow flexion spasticity in stroke survivors: a randomized controlled trail. *Chin J Ethnomed Ethnopharm*. (2018) 27:77–9.

33. Zhang SH, Wang YL, Zhang CX, Zhang CP, Xiao P, Li QF, et al. Effects of interactive dynamic scalp acupuncture on motor function and gait of lower limbs after stroke: a multicenter, randomized, controlled clinical trial. *Chin J Integr Med*. (2022) 28:483–91. doi: 10.1007/s11655-021-3525-0

34. Zhang CX, Wang YL, Zhang SH, Li QF, Liang WR, Pan XH, et al. Impacts of motion-style scalp acupuncture on poststroke upper-limb spasticity: a randomized controlled trail. *Shanghai J Acupunct-Mox*. (2021) 40:937–44. doi: 10.13460/j.issn.1005-0957.2021.08.0937

35. Li ZX, Zhang XX, Lou HJ, Cong DY. Interactive scalp acupuncture for spastic paralysis after stroke: systemic review and meta-analysis. *Lishizhen Med Materia Medica Res*. (2021) 32:1510–4.

36. Sangari S, Perez MA. Imbalanced Corticospinal and Reticulospinal contributions to spasticity in humans with spinal cord injury. *J Neurosci*. (2019) 39:7872–81. doi: 10.1523/JNEUROSCI.1106-19.2019

37. Mukherjee A, Chakravarty A. Spasticity mechanisms - for the clinician. *Front Neurol*. (2010) 1:149. doi: 10.3389/fneur.2010.00149

38. Burke D, Wissel J, Donnan GA. Pathophysiology of spasticity in stroke. *Neurology*. (2013) 80:S20–6. doi: 10.1212/WNL.0b013e31827624a7

39. Gracies JM. Pathophysiology of spastic paresis. II: emergence of muscle overactivity. *Muscle Nerve*. (2005) 31:552–71. doi: 10.1002/mus.20285

40. Ko SH, Kim T, Min JH, Kim M, Ko HY, Shin YI. Corticoreticular pathway in post-stroke spasticity: a diffusion tensor imaging study. *J Pers Med*. (2021) 11:1151. doi: 10.3390/jpm11111151

41. Trompetto C, Marinelli L, Mori L, Pelosin E, Currà A, Molfetta L, et al. Pathophysiology of spasticity: implications for neurorehabilitation. *Biomed Res Int*. (2014) 2014:354906. doi: 10.1155/2014/354906

42. Schreiner LH, Lindsley DB, Magoun HW. Role of brain stem facilitatory systems in maintenance of spasticity. *J Neurophysiol*. (1949) 12:207–16. doi: 10.1152/jn.1949.12.3.207

43. Li S, Chen YT, Francisco GE, Zhou P, Rymer WZ. A unifying pathophysiological account for post-stroke spasticity and disordered motor control. *Front Neurol*. (2019) 10:468. doi: 10.3389/fneur.2019.00468

44. Katz RT, Rymer WZ. Spastic hypertonia: mechanisms and measurement. *Arch Phys Med Rehabil*. (1989) 70:144–55.

45. Nielsen JB, Crone C, Hultborn H. The spinal pathophysiology of spasticity--from a basic science point of view. *Acta Physiol (Oxf)*. (2007) 189:171–80. doi: 10.1111/j.1748-1716.2006.01652.x

46. Sist B, Fouad K, Winship IR. Plasticity beyond peri-infarct cortex: spinal up regulation of structural plasticity, neurotrophins, and inflammatory cytokines during recovery from cortical stroke. *Exp Neurol*. (2014) 252:47–56. doi: 10.1016/j.expneurol.2013.11.019

47. Wang JX, Yang X, Zhang JJ, Zhou TT, Zhu YL, Wang LY. Effects of Shaoyao Gancao decoction on contents of amino acids and expressions of receptors in brains of spastic paralysis rats. *Zhongguo Zhong Yao Za Zhi*. (2016) 41:1100–6. doi: 10.4268/cjcmm20160621

48. Kowalczyk P, Kulig K. GABA system as a target for new drugs. *Curr Med Chem*. (2014) 21:3294–309. doi: 10.2174/0929867321666140601202158

49. Gilbert SL, Zhang L, Forster ML, Anderson JR, Iwase T, Soliven B, et al. Trak1 mutation disrupts GABA(a) receptor homeostasis in hypertonic mice. *Nat Genet*. (2006) 38:245–50. doi: 10.1038/ng1715

50. Wang JX, Ma LX, Yang Y, Song Y. Research progress on mechanism of acupuncture for the treatment of post-stoke spasticity. *Global Tradit Chin Med*. (2019) 12:470–5.

51. Flores AE, Pascotini ET, Kegler A, Broetto N, Gabbi P, Duarte T, et al. Worst spasticity in patients post-stroke associated with MNSOD ALA16VAL polymorphism and interleukin-1β. *Gene*. (2022) 847:146880. doi: 10.1016/j.gene.2022.146880

52. Qi YC, Xiao XJ, Duan RS, Yue YH, Zhang XL, Li JT, et al. Effect of acupuncture on inflammatory cytokines expression of spastic cerebral palsy rats. *Asian Pac J Trop Med*. (2014) 7:492–5. doi: 10.1016/S1995-7645(14)60081-X

53. Lieber RL, Steinman S, Barash IA, Chambers H. Structural and functional changes in spastic skeletal muscle. *Muscle Nerve*. (2004) 29:615–27. doi: 10.1002/mus.20059

54. Jin Y, Jin X, Li J. Fu's subcutaneous needling and constraint-induced movement therapy for a patient with chronic stroke: one-year follow-up case report. *Medicine*. (2019) 98:e13918. doi: 10.1097/MD.0000000000013918

55. Fu ZH, Chen XY, Lu LJ, Lin J, Xu JG. Immediate effect of Fu's subcutaneous needling for low back pain. *Chin Med J*. (2006) 119:953–7. doi: 10.1097/00029330-200606010-00014

56. Wang XW, Yu XZ. Waggle needling combines with wheat-moxibustion for flexor surface electromyography and upper limb F wave in stroke patients with spastic paralysis: a randomized controlled trail. *Acta Chin Med*. (2017) 32:2558–61. doi: 10.16368/j.issn.1674-8999.2017.12.664

57. Fu ZH, Wang JH, Sun JH, Chen XY, Xu JG. Fu's subcutaneous needling: possible clinical evidence of the subcutaneous connective tissue in acupuncture. *J Altern Complement Med*. (2007) 13:47–52. doi: 10.1089/acm.2006.6125

58. Ge RJ, Hu XS, Zeng KX. Preliminary exploration on the effect and mechanism of Dongqi needling at extraordinary points for spastic lower limb paralysis in stroke patients. *J Liaoning Univ Tradit Chin Med*. (2022) 24:140–5. doi: 10.13194/j.issn.1673-842x.2022.07.031

59. Min YJ, Yao HH, Shao SJ, He XW, Wang HS, Yan ZG, et al. Brief analysis on scientifity of the international scalp acupuncture. *Zhongguo Zhen Jiu*. (2007) 27:612–6.

60. Tao R, Yin HN, Liu SL, Lv XL, Zeng XX, Sun ZR. Waggle needling for knee hyperextension during spastic phases and neurotransmitters and inflammatory cytokines in stroke patients: a randomized controlled trail. *Chin J Integr Med Cardio-Cerebrovscu Dis*. (2021) 19:1569–72.

61. Xiao AJ, Xia YB, Fu ZH, Guo J, Liang S. Review on the role of Fu's subcutaneous needling (FSN) in pain relieving. *Zhongguo Zhen Jiu*. (2013) 33:1143–6.

62. Song WP, Zhang SM, Wang J, Yang J. Fu's subcutaneous needling combined with motor training for stroke patients with spastic hand: a randomized controlled trial. *J Pract Tradit Chin Med*. (2022) 38:1035–7.

63. Dai MY, Zhou MY, Wu LX, Yu KQ, Xu R. Fu's subcutaneous needling integrated with muscle activation therapy for triceps muscle spasm during stroke recovery period: a randomized controlled trail. *New Chin Med*. (2021) 53:124–7. doi: 10.13457/j.cnki.jncm.2021.11.033

64. Liu SL, Zhang HY, Man HJ. Impacts of motion-style scalp acupuncture on neurological deficit, somatosensory evoked potential and motor ability in stroke patients with spastic paralysis: a randomized controlled trial. *Mod J Integr Tradit Chin West Med*. (2018) 27:929–33.

65. Tang TJ, Sun KX, Deng RC, et al. Motion-style scalp acupuncture for the temporal and spatial parameters of gait in patients with spastic cerebral palsy: a randomized controlled trail. *Shanghai J Acupunct-Mox*. (2016) 35:1190–3.

66. Li JT, Wu P, Wang RH. Motion-style scalp acupuncture for stroke patients with spastic hemiplegia: a randomized controlled trail. *Chin J Integr Med Cardio-Cerebrovesc Dis*. (2020) 18:1297–300.

67. Zhang J, Zhu L, Tang Q. Electroacupuncture with rehabilitation training for limb spasticity reduction in post-stroke patients: a systematic review and meta-analysis. *Top Stroke Rehabil*. (2021) 28:340–61. doi: 10.1080/10749357.2020.1812938

68. Qi LL, Han ZX, Zhou YX, Jiang SY, Shen M, Lu JY, et al. Dynamic scalp acupuncture combined with PNF therapy for upper limb motor impairment in ischemic stroke spastic hemiplegia. *Zhongguo Zhen Jiu*. (2018) 38:234–8. doi: 10.13703/j.0255-2930.2018.03.002

69. Shi GX, Yang CY, Wu MM, Guan LP, Wang LP, Liu CZ. Muscle hypertonia after permanent focal cerebral ischemia in rats: a qualitative and quantitative behavioral and electrophysiological study. *Int J Neurosci*. (2013) 123:575–81. doi: 10.3109/00207454.2013.783578

70. Wang JX, Mu JD, Ma LX, Sun TY, Qian X, Yu WY, et al. Waggle needling wields preferable neuroprotective and anti-spastic effects on post-stroke spasticity rats by attenuating γ-aminobutyric acid transaminase and enhancing γ-aminobutyric acid. *Neuroreport*. (2020) 31:708–16. doi: 10.1097/WNR.0000000000001471

71. Wang JX, Ma LX, Mu JD, Sun TY, Qian X, Yu WY, et al. Anti-spastic effect induced by waggle needling correlates with KCC2-GABAA pathway in post-stroke spasticity rats. *Neurosci Lett*. (2021) 750:135810. doi: 10.1016/j.neulet.2021.135810

72. Sun TY, Ma LX, Mu JD, Zhang Z, Yu WY, Qian X, et al. Acupuncture improves the structure of spastic muscle and decreases spasticity by enhancing GABA, KCC2, and GABAAγ2 in the brainstem in rats after ischemic stroke. *Neuroreport*. (2022) Publish Ahead of Print:399–407. doi: 10.1097/WNR.0000000000001798

73. Qian X, Ma LX, Mu JD, Zhang Z, Sun TY, Yu WY, et al. Study on the central mechanism of acupuncture for post-stroke spasticity based on the Na^+/K^+-ATPase-EAATs-Glu pathway. *Zhen Ci Yan Jiu*. (2022) 47:283–9. doi: 10.13702/j.1000-0607.20210922

74. Mu JD, Ma LX, Zhang Z, Yu WY, Sun TY, Qian X, et al. Acupuncture alleviates spinal hyperreflexia and motor dysfunction in post-ischemic stroke rats with spastic hypertonia via KCC2-mediated spinal $GABA_A$ activation. *Exp Neurol*. (2022) 354:114027. doi: 10.1016/j.expneurol.2022.114027

75. Akai M, Nishimura R, Fujita N. The swimming test is effective for evaluating spasticity after contusive spinal cord injury. *PLoS One*. (2017) 12:e0171937. doi: 10.1371/journal.pone.0171937

76. Ward NS, Brown MM, Thompson AJ, Frackowiak RS. Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain*. (2003) 126:1430–48. doi: 10.1093/brain/awg145

77. Di Pino G, Pellegrino G, Assenza G, Capone F, Ferreri F, Formica D, et al. Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol*. (2014) 10:597–608. doi: 10.1038/nrneurol.2014.162

78. Floel A, Hummel F, Duque J, Knecht S, Cohen LG. Influence of somatosensory input on interhemispheric interactions in patients with chronic stroke. *Neurorehabil Neural Repair*. (2008) 22:477–85. doi: 10.1177/1545968308316388

79. Coleman ER, Moudgal R, Lang K, Hyacinth HI, Awosika OO, Kissela BM, et al. Early rehabilitation after stroke: a narrative review. *Curr Atheroscler Rep*. (2017) 19:59. doi: 10.1007/s11883-017-0686-6

80. Jackman K, Iadecola C. Neurovascular regulation in the ischemic brain. *Antioxid Redox Signal*. (2015) 22:149–60. doi: 10.1089/ars.2013.5669

81. Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. *Ann Neurol*. (2006) 59:735–42. doi: 10.1002/ana.20845

82. Wei L, Erinjeri JP, Rovainen CM, Woolsey TA. Collateral growth and angiogenesis around cortical stroke. *Stroke*. (2001) 32:2179–84. doi: 10.1161/hs0901.094282

83. Hong M, Kim M, Kim TW, Park SS, Kim MK, Park YH, et al. Treadmill exercise improves motor function and short-term memory by enhancing synaptic plasticity and neurogenesis in Photothrombotic stroke mice. *Int Neurourol J*. (2020) 24:S28–38. doi: 10.5213/inj.2040158.079

84. Chen YT, Li S, DiTommaso C, Zhou P, Li S. Possible contributions of ipsilateral pathways from the Contralesional motor cortex to the voluntary contraction of the spastic elbow flexors in stroke survivors: a TMS study. *Am J Phys Med Rehabil*. (2019) 98:558–65. doi: 10.1097/PHM.0000000000000114

85. Nudo RJ, Wise BM, SiFuentes F, Milliken GW. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science*. (1996) 272:1791–4. doi: 10.1126/science.272.5269.1791

86. Liu H, Jiang Y, Wang N, Yan H, Chen L, Gao J, et al. Scalp acupuncture enhances local brain regions functional activities and functional connections between cerebral hemispheres in acute ischemic stroke patients. *Anat Rec (Hoboken)*. (2021) 304:2538–51. doi: 10.1002/ar.24746

87. Schaechter JD, Connell BD, Stason WB, Kaptchuk TJ, Krebs DE, Macklin EA, et al. Correlated change in upper limb function and motor cortex activation after verum and sham acupuncture in patients with chronic stroke. *J Altern Complement Med*. (2007) 13:527–32. doi: 10.1089/acm.2007.6316

88. Dhond RP, Kettner N, Napadow V. Neuroimaging acupuncture effects in the human brain. *J Altern Complement Med*. (2007) 13:603–16. doi: 10.1089/acm.2007.7040

89. Chen J, Wang J, Huang Y, Lai X, Tang C, Yang J, et al. Modulatory effect of acupuncture at Waiguan (TE5) on the functional connectivity of the central nervous system of patients with ischemic stroke in the left basal ganglia. *PLoS One*. (2014) 9:e96777. doi: 10.1371/journal.pone.0096777

90. Howard JJ, Herzog W. Skeletal muscle in cerebral palsy: from belly to myofibril. *Front Neurol*. (2021) 12:620852. doi: 10.3389/fneur.2021.620852

91. Singer B, Dunne J, Allison G. Reflex and non-reflex elements of hypertonia in triceps surae muscles following acquired brain injury: implications for rehabilitation. *Disabil Rehabil*. (2001) 23:749–57. doi: 10.1080/09638280110060466

92. Kalkman BM, Bar-On L, O'Brien TD, Maganaris CN. Stretching interventions in children with cerebral palsy: why are they ineffective in improving muscle function and how can we better their outcome? *Front Physiol*. (2020) 11:131. doi: 10.3389/fphys.2020.00131

93. Miczak K, Padova J. Muscle overactivity in the upper motor neuron syndrome: assessment and problem solving for complex cases: the role of physical and occupational therapy. *Phys Med Rehabil Clin N Am*. (2018) 29:529–36. doi: 10.1016/j.pmr.2018.03.006

94. Tian DS, Xiong J, Pan Q, Liu F, Wang L, Xu SB, et al. De qi, a threshold of the stimulus intensity, elicits the specific response of acupoints and intrinsic change of human brain to acupuncture. *Evid Based Complement Alternat Med*. (2014) 2014:914878. doi: 10.1155/2014/914878

95. Yuan HW, Ma LX, Qi DD, Zhang P, Li CH, Zhu J. The historical development of *Deqi* concept from classics of traditional Chinese medicine to modern research: exploitation of the connotation of *Deqi* in Chinese medicine. *Evid Based Complement Alternat Med*. (2013) 2013:639302. doi: 10.1155/2013/639302

96. Shi GX, Yang XM, Liu CZ, Wang LP. Factors contributing to therapeutic effects evaluated in acupuncture clinical trials. *Trials*. (2012) 13:42. doi: 10.1186/1745-6215-13-42

97. Nierhaus T, Chang Y, Liu B, Shi X, Yi M, Witt CM, et al. Somatosensory stimulation with XNKQ acupuncture modulates functional connectivity of motor areas. *Front Neurosci*. (2019) 13:147. doi: 10.3389/fnins.2019.00147

98. Konofagou EE, Langevin HM. Using ultrasound to understand acupuncture. *IEEE Pulse*. (2005) 24:41–6. doi: 10.1109/memb.2005.1411347

99. Lu Z, Briley A, Zhou P, Li S. Are there trigger points in the spastic muscles? Electromyographical evidence of dry needling effects on spastic finger flexors in chronic stroke. *Front Neurol*. (2020) 11:78. doi: 10.3389/fneur.2020.00078

100. Theodosiadou A, Henry M, Duchateau J, Baudry S. Revisiting the use of Hoffmann reflex in motor control research on humans. *Eur J Appl Physiol*. (2022). doi: 10.1007/s00421-022-05119-7

101. Liu QG, Liu L, Huang QM, Nguyen TT, Ma YT, Zhao JM. Decreased spontaneous electrical activity and acetylcholine at Myofascial trigger spots after dry needling treatment: a pilot study. *Evid Based Complement Alternat Med*. (2017) 2017:3938191–7. doi: 10.1155/2017/3938191

102. Krakauer JW, Carmichael ST, Corbett D, Wittenberg GF. Getting neurorehabilitation right: what can be learned from animal models? *Neurorehabil Neural Repair*. (2012) 26:923–31. doi: 10.1177/1545968312440745

103. Li F, Pendy JT Jr, Ding JN, Peng C, Li X, Shen J, et al. Exercise rehabilitation immediately following ischemic stroke exacerbates inflammatory injury. *Neurol Res*. (2017) 39:530–7. doi: 10.1080/01616412.2017.1315882

104. Zhang Q, Zhang J, Yan Y, Zhang P, Zhang W, Xia R. Proinflammatory cytokines correlate with early exercise attenuating anxiety-like behavior after cerebral ischemia. *Brain Behav*. (2017) 7:e00854. doi: 10.1002/brb3.854

105. Zhang L, Hu X, Luo J, Li L, Chen X, Huang R, et al. Physical exercise improves functional recovery through mitigation of autophagy, attenuation of apoptosis and enhancement of neurogenesis after MCAO in rats. *BMC Neurosci*. (2013) 14:46. doi: 10.1186/1471-2202-14-46

106. AVERT Trial Collaboration group. Efficacy and safety of very early mobilisation within 24 h of stroke onset (AVERT): a randomised controlled trial. *Lancet*. (2015) 386:46–55. doi: 10.1016/S0140-6736(15)60690-0

107. Momosaki R, Yasunaga H, Kakuda W, Matsui H, Fushimi K, Abo M. Very early versus delayed rehabilitation for acute ischemic stroke patients with intravenous recombinant tissue plasminogen activator: a Nationwide retrospective cohort study. *Cerebrovasc Dis*. (2016) 42:41–8. doi: 10.1159/000444720

108. Wang Q, Peng Y, Chen S, Gou X, Hu B, Du J, et al. Pretreatment with electroacupuncture induces rapid tolerance to focal cerebral ischemia through regulation of endocannabinoid system. *Stroke*. (2009) 40:2157–64. doi: 10.1161/STROKEAHA.108.541490

109. Zhang BY, Wang GR, Ning WH, Liu J, Yang S, Shen Y, et al. Electroacupuncture pretreatment elicits tolerance to cerebral ischemia/reperfusion through inhibition of the GluN2B/m-Calpain/p38 MAPK Proapoptotic pathway. *Neural Plast*. (2020) 2020:8840675–14. doi: 10.1155/2020/8840675

110. Gao Z, Liu Q, Yang L, Zhu X. Identification of high-risk factors for prehospital delay for patients with stroke using the risk matrix methods. *Front Public Health*. (2022) 10:858926. doi: 10.3389/fpubh.2022.858926

111. White A, Cummings M, Filshie J. (2008). *An introduction to western medical acupuncture*. London: Churchill Livingstone Elsevier. p.7–76.

112. Tian L, Wang JH, Sun RJ, Zhang XH, Yuan B, Du XZ. Development of researches on scalp acupuncture for ischemic stroke. *Zhen Ci Yan Jiu*. (2016) 41:93.

113. Wang WW, Xie CL, Lu L, Zheng GQ. A systematic review and meta-analysis of Baihui (GV20)-based scalp acupuncture in experimental ischemic stroke. *Sci Rep*. (2014) 4:3981. doi: 10.1038/srep03981

114. Kelso JA, Holt KG, Rubin P, Kugler PN. Patterns of human interlimb coordination emerge from the properties of non-linear, limit cycle oscillatory processes: theory and data. *J Mot Behav*. (1981) 13:226–61. doi: 10.1080/00222895.1981.10735251

115. Li A, Yan T, Chen Y. Impacts of unilateral needling and bilateral needling on the somatosensory evoked potential on patients with acute ischemic stroke: a randomized controlled trail. *Chin J Rehabil Med.* (2007) 22:81–2.

116. Liu L, Chen SQ, Wei J, Xu XB, Jing XH, Wang LP. The effect of acupuncture at Wang's twelve points in the hands and feet on the plasticity of primary motor cortex in patients with ischemic stroke. *Global Tradit Chin Med.* (2019) 12:385–9.

117. Shen TL. Influence of scalp acupuncture at unilateral side and bilateral sides on TCD in acute cerebral infarction: a randomized controlled trail. *Shanghai J Acupunct-Mox*. (2002) 1:8–10. doi: 10.13460/j.issn.1005-0957.2002.01.007

118. McCombe Waller S, Forrester L, Villagra F, Whitall J. Intracortical inhibition and facilitation with unilateral dominant, unilateral nondominant and bilateral movement tasks in left- and right-handed adults. *J Neurol Sci*. (2008) 269:96–104. doi: 10.1016/j.jns.2007.12.033

119. Cauraugh JH, Lodha N, Naik SK, Summers JJ. Bilateral movement training and stroke motor recovery progress: a structured review and meta-analysis. *Hum Mov Sci*. (2010) 29:853–70. doi: 10.1016/j.humov.2009.09.004

120. Khan F, Amatya B, Bensmail D, Yelnik A. Non-pharmacological interventions for spasticity in adults: an overview of systematic reviews. *Ann Phys Rehabil Med*. (2019) 62:265–73. doi: 10.1016/j.rehab.2017.10.001

121. Han P, Zhang W, Kang L, Ma Y, Fu L, Jia L, et al. Clinical evidence of exercise benefits for stroke. *Adv Exp Med Biol*. (2017) 1000:131–51. doi: 10.1007/978-981-10-4304-8_9

122. Brusola G, Garcia E, Albosta M, Daly A, Kafes K, Furtado M. Effectiveness of physical therapy interventions on post-stroke spasticity: an umbrella review. *NeuroRehabilitation*. (2023):1–15. doi: 10.3233/NRE-220275

123. Coronado RA, Simon CB, Valencia C, George SZ. Experimental pain responses support peripheral and central sensitization in patients with unilateral shoulder pain. *Clin J Pain*. (2014) 30:143–51. doi: 10.1097/AJP.0b013e318287a2a4

124. Zorowitz RD, Gillard PJ, Brainin M. Poststroke spasticity: sequelae and burden on stroke survivors and caregivers. *Neurology*. (2013) 80:S45–52. doi: 10.1212/WNL.0b013e3182764c86

125. Glaess-Leistner S, Ri SJ, Audebert HJ, Wissel J. Early clinical predictors of post stroke spasticity. *Top Stroke Rehabil.* (2021) 28:508–18. doi: 10.1080/10749357.2020.1843845

126. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: an overview of clinical and preclinical studies. *Exp Neurol*. (2021) 335:113518. doi: 10.1016/j.expneurol.2020.113518

127. Chen H, He Y, Chen S, Qi S, Shen J. Therapeutic targets of oxidative/nitrosative stress and neuroinflammation in ischemic stroke: applications for natural product efficacy with omics and systemic biology. *Pharmacol Res*. (2020) 158:104877. doi: 10.1016/j.phrs.2020.104877

128. Hu C, Qin X, Ye R, Jiang M, Lu Y, Lin C. The role of traditional Chinese medicine nursing for stroke: an umbrella review. *Evid Based Complement Alternat Med*. (2021) 2021:9918687–12. doi: 10.1155/2021/9918687

129. Huppertz V, Guida S, Holdoway A, Strilciuc S, Baijens L, Schols JMGA, et al. Impaired nutritional condition after stroke from the Hyperacute to the chronic phase: a systematic review and meta-analysis. *Front Neurol*. (2022) 12:780080. doi: 10.3389/fneur.2021.780080

130. Briggs JP, Shurtleff D. Acupuncture and the complex connections between the mind and the body. *JAMA*. (2017) 317:2489–90. doi: 10.1001/jama.2017.7214

131. Zhao L, Liang FR, Li Y, Zhang FW, Zheng H, Wu X. Improved quality monitoring of multi-center acupuncture clinical trials in China. *Trials*. (2009) 10:123. doi: 10.1186/1745-6215-10-123

132. Yuan HW, Ma LX, Zhang P, Lin C, Qi DD, Li J, et al. An exploratory survey of deqi sensation from the views and experiences of chinese patients and acupuncturists. *Evid Based Complement Alternat Med*. (2013) 2013:430851. doi: 10.1155/2013/430851

133. Davis LC, Baumer TG, Bey MJ, Holsbeeck MV. Clinical utilization of shear wave elastography in the musculoskeletal system. *Ultrasonography*. (2019) 38:2–12. doi: 10.14366/usg.18039

134. Boddington LJ, Reynolds JNJ. Targeting interhemispheric inhibition with neuromodulation to enhance stroke rehabilitation. *Brain Stimul*. (2017) 10:214–22. doi: 10.1016/j.brs.2017.01.006

135. Fawcett TJ, Longenecker RJ, Brunelle DL, Berger JI, Wallace MN, Galazyuk AV, et al. Universal automated classification of the acoustic startle reflex using machine learning. *Hear Res*. (2023) 428:108667. doi: 10.1016/j.heares.2022.108667

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Effect of acupuncture in the acute phase of intracerebral hemorrhage on the prognosis and serum BDNF: a randomized controlled trial

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Background: Intracerebral hemorrhage (ICH) is a common cerebrovascular disease, with a high rate of disability. In the literature on Chinese traditional medicine, there is increasing evidence that acupuncture can help hematoma absorption and improve neurological deficits after cerebral hemorrhage. Brain-derived neurotrophic factor (BDNF), one of the most studied neurotrophic factors, is involved in a variety of neurological functions and plays an important role in brain injury recovery. We investigated the effect of acupuncture intervention in the acute phase of ICH on the prognosis and serum BDNF levels of several patient groups.

Objective: To investigate the influence of acupuncture on the prognosis and brain-derived neurotrophic factor (BDNF) levels in patients in the acute phase of ICH.

Methods: From November 2021 to May 2022, 109 subjects were consecutively enrolled, including patients with ICH, who were randomized into the acupuncture group (AG) and sham acupuncture group (SAG), and a control group (CG). The CG received the same acupuncture intervention as the AG, and the SAG received sham acupuncture, with 14 interventions in each group. The level of consciousness of patients with ICH was assessed and serum BDNF levels were measured in all three groups before the intervention and at 3 weeks after onset, and the level of consciousness and outcomes were assessed at 12 weeks after onset.

Results: After the intervention, the level of consciousness of the AG improved significantly ($P < 0.05$); the BDNF level of only the AG increased significantly ($P < 0.05$); the changes in Glasgow Coma Scale (GCS) score and BDNF level were significantly greater in the AG than in the SAG ($P < 0.05$), especially for locomotion ($P < 0.05$). At 12 weeks post-onset, the AG showed better outcomes and recovery of consciousness than the SAG ($P < 0.05$).

KEYWORDS

acupuncture, intracerebral hemorrhage, brain-derived neurotrophic factor (BDNF), stroke, prognosis

1. Introduction

Intracerebral hemorrhage (ICH) refers to the rupture of blood vessels in the brain, resulting in bleeding in the brain parenchyma, ventricles, and subarachnoid space; it accounts for 10–15% of all stroke types (Al-Kawaz et al., 2020).

Brain-derived neurotrophic factor (BDNF) is a peptide growth factor involved in a variety of neurological functions, such as cell growth, differentiation, and plasticity (Mang et al., 2013; Polacchini et al., 2015), thereby affecting cognition, memory, and other functions. It is derived mainly from central nervous system neurons and to a lesser extent from peripheral blood cells, among others (Polacchini et al., 2015). It has been shown that intracranially produced BDNF can cross the blood-brain barrier (Pan et al., 1998); therefore, the level of peripheral BDNF after stroke may reflect central BDNF levels. After stroke onset, BDNF can enhance local anti-inflammatory action by upregulating IL-10 and downregulating TNF-α (Jiang et al., 2010) expression.

In Chinese medical theory, intracerebral hemorrhage (known as Zhongfeng in Chinese medicine) is based on an internal deficiency of Qi (the potential energy that maintains your life and every body system) and blood, and is triggered by exertion, worry and poor diet, resulting in an imbalance of internal Yin and Yang, and thus cerebral vascular blockage or blood overflow. Therefore, in terms of treatment, it is important to differentiate according to Yin and Yang so as to "treat the same disease differently" or "treat different diseases with the same treatment." Acupuncture is an important treatment tool, and several previous studies (Fan and Liu, 1997) have found that acupuncture in the acute phase of intracerebral hemorrhage has better outcomes than in other phases. Acupuncture is well accepted in China as an alternative and complementary strategy after stroke, as recommended by the World Health Organization (Lehmann, 2013). Some studies have suggested that acupuncture can improve post-stroke dysfunction through a variety of mechanisms, including anti-apoptosis in ischemic areas (Chavez et al., 2017). A randomized controlled trial found that acupuncture reversed the downregulation of serum BDNF (Jiang et al., 2018). Xiong et al. (2020) found that head acupuncture combined with cognitive function training resulted in a pronounced increase in BDNF levels and improved post-stroke outcomes. These findings suggest that one of the mechanisms by which acupuncture improves functional impairment after stroke may involve serum BDNF levels.

Regarding the timing of rehabilitation interventions, overseas recommendations suggest starting rehabilitation within 24–48 h after the onset of stroke (Greenberg et al., 2022), whereas Chinese guidelines usually suggest starting rehabilitation 48 h to 1 week after the onset of stroke or after the stabilization of vital signs (Yu et al., 2020). The Xingnao-Kaiqiao acupuncture method is commonly used in clinical practice to treat stroke. It has been known to revive the human brain, and can improve the hypoxic state of brain tissue, leading to functional recovery (Li and Han, 2015).

In this trial, acupuncture was applied starting from the acute phase [generally considered to be 2–7 days after onset (Manning et al., 2014)] of ICH, and the participants were divided into acupuncture (AG), sham acupuncture (SAG), and control groups (CG) to compare BDNF levels in each group. The relationship between acupuncture and BDNF was investigated by comparing the changes in BDNF levels in each group and by analyzing the outcome between the groups. The efficacy of acupuncture for outcomes of ICH was determined in order to provide a reference for acupuncture treatment protocols in the acute phase of ICH.

2. Subjects and methods

2.1. Trial design

This double-blind, randomized controlled trial was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Yongchuan Hospital of Chongqing Medical University (No. 110, 2021). This study was registered at ClinicalTrials.org (NCT05479903). Patients with ICH who met the inclusion criteria were randomly divided into the AG or SAG using the SAS software (version 9.4). All patients with ICH underwent poststroke secondary prevention, treatment of underlying diseases, and comprehensive rehabilitation training. Accordingly, the AG was administered an acupuncture intervention and the SAG was administered sham acupuncture intervention. When the medical condition changed and the intervention was no longer appropriate, it was suspended; it was resumed after the condition had stabilized. Fourteen interventions were performed in all groups, with the first intervention administered no longer than 7 days after onset and the last intervention administered no longer than 3 weeks after onset. The CG was administered the same intervention protocol as the AG. Serum BDNF levels were measured in all three groups before and after the interventions, changes in patient consciousness were assessed using the Glasgow Coma Scale (GCS), and patient outcomes were assessed using the modified Rankin Scale (mRS) and GCS at week 12 after onset. All included patients or their guardians signed an informed consent form before entering the trial.

2.2. Participants

From November 2021 to May 2022, patients with ICH were consecutively enrolled from the Department of Neurosurgery or Department of Rehabilitation Medicine of Yongchuan Hospital of Chongqing Medical University. Patients who visited the physical examination center during the same period were enrolled to the CG.

2.2.1. ICH inclusion criteria

The incision criteria for the AG and SAG were as follows: (1) age $\geq$ 18 years, (2) confirmed lesion on brain CT or MRI, (3) mRS $\leq$ 1 before onset, (4) time between symptom recognition and admission $\leq$ 72 h, (5) diagnosis of intracerebral hemorrhage by two regular physicians according to the Chinese Guidelines for the Diagnosis and Treatment of Cerebral Hemorrhage (2019), and (6) stable vital signs within 7 days after onset. The inclusion criteria for the CG were (1) age $\geq$ 50 years; (2) mRS $\leq$ 1; and (3) normal cognitive, motor, and speech function.

2.2.2. Exclusion criteria for all subjects

The exclusion criteria were as follows: (1) History of intracranial surgery or trauma, (2) neurological or psychiatric disease, (3) stroke; (4) abnormal coagulation function; (5) physical disability prior to onset; and (6) participation in other clinical trials within the previous 3 months.

2.2.3. Dropping out

Subjects with worsening medical conditions or serious complications that made them unfit for continuing the trial, subjects with poor compliance who failed to complete the 14 interventions within 3 weeks of onset, and subjects who discontinued participation for personal reasons were dropped from the analysis, and the reasons for withdrawal and dropping out were recorded.

2.3. Intervention

The basic disease treatment for AG and SAG, secondary prevention interventions after stroke, and comprehensive rehabilitation training were carried out with reference to relevant guidelines (Zhang et al., 2019; Al-Kawaz et al., 2020). The AG and CG were administered the Xingnao-Kaiqiao Acupuncture Method; the SAG was administered sham acupuncture.

2.3.1. Selection of acupoints

Neiguan (PC06), Shuigou (GV26), and Sanyinjiao (SP06) were the main acupoints, with Jiquan (HT01), Weizhong (BL40), and Chize (LU05) on the affected side as the secondary acupoints. All the acupoints were selected and positioned according to GB/T123462006 (Gb/T12346-2006, 2006), and the manipulation techniques refer to the research (Shi, 2005) of Academician Shi Xuemin.

2.3.2. Intervention steps

The subject was placed in a supine position, and the acupuncture operator disinfected both hands and the skin at the acupoint using povidone iodine solution (concentration 5%, Chongqing Xieran Pharmaceutical Co., Ltd., China). Then, a disposable electrode piece (LHJ-5680, Chongqing Younaite Medical Equipment Co., Ltd., China) was used to cover the skin at the selected acupoint, with or without piercing the electrode piece with disposable acupuncture needles (0.30 × 50 mm, Suzhou Dongbang Medical Devices Co., China). The location and manipulation of the AG and CG acupoints are shown in **Table 1**. Subjects suitable for acupuncture were given the intervention once a day for 30 min each.

The SAG received sham acupuncture intervention using the same procedure and equipment as the AG, with the difference that the acupuncture needle did not pierce the electrode piece, that is, it did not pierce the skin, and the needle was fixed to the electrode piece.

2.3.3. Intervention personnel

All of the acupuncture therapists have completed a relevant medical course, graduated in the field of acupuncture and have obtained a national qualification. They had all been working for more than 5 years and were trained in both the Xingnao-Kaiqiao acupuncture and the sham acupuncture method before the intervention to ensure that each therapist was able to perform the intervention correctly. If bleeding occurred at the acupoints during the intervention, pressure was applied to the points with a sterile cotton swab until the bleeding stopped. If the patient's condition changed (fever, epilepsy, etc.) such that the intervention was no longer appropriate, the intervention was suspended until the condition had stabilized (**Figure 1**).

2.4. Follow-up and outcomes

2.4.1. Time points for follow-up

The day of onset was defined as d1, the first follow-up (pre-intervention, t1) and the first intervention were completed at d1–d7, the last intervention was completed at d14–d20, the second follow-up (3 weeks after onset, t2) was completed at d20–d22, and the third follow-up (12 weeks after onset, t3) was completed at d82–d86. As far as possible, the intervention and follow-up were carried out with all patients at similar time points of the disease course, and all interventions were completed at t2 and t3 follow-up visits.

2.4.2. Clinical follow-up

Relevant training is provided to all assessors. The assessment of one subject was carried out by the same assessor.

The main outcome indicators were the change in the GCS score and the mRS score. The GCS assesses the patient's ability to open their eyes and their responses to verbal and motor tasks on a scale of 3–15, with higher scores representing a better state of consciousness. The mRS assesses the ability to perform daily activities on a scale of 0–6, with higher scores indicating poorer ability to take care of oneself. In this trial, mRS scores of 0–2 were defined as "good outcome" and mRS scores of 3–6 were defined as "poor outcome" according to the American Stroke Association guidelines (Lin et al., 2021).

Secondary outcome indicators were changes in BDNF levels and each GCS subcategory. Blood samples were collected from all subjects at t1 and t2. Venous blood (2 mL) was drawn and transferred into tubes containing procoagulant (procoagulant 5 mL, Weihai Weigao Medical Devices Ltd., Weihai, Shandong, China), shaken gently, and allowed to coagulate at room temperature for at least 30 min. Serum was then collected by centrifugation (Neofuge15R, Heal Force Development Ltd.) at 3,000 rpm for 10 min and stored at −80°C until assay. Serum BDNF levels were measured using an ELISA kit (R&D Systems, Inc., USA) by experienced laboratory staff who were blinded to the rest of the trial, and laboratory results were not known to the assessors until the end of the study.

2.5. Sample size

This study was a randomized controlled trial, and the sample size was estimated by comparing the means of the two samples, where n1 and n2 are the required numbers of the two samples, δ is the difference between the means of the two groups, and σ is the overall standard deviation: $\alpha = 0.05$ and $\beta = 0.20$. After reviewing the relevant literature (Liu et al., 2016), we set $\delta/\sigma = 0.77$. Using the tables of two-side tolerance factors, we confirmed that $\mu\alpha = 1.96$ and $\mu\beta = 0.84$, and substituted these into the formula to

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obtain n≈28, meaning that 28 cases were needed in each group. Considering withdrawals during the trial, the sample size was increased by 20% so that at least 34 subjects needed to be included in each group.

$$n_1 = n_2 = 2\left[\frac{\mu_\alpha + \mu_\beta}{\delta/\sigma}\right]^2 + \frac{1}{4}\mu_\alpha^2$$

2.6. Randomization

Random numbers were generated by an investigator who was not involved in other parts of the trial using SAS 9.4, and all numbers were assigned to the groups according to prespecified rules. The grouping results were stored in a sealed opaque envelope with the generated order written on its surface. The allocation results were kept by another investigator (Chen) who was not involved in the rest of the trial; when a subject was enrolled, Chen allocated the grouping result according to the generated order and informed the acupuncture operator.

2.7. Allocation and blinding

Only the necessary medical staff will be on site during the intervention to reduce the possibility of blind failure of the patients' families. Until the end of the trial, only Chen and the acupuncture operators were aware of the allocation results; the subjects, assessors, and other investigators were not informed of the grouping results.

2.8. Statistical methods

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 26.0 (Chicago, Armonk, NY, USA). The data were assessed for normality using the Kolmogorov–Smirnov test. Measurement data were expressed as median (interquartile range) or mean ± standard deviation, and categorical, as number (rate).

Comparisons between two groups of normally distributed count data were made using the Student *t*-test and among three groups using one-way ANOVA; comparisons between two groups of non-normally distributed count data were made using the Mann–Whitney U test and among three groups using the Kruskal–Wallis test. Multiple time points of count data were compared using the Mauchly's test of sphericity and Two-way Repeated Measures ANOVA.

The Pearson or fisher chi-square test was used for comparisons of unordered categorical information, the Mann-Whitney U test for comparisons of two groups of ordered categorical information, the Kruskal–wallis for comparisons of three groups of ordered categorical information, and the Pearson chi-square test for cross-tabulations when comparing inter-group outcomes before and after the intervention.

The GCS has three sub-categories with full marks of 4, 5, and 6. To compare the changes in scores between subscales, the absolute value of a given change was divided by the full score of that subscale. For example, the absolute value of the change in eye opening was divided by 4. Statistical analyses were performed as described previously. All results were considered statistically significant at $P < 0.05$.

2.9. Reasons for dropped out and adverse reactions

Adverse effects: pain, 20 in CG, 2 in AG and 1 in SAG reported that the intervention caused pain, but all disappeared within 1 h of the end of the intervention. Bleeding, 6 in CG, 19 in AG and 0 in SAG had bleeding at the end of the intervention at the

TABLE 1 Acupoints selected in the study.

	Location	Manipulation
Neiguan (PC06)	On the forearm: 6.5 cm above the transverse wrist palmaris, between the palmaris longus tendon and the radial carpal flexor tendon	Vertical insertion at a depth of 10–15 mm applied with the twisting, lifting, and thrusting technique of the reducing method[1] for 1 min.
Shuigou (GV26)	On the face: median depression above the upper lip	Oblique insertion at a depth of 5–10 mm toward the nasal septum; heavy bird-pecking needling[2] is applied until tear formation is observed in the patient's eyes.
Sanyinjiao (SP06)	On the crus: 10 cm directly above the tip of the medial malleolus, posterior to the medial border of the tibia	Inserted obliquely along the inner edge of the tibia at an angle of 45° to the skin to a depth of 10–15 mm and applied with the twisting technique of the reinforcing method[3]; three twitches of the lower limbs are considered appropriate.
Jiquan (HT01)	Apex of the armpit	Insert vertically 15–20 mm next to the Jiuquan at a depth of 10–15 mm, using the twist, lift and reducing method,[4] with three upper limb twitches as appropriate.
Weizhong (BL40)	Midpoint of the transverse popliteal line	Straight leg elevation of the affected limb, vertical insertion at a depth of 5–10 mm; applied with the twisting, lifting, and reducing method[4]; three twitches of the lower limbs are considered appropriate.
Chize (LU05)	Intersection of the radial recess of the biceps tendon with the transverse elbow stripe	Elbow is bent at an angle of 120° and stabbed straight to a depth of 10–15 mm; applied with the twisting, lifting, and reducing method[4]; three twitches of the forearm and fingers are considered appropriate.

[1]The thumb, middle, and index fingers hold the needle handle and twist it back and forth in a forward and backward rotation, then release the needle handle, lift it slightly when it returns to its natural position and then pierce it vertically.
[2]The needle handle is rotated 360 degrees and the needle is then lifted or inserted into the acupuncture point in small, rapid movements, similar to a sparrow pecking at rice.
[3]The needles are lifted slowly, but not completely out of the acupuncture point, and then inserted quickly and forcefully into the point.
[4]The needles are lifted quickly, but not completely withdrawn, and then slowly and gently inserted into the acupuncture point.

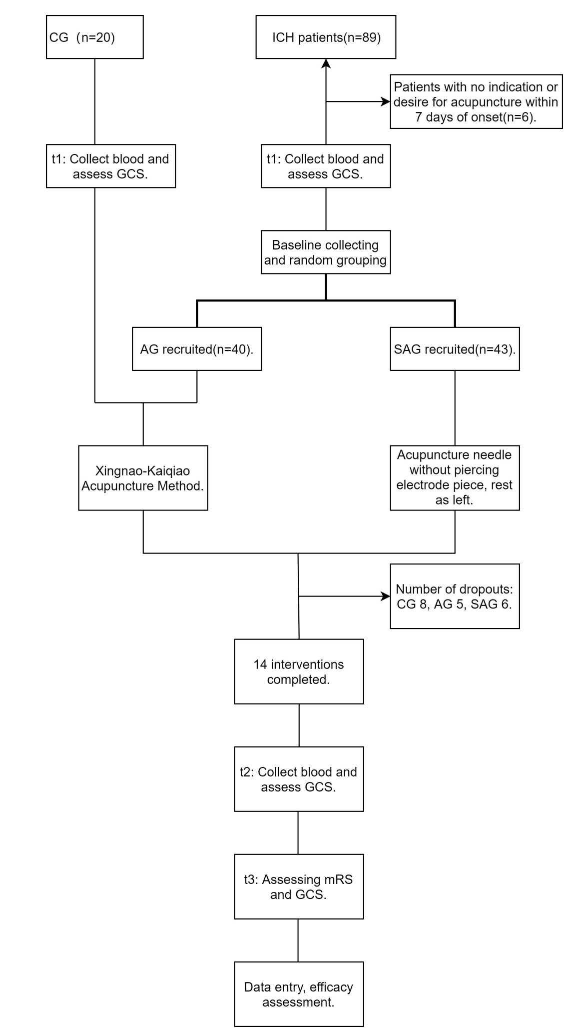

FIGURE 1
Flow chart of subjects.

pinpoint, which stopped within 10 min after applying sterile swab pressure. Some ICH patients are unconscious for part or all of the intervention, so that their feelings are not known. No other adverse effects were observed during this trial.

Subjects dropped out for the following reasons: pain, CG = 7; personal reasons, CG = 1, AG = 3, and SAG = 2; failure to complete the 14 interventions on time, AG = 2 and SAG = 4.

3. Results

3.1. Overall baseline characteristics

Overall, 84 subjects finally completed the full trial and were included in the analysis; the basic information is shown in Table 2. There were no statistically significant differences in any basic information between the 84 subjects who completed the trial and the 109 enrolled subjects ($P > 0.05$). There were no statistically significant differences in gender, age, comorbidities, lifestyle habits, or baseline BDNF levels among the AG, SAG, and CG ($P > 0.05$). There were no statistically significant differences in time from symptom recognition to admission, proportion treated surgically, admission systolic blood pressure, or pre-intervention GCS score between AG and SAG ($P > 0.05$). There was a statistically significant difference in systolic blood pressure between CG and ICH patients on admission ($p < 0.05$).

3.2. BDNF before and after the intervention

Table 3 demonstrates the levels of BDNF before and after the intervention (t1 and t2). As shown in the table, only the BDNF levels of AG were significantly higher after the intervention compared to before ($P < 0.05$), while the BDNF levels of SAG and CG were not statistically significantly changed compared to before the intervention ($P > 0.05$). In terms of BDNF change values, AG was more significantly elevated than CG or SAG ($P < 0.01$).

3.3. GCS before and after the intervention

The Mauchly's test of sphericity $P = 0.003$. Using two-way Repeated Measures ANOVA, it was suggested that GCS rose over time in both AG and SAG ($P < 0.001$), but even though both increased over time, the rise in GCS was significantly higher in AG than in SAG ($P < 0.01$). As shown in Table 4, baseline GCS was found to be comparable between AG and SAG ($P > 0.05$). Both at the end of the intervention and 12 weeks after onset, GCS was significantly higher in AG than in SAG ($P < 0.05$).

3.4. Change in GCS subscale values and outcomes 12 weeks after onset

Table 5 suggests that the outcome evaluation of AG and SAG using the mRS at 12 weeks after onset was better for AG than for SAG ($p < 0.05$). Of the three aspects evaluated by the GCS, only motor aspects were better recovered in AG than in SAG ($p < 0.05$), while there was no statistical difference in the degree of improvement in eye opening and verbal response between the two ($p > 0.05$).

4. Discussion

To the best of our knowledge, this study is one of the rare studies to focus on the efficacy of acupuncture in the acute phase of ICH, which is characterized by high mortality and disability as a subtype of stroke. In designing the trial, we simultaneously explored changes in BDNF levels after the intervention and ICH outcomes, while adjusting for confounding factors such as natural disease progression and physiological effects of acupuncture in healthy humans by employing the SAG and CG.

For the timing of needle intervention in patients with cerebral hemorrhage, previous guidelines (Lin et al., 2019) or meta-analyses (Liu et al., 2017) have recommended intervention in the acute phase, but some clinicians have abandoned needle treatment in the acute phase to prevent coagulation disorders in patients after invasive stimulation. There were no severe adverse effects of acupuncture in this trial, and none of the subjects withdrew owing to dissatisfaction with the efficacy, suggesting that the safety of acupuncture is reliable. Our study suggests that acupuncture shows positive effects in both short-term recovery of consciousness and long-term improvement in quality of life. In clinical practice, acupuncture, which has the advantages of being inexpensive and available at home, is less difficult to promote, and Xingnao-Kaiqiao acupuncture in particular may bring significant benefits to patients.

We also found that the CG had a 40% dropout rate, with seven of the eight dropouts withdrawing because of pain. To ensure a control effect, we administered the same interventions to the CG and AG, that is, Xingnao-Kaiqiao acupuncture. The majority of patients in the AG were in a coma, and this intervention was helpful in promoting awakening, but the strength of the intervention was too strong for conscious people to tolerate; therefore, only 12 people in the CG completed the full intervention, which in turn resulted in a small number of CG subjects and may have increased the sample bias. In the future, if similar trials are conducted, the high dropout rate of the CG due to pain will need to be addressed or more CGs need to be included.

Over half of the patients were unconscious during the intervention, which improved the blinding reliability of the interventions. However, it is worth noting that although the SAG was not affected by the direct penetration of the needles into the skin, this does not mean that sham needling would not have a therapeutic effect. Chae et al. (2018) argued that sham needling could have some efficacy, as it generates tactile sensation. After the end of all interventions, consciousness improved in both groups of patients with ICH compared to the pre-intervention

TABLE 2 Baseline characteristics.

	AG (*n* = 35)	SAG (*n* = 37)	CG (*n* = 12)	*P*-value
Female [*n* (%)]	14 (40.0)	15 (40.5)	5 (41.7)	>0.05
Age, years, mean ± standard deviation	58.2 ± 12.7	60.6 ± 11.2	60.8 ± 6.8	>0.05
Cardiac disease [*n* (%)]	5 (14.3)	3 (8.1)	2 (16.7)	>0.05
Hypertension [*n* (%)]	29 (82.9)	29 (78.4)	3 (25.0)	0.000
Diabetes [*n* (%)]	6 (17.1)	4 (10.8)	2 (16.7)	>0.05
Smoking [*n* (%)]	19 (54.3)	16 (43.2)	6 (50.0)	>0.05
Drinking [*n* (%)]	15 (42.9)	16 (43.2)	5 (41.7)	>0.05
Time from symptom recognition to admission, hours, median (interquartile range)	8.0 (5.0–3.0)	6.0 (4.0–17.0)	/	>0.05
Surgical treatment [*N* (%)]	23 (65.7)	26 (70.3)	/	>0.05
Systolic blood pressure at admission >180 mmHg [*n* (%)]	16 (45.7)	15 (40.5)	0 (0.0)	
Systolic blood pressure at admission 160–180 mmHg [*n* (%)]	5 (14.3)	9 (24.3)	2 (16.7)	0.001
Systolic blood pressure at admission 140–159 mmHg [*n* (%)]	8 (22.9)	5 (13.5)	1 (8.3)	
Systolic blood pressure at admission <140 mmHg [*n* (%)]	6 (17.1)	8 (21.6)	9 (75)	
Baseline BDNF levels, ng/mL, median (interquartile range)	24.5 (17.8–30.2)	21.6 (13.8–31.2)	21.7 (17.3–24.3)	>0.05
GCS score pre-intervention, median (interquartile range)	10.0 (8.0–11.5)	9.0 (7.0–12.0)	/	>0.05

BDNF, brain-derived neurotrophic factor.

TABLE 3 BDNF levels before and after intervention.

BDNF levels, ng/ml, median (interquartile range)	t1	t2	*P*-value
AG	24.5 (17.8∼30.2)	28.0 (26.2∼37.1)	0.035
SAG	21.6 (13.8∼31.2)	19.4 (15.0∼26.3)*	>0.05
CG	21.7 (17.3∼24.3)	22.5 (15.8∼26.7)*	>0.05

*BDNF change values (from t1 to t2) were statistically different from those of AG, $p < 0.01$.
BDNF, brain-derived neurotrophic factor.

TABLE 4 GCS before and after intervention.

GCS, median (interquartile range)	t1	t2	t3	*P*-value
AG	10.0 (8.0∼11.5)	12.0 (10.0∼15.0)*	15.0 (13.0∼15.0)*	0.000
SAG	9.0 (7.0∼12.0)	11.0 (8.0∼13.0)*	12.0 (10.0∼15.0)*	0.000
P-value	>0.05	0.04	0.01	/

*Statistically different compared to GCS at t1 within the group.
GCS, Glasgow Coma Scale.

TABLE 5 Change from t1 to t3 in GCS subscale values and outcomes in t3.

	AG (*n* = 35)	SAG (*n* = 37)	*P*-value
Good outcome (mRS ≤ 2) [*n* (%)]	18 (51.4)	9 (24.3)	0.018*
ΔGCS-E/4 (eye opening)	0.3 (0.0∼0.3)	0.0 (0.0∼0.3)	0.212
ΔGCS-V/5 (verbal response)	0.2 (0.2∼0.5)	0.2 (0.0∼0.4)	0.453
ΔGCS-M/6 (motor response)	0.3 (0.2∼0.5)	0.2 (0.0∼0.3)	0.016

*The *P*-value was obtained by Pearson χ^2 test, $\chi^2 = 5.638$.

levels, indicating that there may be other mechanisms of recovery from ICH besides the effect of acupuncture. The recovery of consciousness and the magnitude of BDNF changes were more pronounced in the AG than in the SAG, suggesting that acupuncture likely helped patients recover more quickly by enhancing mechanisms related to the production of BDNF. Among the three dimensions of the GCS assessment, we found greater improvements in motor response in the AG than in the SAG, suggesting a specific modulatory effect of acupuncture on the motor-related network of stroke patients (Zhang et al., 2021). In addition to this, Zhang et al. (2021) suggested that acupuncture promotes the recovery of verbal functions by regulating neuroplasticity, which can help patients return to society as soon as possible. After the intervention, only the BDNF level of the AG changed significantly after the intervention, suggesting that there may not be a significant effect of sham acupuncture or acupuncture directed to BDNF levels in healthy bodies. In other words, the specific changes that occurs after ICH may be a prerequisite for acupuncture to have an effect on BDNF levels.

This study has some limitations. First, as a product of secretion, peripheral levels of BDNF may be influenced by drugs, and although the effect of interfering factors could be reduced in the AG in comparison with the SAG, we did not restrict patient drug intake during basal treatment, and it is unclear whether changes in BDNF levels in the AG were caused by specific drugs or other unknown factors. Second, this was a single-center trial, and the number of subjects who completed the full trial was 84; therefore, selection bias is inevitable. Third, although we trained each acupuncture operator, differences in techniques between operators may have led to individual differences in efficacy. Fourth, previous studies have suggested that changes in BDNF levels differ at different stages after stroke (Di Lazzaro et al., 2007; Jimenez et al., 2009; Chan et al., 2015; Zhang et al., 2017), and although we controlled the analysis for time from symptom recognition to admission and time from enrolment to onset, we may still have missed the observation time window for BDNF changes in some patients.

5. Conclusion

Acupuncture in the acute phase of ICH has a significant positive effect on both short-term recovery of consciousness and long-term outcomes in patients with ICH, and the production of BDNF may be associated with this effect.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Yongchuan Hospital of Chongqing Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZW designed the trial protocol. YC kept the grouping results until the end of the trial. XW and JG recruited and followed the subjects. LL was responsible for analyzing the data and writing the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Al-Kawaz, M., Hanley, D., and Ziai, W. (2020). Advances in therapeutic approaches for spontaneous intracerebral hemorrhage. *Neurotherapeutics* 17, 1757–1767. doi: 10.1007/s13311-020-00902-w

Chae, Y., Lee, Y., and Enck, P. (2018). How placebo needles differ from placebo pills? *Front. Psychiatry* 9:243. doi: 10.3389/fpsyt.2018.00243

Chan, A., Yan, J., Csurhes, P., Greer, J., and McCombe, P. (2015). Circulating brain derived neurotrophic factor (BDNF) and frequency of BDNF positive T cells in peripheral blood in human ischemic stroke: effect on outcome. *J. Neuroimmunol.* 286, 42–47. doi: 10.1016/j.jneuroim.2015.06.013

Chavez, L., Huang, S., MacDonald, I., Lin, J., Lee, Y., and Chen, Y. (2017). Mechanisms of acupuncture therapy in ischemic stroke rehabilitation: a literature review of basic studies. *Int. J. Mol. Sci.* 18:11. doi: 10.3390/ijms18112270

Di Lazzaro, V., Profice, P., Pilato, F., Dileone, M., Florio, L., Tonali, P., et al. (2007). BDNF plasma levels in acute stroke. *Neurosci. Lett.* 422, 128–130. doi: 10.1016/j.neulet.2007.06.001

Fan, G., and Liu, G. (1997). Timing of acupuncture in the treatment of stroke. *J. Tradit. Chin. Med.* 1997, 178–179.

Gb/T12346-2006, (2006). *The Nomenclature and Location of Acupuncture Points.* Beijing: Standardization Administration of the People's Republic of China.

Greenberg, S., Ziai, W., Cordonnier, C., Dowlatshahi, D., Francis, B., Goldstein, J., et al. (2022). 2022 guideline for the management of patients with spontaneous intracerebral hemorrhage: a guideline from the american heart association/american stroke association. *Stroke* 53, e282–e361. doi: 10.1161/STR.0000000000000407

Jiang, H., Zhang, X., Lu, J., Meng, H., Sun, Y., Yang, X., et al. (2018). Antidepressant-like effects of acupuncture-insights from DNA methylation and histone modifications of brain-derived neurotrophic factor. *Front. Psychiatry* 9:102. doi: 10.3389/fpsyt.2018.00102

Jiang, Y., Wei, N., Zhu, J., Lu, T., Chen, Z., Xu, G., et al. (2010). Effects of brain-derived neurotrophic factor on local inflammation in experimental stroke of rat. *Med. Inflam.* 2010:372423. doi: 10.1155/2010/372423

Jimenez, I., Sobrino, T., Rodriguez-Yanez, M., Pouso, M., Cristobo, I., Sabucedo, M., et al. (2009). High serum levels of leptin are associated with post-stroke depression. *Psychol. Med.* 39, 1201–1209. doi: 10.1017/S0033291709005637

Lehmann, H. (2013). Acupuncture in ancient China: how important was it really? *J. Integr. Med.* 11, 45–53. doi: 10.3736/jintegrmed2013008

Li, H., and Han, B. (2015). Treatment of 60 cases of post-stroke hemiplegia with the Xingnao Kaiqiao acupuncture method combined with blood light quantum therapy. *Western Chin. Med.* 28, 104–106.

Lin, C., Lee, J., Hurt, C., Lazar, R., Arevalo, Y., Prabhakaran, S., et al. (2021). Gait measures at admission to inpatient rehabilitation after ischemic stroke predict 3-month quality of life and function. *PM R* 13, 258–264. doi: 10.1002/pmrj.12402

Lin, Z., Xue, X., Jiang, Y., You, Y., Wang, J., Zhan, Z., et al. (2019). Clinical practice guidelines for chinese medicine rehabilitation – stroke. *J. Rehabilit.* 29:5.

Liu, C., Hsieh, Y., Tseng, H., Lin, H., Lin, C., Wu, T., et al. (2016). Acupuncture for a first episode of acute ischaemic stroke: an observer-blinded randomised controlled pilot study. *Acupunct. Med.* 34, 349–355. doi: 10.1136/acupmed-2015-010825

Liu, F., Yu, K., Song, C., Liu, W., Jiang, Z., and Zhu, W. (2017). Effect of acupuncture for different stage of stroke on motor function: a systematic review and meta-analysis. *J. Clin. Acupunct. Moxib.* 33, 64–68.

Mang, C., Campbell, K., Ross, C., and Boyd, L. (2013). BDNF promoting neuroplasticity for motor rehabilitation after stroke: considering the effects of aerobic exercise and genetic variation on brain-derived neurotrophic factor. *Phys. Ther.* 93, 1707–1716. doi: 10.2522/ptj.20130053

Manning, L., Hirakawa, Y., Arima, H., Wang, X., Chalmers, J., Wang, J., et al. (2014). Blood pressure variability and outcome after acute intracerebral haemorrhage: a post-hoc analysis of INTERACT2, a randomised controlled trial. *Lancet Neurol.* 13, 364–373. doi: 10.1016/S1474-4422(14)70018-3

Pan, W., Banks, W., Fasold, M., Bluth, J., and Kastin, A. (1998). Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology* 37, 1553–1561. doi: 10.1016/S0028-3908(98)00141-5

Polacchini, A., Metelli, G., Francavilla, R., Baj, G., Florean, M., Mascaretti, L., et al. (2015). A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. *Sci. Rep.* 5:17989. doi: 10.1038/srep17989

Shi, X. (2005). Clinical study on 9005 cases of stroke disease treated by Xingnao-Kaiqiao acupuncture method. *Chin. Med. Herald* 2005, 3–5.

Xiong, J., Zhang, Z., Ma, Y., Li, Z., Zhou, F., Qiao, N., et al. (2020). The effect of combined scalp acupuncture and cognitive training in patients with stroke on cognitive and motor functions. *NeuroRehabilitation* 46, 75–82. doi: 10.3233/NRE-192942

Yu, Z., Tao, C., Xiao, A., Wu, C., Fu, M., Dong, W., et al. (2020). Yu Xuezhong, Tao Chuan Yuan, Xiao Anqi, Wu Cong: Chinese multidisciplinary guidelines for the management of hypertensive cerebral hemorrhage. *Chin. Emer. Med.* 40, 689–702.

Zhang, J., Lu, C., Wu, X., Nie, D., and Yu, H. (2021). Neuroplasticity of acupuncture for stroke: an evidence-based review of MRI. *Neural. Plast.* 2021:2662585. doi: 10.1155/2021/2662585

Zhang, J., Mu, X., Breker, D., Li, Y., Gao, Z., and Huang, Y. (2017). Atorvastatin treatment is associated with increased BDNF level and improved functional recovery after atherothrombotic stroke. *Int. J. Neurosci.* 127, 92–97. doi: 10.3109/00207454.2016.1146882

Zhang, T., Zhao, J., Bai, Y., Li, X., Qu, Y., Wang, B., et al. (2019). Chinese clinical management guidelines for cerebrovascular disease (abridged version) – Stroke rehabilitation management. *Chinese J. Neurol.* 14, 823–831. doi: 10.1136/svn-2019-000321

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Acupuncture and other traditional Chinese medicine therapies in the treatment of children's tic syndrome: A network meta-analysis

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Background: Tic disorders (TD) are a kind of neuropsychiatric disease that frequently occur among preschool and school-age children, mainly characterized by motor tics or sometimes accompanied by vocal tics, and its pathogenesis is still unclear. The clinical manifestations are mainly characterized by chronic multiple movements, rapid muscle twitching, involuntary occurrence, and language disorder. Acupuncture, tuina, traditional Chinese medicine, and other methods are commonly used in clinical treatments, which have unique therapeutic advantages but have not been recognized and accepted by the international community. This study conducted a quality evaluation and meta-analysis of the currently published randomized controlled trials (RCTs) of acupuncture for TD in children in order to provide reliable evidence-based medical evidence for acupuncture for TD.

Methods: All the randomized controlled trials (RCTs) using the intervention methods acupuncture + traditional Chinese medical herbs, acupuncture + tuina, and acupuncture, and the control group using Western medicine were included in the analysis. The main outcomes were obtained by using the Yale Global Tic Severity Scale (YGTSS), the Traditional Chinese medicine (TCM) syndrome score scale, and clinical treatment efficiency. Secondary outcomes included adverse events. The risk of bias in the included studies was assessed according to the tool recommended by Cochrane 5.3. The risk of bias assessment chart, risk of bias summary chart, and evidence chart in this study will be produced using R and Stata software.

Results: There were 39 studies that met the inclusion criteria, including 3,038 patients. In terms of YGTSS, the TCM syndrome score scale changes and shows a clinically effective rate, and we found that acupuncture combined with Chinese medicine is the best treatment.

Conclusion: Acupuncture+traditional Chinese medical herbs may be the best therapy to improve TD in children. At the same time, compared with Western medicine commonly used in clinical practice, acupuncture and acupuncture combined with tuina therapy have better effects on improving TD in children.

KEYWORDS

stroke, children's tic syndrome, neural mechanism, acupuncture, acupuncture and moxibustion, network meta-analysis

1. Introduction

Children's tic disorder is a common movement disorder in childhood, and its incidence has gradually increased in recent years (Knight et al., 2012; Cubo et al., 2017). Its prevalence rate among preschool children has reached as high as 6.1% (Yang et al., 2016), with a male-to-female ratio of about 4:1 (Robertson et al., 2009). The prevalence of this disease in children and adolescents is 0.1–0.6%, and the male-to-female ratio is (3–4):1. After treatment, approximately two-thirds of the children can achieve control or symptom relief, and approximately one-third of the children's symptoms still fluctuate and can continue to adulthood. Clinical symptoms are mainly manifested as multiple, involuntary muscle production in one or more parts, and repeated rapid motor twitch and vocal twitch (Freeman et al., 2000). Sometimes, attention deficit hyperactivity disorder (ADHD), obsessive–compulsive disorder, and other mental complications can be accompanied clinically, thus increasing the clinical treatment difficulty of children with TD (Scharf et al., 2012; Zinner et al., 2012).

Currently, in the Western medicine treatment system, the main treatment methods for children with tic disorders are oral Western drugs such as haloperidol, thioperide, and risperidone (Weisman et al., 2013). Although these drugs have certain curative effects, children and their parents are more inclined to use non-drug therapy due to adverse reactions such as drowsiness and nausea and the instability of the curative effect (McHugh et al., 2013; Mogwitz et al., 2013).

At present, a large number of studies have shown that perinatal adverse factors may lead to the onset of TD in children (Pasamanick and Kawi, 1956; Bos-Veneman et al., 2010; Hoekstra et al., 2013). Bad habits are also an important factor leading to the onset of TD. According to previous studies, cola, coffee, black tea, preservatives, and sweeteners can directly or indirectly affect the dopamine system in the brain, thereby causing or aggravating tic symptoms in children (Müller-Vahl et al., 2008). At the same time, with the development of social science and technology, the long-term use of mobile phones, televisions, and computers leads to the long-term exposure of children to radiofrequency radiation and magnetic field radiation, which may change the function of the central nervous system by changing the structure of nerve cell membranes (Maier et al., 2004). Other studies believe that the incidence and recurrence of TD in children may be partly associated with the infection of pathogenic microorganisms. Studies have found (Krause et al., 2012) that the decline of immune function and the abnormal production of autoantibodies after infection in some children are caused by the imbalance of the autoimmune system. Repeated viral or bacterial infection is an important cause of immune dysfunction and autoimmune pathological damage. Generally, simple TD only damages the subcortical tissue structure such as the basal ganglia, but the hyperactivity disorder caused by TD may also cause damage to the frontal lobe, resulting in patients being more prone to problems such as attention deficit or aggression.

Traditional Chinese medicine theory related to the disease is often relegated to emotion, diet, innate endowment, and external sensation. Although there is no significant organic disease and damage, the motor twitch and vocal twitch caused by this disease seriously affect the growth and development of children, life and learning, and mental state. According to the traditional Chinese medicine theory, the treatment of this disease is mainly related to "jiang huo" (reduce the fire in the body), "xi feng" (extinguish the wind inside), "hua tan" (reduce phlegm in the body), and other treatment methods such as acupuncture and other traditional Chinese medicine means of comprehensive treatment, the effect is very significant.

2. Methods

2.1. Protocol and registration

The protocol of this systematic review and meta-analysis is registered in PROSPERO, under the registration number CRD42022376370. It is available from the following website.[1]

2.2. Inclusion criteria

The studies included in this study should be randomized controlled trials (RCTs). In the treatment group, acupuncture combined with tuina, acupuncture combined with traditional Chinese medical herbs, and simple acupuncture (needle materials, acupoints, and techniques do not limit the treatment means) were included. The control group was treated with Western drugs that were more commonly used in clinical practice, including but not limited to haloperidol, thioperide, and risperidone.

2.3. Exclusion criteria

In this analysis, we excluded the following criteria: (1) non-randomized controlled trials; (2) trials without baseline data

1 http://h-p.www.crd.york.ac.uk.hnucm.opac.vip/PROSPERO/

assessment; (3) animal experimental studies, reviews, meeting minutes, case reports, or expert experience summaries, and other non-randomized controlled trial studies; (4) the number of people in the experimental group and the control group was significantly different; (5) usage of the blank control test; (6) in the same experiment, the two groups had the same type of intervention, such as millimeter needle treatment in both groups; (7) only abstract available and the lack of full text or important information report is incomplete and the contact author did not respond; (8) the original text is not standard, and there are obvious mistakes.

2.4. Outcomes

2.4.1. Primary outcomes

1. Motor tic, vocal tic, and tic total scores on the Yale Global Tic Severity Scale (YGTSS);
2. The changes of nodding and shrugging, the voice in the larynx, upset, and main symptom scores in the Traditional Chinese medicine (TCM) syndrome score scale.

2.4.2. Secondary outcomes

1. Clinical effective rate;
2. Adverse reactions.

TABLE 1 Search strategy.

No.	Literature search term
1	randomized controlled trial [pt]
2	controlled clinical trial [pt]
3	randomized [tiab]
4	placebo [tiab]
5	clinical trials as topic [mesh:noexp]
6	randomly [tiab]
7	trial [ti]
8	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7
9	animals [mh] NOT humans [mh]
10	#8 NOT #9
11	tic disorder [mesh]
12	tourette syndrome [tiab]
13	#11 OR #12
14	acupuncture [mesh]
15	acupuncture therapy [mesh]
16	massage [tiab]
17	tuina [tiab]
18	herb [tiab]
19	#14 OR #15 OR #16 OR #17 OR #18
20	#8 AND #13 AND #19
21	remove duplicates from #20

2.5. Literature search

The literature search time of this study was from the establishment date of each database to 15 November 2022, and the following databases were searched: China Biology Medicine, China National Knowledge Infrastructure, Wan Fang Data, the Chinese Science and Technology Periodical Database, Medline, Excerpt Medical Database (EMBASE), Web of Science, and the Cochrane Library, which has no national or language restrictions. The search strategy includes a combination of free-text terms, synonyms, and subject headings related to Tourette syndrome in children and its associated subjects (Table 1).

2.6. Study selection and data extraction

2.6.1. Selection of studies

Literature screening and data extraction were carried out independently by two professionally trained researchers, and the results were cross-checked by two persons. In case of disagreement or dispute, they were resolved through discussion or consultation with a third party. Data extraction included author, year, age, sample size, intervention measures, course of treatment, outcome indicators, adverse reactions, and literature type. The study selection process is illustrated in Figure 1.

2.6.2. Data extraction and management

According to the bias risk assessment tool recommended by the Cochrane Handbook 5.1, our team members conducted a bias analysis of the included articles. As acupuncture and tuina are non-drug treatments, some participants and researchers involved in these studies could not be blinded (Table 2).

2.6.3. Assessment of risk of bias

Two independent reviewers assessed the risk of bias using the Cochrane Collaboration's bias risk assessment tool. Specifically, evaluators assessed the risk of bias associated with random sequence generation (selection bias), assignment hiding (selection bias), subject and researcher blindness (implementation bias), outcome rater blindness (detection bias), integrity of reported outcome data (loss of follow up bias), and selective outcome reporting (report bias). Any disagreements about the risk of bias were resolved through discussions within the review group.

2.7. Assessment of trial quality and statistical analysis

The Grades of Recommendations Assessment, Development and Evaluation (GRADE) classification was used to grade the six outcome indicators. Stata 14.2 software was used to perform mesh meta-analysis (Zhang et al., 2014). The interrelationship between the interventions was represented by evidence network plots. Continuous variables were represented by average difference (MD) and 95% confidence intervals, while dichotomous variables were represented by odds ratio (OR) and 95% CI. A network evidence graph was drawn for outcome indicators. When closed rings were formed in the network diagram, the inconsistency test was needed to be carried out, and the inconsistency factor (IF)

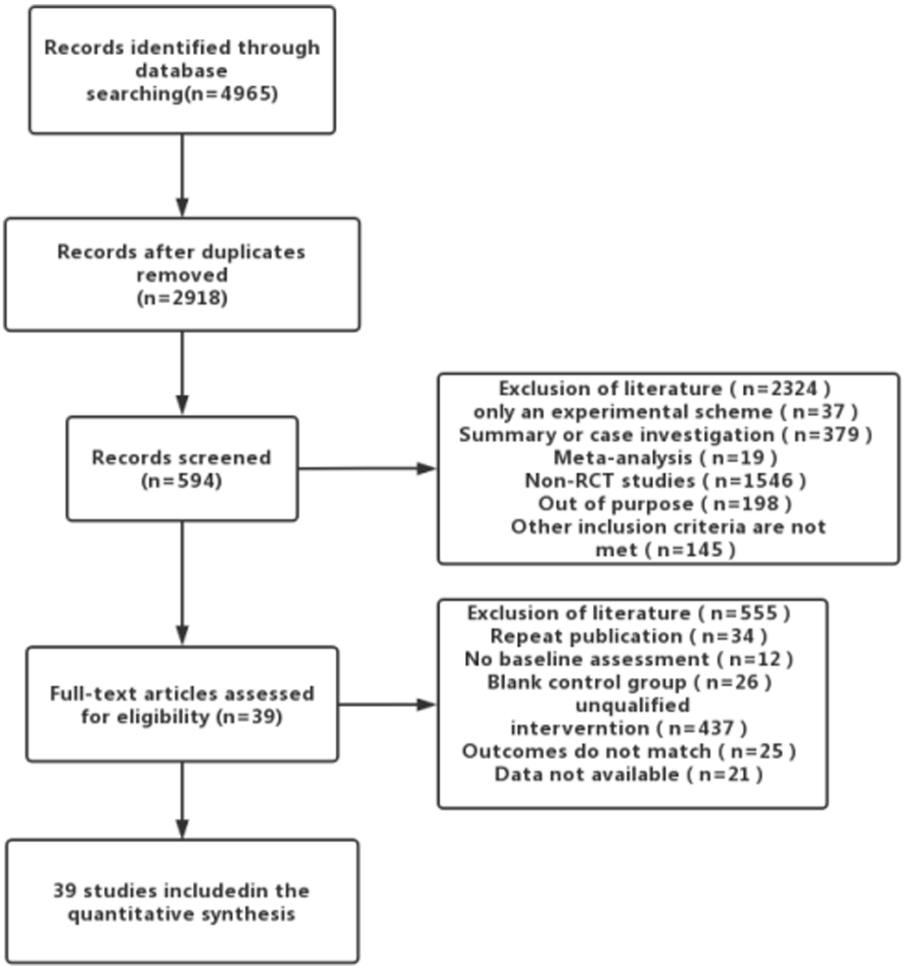

FIGURE 1
Process of literature search results and study selection.

was calculated. IF value and *p*-value were used to determine any inconsistency. IF results of direct and indirect comparisons are consistent; the results of IF are close to 0% and the 95% confidence interval (CI) starts at 0, with a *p*-value of >0.05. The cumulative ranking curve (SUCRA) and the surface under the cumulative ranking curve surface under the cumulative ranking curve (SUCRA) were compared for different interventions, and the cumulative ranking curve (0 ~ 100) was used to rank the efficacy of various interventions. The larger the value is and the larger the area under the curve, indicating that the intervention is more likely to be the best intervention (Salanti et al., 2011; Chaimani et al., 2013). Meanwhile, the comparison-correction funnel plot was drawn to determine whether there was a publication bias or a small sample effect (Dias et al., 2013).

3. Results

3.1. Characteristics of included studies

Overall, relevant studies were retrieved from the database, and 39 RCT experiments were finally included in the analysis after one study was removed. All the studies are from China. It involves four intervention measures: acupuncture + tuina, acupuncture + traditional Chinese medical herbs, ordinary acupuncture, and commonly used Western medicine (Table 3).

3.2. Assessment of risk of bias

The risk analysis and summary of bias for all the studies included in this review are shown in Figures 2, 3.

3.3. Meta-analyses of the outcomes

3.3.1. Results of pairwise meta-analyses

The results of Pairwise Meta-Analyses are shown in Figure 4. The interrelationship between the interventions was represented by evidence network plots. Continuous variables were represented by average difference (MD) and 95% confidence intervals, while dichotomous variables were represented by odds ratio (OR) and 95% CI. A network evidence graph was drawn for outcome indicators.

TABLE 2 Result of data extraction and management.

	Study ID	Random sequence generation selection bias	Allocation concealment selection bias	Blinding of participants and personnel performance bias	Blinding of outcome assessment detection bias	Incomplete outcome data attrition bias	Selective reporting bias	Other bias	Weight
1	Shuai Sun 2021	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	60
2	Zhonghua Qin 2017	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	110
3	Yiyi Zeng 2016	High	Unclear	Unclear	Unclear	Low	Unclear	Low	60
4	Jinng Huang 2022	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	70
5	Xueyuan Jiang 2009	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	70
6	Lili Zhang 2021	Low	Low	Unclear	Unclear	Low	Unclear	Low	60
7	Wang Luo 2021	Low	Low	Unclear	Unclear	Low	Unclear	Low	60
8	Tao Xu 2014	Low	Low	Unclear	Unclear	Low	Unclear	Low	140
9	Xiaocheng Shi 2012	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	84
10	Xiaowei Wei 2005	High	Unclear	Unclear	Unclear	Low	Unclear	Low	120
11	Wei Wang 2019	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	89
12	Ying Tang 2015	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	47
13	Liping Cui 2021	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	68
14	Lingzhi Wu 2018	Low	Low	Unclear	Unclear	Low	Unclear	Low	65
15	Wenqing Zou 2011	High	Low	Unclear	Unclear	Low	Unclear	Low	65
16	Yan Li 2021	Unclear	Low	Unclear	Unclear	Low	Unclear	Low	64
17	Xiaoshu Xie 2021	Low	Low	Unclear	Unclear	Low	Unclear	Low	100
18	Qiang Li 2019	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	65
19	Qinghua Zhu 2022	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	66
20	Lingzhe Li 2021	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	80
21	Kaipeng Liang 2016	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	149
22	Zusen Guo 2014	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	60
23	Yu Xia 2022	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	62
24	Lanzhi Huang 2019	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	60
25	Liping Shen 2019	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	80
26	Yulin Chen 2016	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	86
27	Lezhong He 2012	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	56
28	Ning Xu 2005	High	Unclear	Unclear	Unclear	Low	Unclear	Low	110

(Continued)

TABLE 2 (Continued)

	Study ID	Random sequence generation selection bias	Allocation concealment selection bias	Blinding of participants and personnel performance bias	Blinding of outcome assessment detection bias	Incomplete outcome data attrition bias	Selective reporting bias	Other bias	Weight
29	Aiqun Mo 2018	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	64
30	Xijuan Zhang 2015	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	76
31	Chaying Hu 2022	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	48
32	Yan Zhang 2019	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	70
33	Wei Ni 2017	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	57
34	Dongwei Sun 2005	High	Unclear	Unclear	Unclear	Low	Unclear	Low	49
35	Haisheng Wu 2021	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	98
36	Tianhong Hu 2018	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	80
37	Xiong Qian 2017	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	72
38	Jianxi Wang 2013	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	60
39	Donglan Ye 2013	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	82

3.4. Primary outcome

3.4.1. YGTSS-motor tics

A total of 24 articles reported changes in YGTSS motor twitch involving 1,767 patients. The mesh meta-analysis showed results of acupuncture + tuina (MD = 3.77, 95% CI [2.12, 5.42]), acupuncture + traditional Chinese medical herbs (MD = 4.90, 95% CI [3.69, 6.10]), and acupuncture (MD = 3.96, 95% CI [1.75, 6.17]) (Supplementary Table S1).

3.4.1.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs (SUCRA = 87.3) > acupuncture + tuina (SUCRA = 59.6) > acupuncture (SUCRA = 53.1) (Supplementary Figure S1).

3.4.2. YGTSS-vocal tics

A total of 21 pieces of literature reported changes in YGTSS vocal tic involving 1,626 patients. The reticular meta-analysis showed results of acupuncture + tuina (MD = 3.50, 95% CI [2.82, 4.18]), acupuncture + traditional Chinese medical herbs (MD = 4.03, 95% CI [3.50, 4.55]), and acupuncture (MD = 3.40, 95% CI [2.58, 4.22]) (Supplementary Table S2).

3.4.2.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs (SUCRA = 92.6) > acupuncture (SUCRA = 56.5) > acupuncture = tuina (SUCRA = 50.9) (Supplementary Figure S2).

3.4.3. YGTSS-total score

A total of 21 pieces of literature reported changes in the total score of YGTSS involving 1,649 patients. The mesh meta-analysis showed results of acupuncture + tuina (MD = 6.25, 95% CI [5.44, 7.05]), acupuncture + traditional Chinese medical herbs (MD = 6.98, 95% CI [6.31, 7.65]), and acupuncture (MD = 5.95, 95% CI [4.23, 7.68]) (Supplementary Table S3).

3.4.3.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs (SUCRA = 92.3) > acupuncture (SUCRA = 57.4) > acupuncture + tuina (SUCRA = 50.3) (Supplementary Figure S3).

3.4.4. Score for TCM syndrome-nod and shrug

A total of nine pieces of literature reported changes in score for the TCM syndrome score scale, nod and shrug, involving 712 patients. The reticular meta-analysis showed results of acupuncture + tuina (MD = 1.48, 95% CI [0.93, 2.03]), acupuncture + traditional Chinese medical herbs (MD = 1.73, 95% CI [1.16, 2.30]), and acupuncture (MD = 1.70, 95% CI [1.15, 2.26]) (Supplementary Table S4).

3.4.4.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs (SUCRA = 75) > acupuncture + tuina (SUCRA = 73.1) > acupuncture (SUCRA = 51.9) (Supplementary Figure S4).

TABLE 3 Characteristics of included RCTs.

Age	Study population		Intervention		Period of treatment		Outcome measures	Adverse events		References
	T	C	T	C	T	C		T	C	
7.7 ± 2.4	8.9 ± 1.5	30	30	Acupuncture+tuina	Western medicine	4 Weeks	①②③⑤⑥⑧	1	6	Shuai (2020)
8.04 ± 2.56	8.05 ± 2.58	54	56	Acupuncture+tuina	Western medicine	24 Weeks	①②③④⑦⑧	0	9	Zhonghua et al. (2017)
4.87 ± 0.78	4.8 ± 0.76	30	30	Acupuncture+tuina	Western medicine	4 Weeks	③⑤⑦⑧	\	\	Yiyi et al. (2016)
7.43 ± 2.35	7.62 ± 2.42	34	36	Acupuncture+tuina	Western medicine	4 Weeks	①②④⑦⑧	0	3	Jing (2022)
8.23 ± 2.31	8.25 ± 1.26	40	30	Acupuncture+tuina	Western medicine	8 Weeks	①②⑥⑦⑧	\	\	Xueyuan (2009)
9.31 ± 2.14	8.67 ± 2.53	30	30	Acupuncture+Chinese medicine	Western medicine	8 Weeks	①②④⑤⑥⑧	\	\	Lili et al. (2021)
7.56 ± 3.32	7.48 ± 2.65	30	30	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①②⑤⑧	0	6	Wang (2021)
8.12 ± 1.45	8.48 ± 3.26	70	70	Acupuncture+Chinese medicine	Western medicine	8 Weeks	①②⑤⑦⑧	\	\	Tao and Jingdong (2014)
6.62 ± 2.14	5.35 ± 2.33	40	40	Acupuncture+Chinese medicine	Western medicine	8 Weeks	①②⑤⑧	\	\	Li Liang and Xiang (2012)
8.51 ± 2.33	8.13 ± 2.32	42	42	Acupuncture+Chinese medicine	Western medicine	6 Weeks	①②⑧	0	6	Xiaocheng et al. (2012)
7.95 ± 2.65	7.46 ± 3.21	60	60	Acupuncture+Chinese medicine	Western medicine	12 Weeks	④⑦⑧	\	\	Xiaowei et al. (2005)
8.08 ± 3.16	8.17 ± 2.97	45	44	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①②③⑥⑦⑧	\	\	Wei and Fei (2019)
7.65 ± 2.31	7.18 ± 2.15	25	22	Acupuncture+Chinese medicine	Western medicine	12 Weeks	④⑥⑧	4	6	Ying et al. (2015)
10.34 ± 3.64	10.38 ± 3.52	34	34	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①④⑤⑦⑧	2	6	Liping and Ningning (2021)
6.58 ± 2.72	5.97 ± 2.48	33	32	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①②③⑦⑧	2	0	Wu Lingzhi et al. (2018)
7.62 ± 3.45	7.45 ± 2.13	33	32	Acupuncture+Chinese medicine	Western medicine	4 Weeks	④⑤⑧	0	10	Wenqing (2011)
8.57 ± 1.96	7.51 ± 1.18	32	32	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①②③⑦⑧	0	12	Yan et al. (2021)
8.05 ± 1.60	8.06 ± 1.58	50	50	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①②③⑦⑧	1	10	Xiaoshu et al. (2021)
9.08 ± 2.10	9.74 ± 2.23	37	38	Acupuncture+Chinese medicine	Western medicine	12 Weeks	④⑤⑧	2	15	Qiang (2019)
9.61 ± 2.67	9.59 ± 2.64	34	32	Acupuncture+Chinese medicine	Western medicine	8 Weeks	①②③⑦⑧	\	\	Qinghua et al. (2022)
8.30 ± 3.53	8.25 ± 3.65	40	40	Acupuncture+Chinese medicine	Western medicine	8 Weeks	①②③⑧	2	8	Li Lingzhe et al. (2021)
8.21 ± 1.23	8.62 ± 1.53	78	71	Acupuncture+Chinese medicine	Western medicine	12 Weeks	①②③⑧	1	3	Kaipeng (2016)
7.15 ± 2.35	7.48 ± 3.41	30	30	Acupuncture+Chinese medicine	Western medicine	8 Weeks	①②③⑧	4	6	Zusen et al. (2014)
7.69 ± 2.17	8.21 ± 2.34	31	31	Acupuncture	Western medicine	4 Weeks	①②③	1	7	Xia and Hongnan (2022)
8.83 ± 2.49	9.47 ± 2.52	30	30	Acupuncture	Western medicine	4 Weeks	①②③	0	6	Lanzhi (2019)
9.23 ± 2.79	8.90 ± 2.45	40	40	Acupuncture	Western medicine	8 Weeks	⑤⑥⑦⑧	4	8	Liping (2019)
8.4 ± 3.3	9.6 ± 4.2	43	43	Acupuncture	Western medicine	4 Weeks	④⑤⑧	0	3	Yulin (2016)
7.84 ± 3.43	7.49 ± 1.68	29	27	Acupuncture	Western medicine	8 Weeks	①②③⑧	\	\	Lezhong et al. (2012)
8.1 ± 2.6	7.9 ± 2.3	55	46	Acupuncture	Western medicine	8 Weeks	④⑦⑧	\	\	Ning and Haizhu (2005)

(Continued)

TABLE 3 (Continued)

Age	Study population		Intervention		Period of treatment		Outcome measures	Adverse events		References
	T	C	T	C	T	C		T	C	
8.54 ± 3.23	8.15 ± 1.58	23	23	Acupuncture	Western medicine	8 Weeks	①③④⑧	/	/	Aiqun and Guiling (2018)
7.62 ± 2.65	7.42 ± 1.20	44	43	Acupuncture	Western medicine	12 Weeks	④⑥⑦⑧	/	/	Xijuan et al. (2015)
7.80 ± 2.34	8.11 ± 1.98	24	24	Acupuncture	Western medicine	16 Weeks	①②③⑧	1	3	Chaying and Hui (2022)
9.23 ± 1.84	8.97 ± 1.74	35	35	Acupuncture	Western medicine	8 Weeks	①②⑧	/	/	Yan (2019)
7.52 ± 2.85	7.624 ± 2.36	29	28	Acupuncture	Western medicine	4 Weeks	①②③⑥⑦⑧	/	/	Wei (2016)
8.26 ± 1.25	8.45 ± 2.32	25	24	Acupuncture	Western medicine	4 Weeks	③⑥⑦⑧	/	/	Dongwei (2005)
8.45 ± 2.33	8.17 ± 2.24	49	49	Acupuncture	Western medicine	8 Weeks	⑥⑦⑧	1	6	Haisheng et al. (2021)
7.14 ± 2.51	7.46 ± 2.33	40	40	Acupuncture	Western medicine	8 Weeks	①②③⑧	5	5	Tianhong (2018)
7.44 ± 2.62	7.15 ± 2.42	37	35	Acupuncture	Western medicine	12 Weeks	⑥⑦⑧	/	/	Xiong et al. (2017)
7.58 ± 1.49	7.62 ± 3.28	30	30	Acupuncture	Western medicine	8 Weeks	①②⑤⑧	1	6	Jianxi (2013)
8.1 ± 2.5	8.1 ± 2.5	82	82	Acupuncture	Western medicine	4 Weeks	⑤⑦⑧	0	9	Donglan (2013)

① YGTSS motor twitch; ② YGTSS vocal twitch; ③ total score of YGTSS; ④ TCM Syndrome Score Scale—nod and shrug; ⑤ TCM Syndrome Score Scale—laryngeal vocalization; ⑥ TCM syndrome score scale—upset; ⑦ TCM Syndrome Score Scale—total score; ⑧ clinical effectiveness.

3.4.5. Score for TCM syndrome-vocalization in the larynx

A total of 10 pieces of literature reported changes in the score for the TCM syndrome score scale, laryngeal vocalization, involving 804 patients. The reticular meta-analysis showed results of acupuncture + tuina (MD = 1.19, 95% CI [0.81, 1.57]), acupuncture + traditional Chinese medical herbs (MD = 1.34, 95% CI [0.88, 1.80]), acupuncture (MD = 1.50, 95% CI [1.06, 1.95]) (Supplementary Table S5).

3.4.5.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + tuina (SUCRA = 84.7) > acupuncture + traditional Chinese medical herbs (SUCRA = 66.8) > acupuncture (SUCRA = 48.4) (Supplementary Figure S5).

3.4.6. Score for TCM syndrome-vexation

A total of 10 pieces of literature reported changes in the score for the TCM syndrome score scale, upset, involving a total of 666 patients. The mesh meta-analysis showed results of acupuncture + tuina (MD = 0.98, 95% CI [0.44, 1.52]), acupuncture + traditional Chinese medical herbs (MD = 1.24, 95% CI [0.76, 1.72]), and acupuncture (MD = 1.17, 95% CI [0.62, 1.73]) (Supplementary Table S6).

3.4.6.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs (SUCRA = 78.1) > acupuncture + tuina (SUCRA = 70) > acupuncture (SUCRA = 51.9) (Supplementary Figure S6).

3.4.7. Score for TCM syndrome-primary symptom

A total of 15 pieces of literature reported changes in the score for the TCM syndrome score scale, main syndrome, involving a total of 1,174 patients. The results of the reticular meta-analysis showed that acupuncture + tuina (MD = 2.82, 95% CI [2.14, 3.51]), acupuncture + traditional Chinese medical herbs (MD = 4.09, 95% CI [3.45, 4.73]), and acupuncture (MD = 3.17, 95% CI [2.41, 3.93]) (Supplementary Table S7).

3.4.7.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs (SUCRA = 98.6) > acupuncture + tuina(SUCRA = 59.4) > acupuncture (SUCRA = 42) (Supplementary Figure S7).

3.5. Secondary outcome

3.5.1. Clinical efficiency

The clinical effective rate was reported in 39 pieces of literature, involving a total of 3,038 patients. The mesh meta-analysis showed results of acupuncture + tuina (OR = 1.97, 95% CI [1.10, 3.51]), acupuncture + traditional Chinese medical herbs (OR = 3.06, 95% CI [2.07, 4.51]), and acupuncture (OR = 1.72, 95% CI [1.29, 2.30]) (Supplementary Table S8).

3.5.1.1. Sorting of network meta-analysis results

The sorting of network meta-analysis results showed that acupuncture + traditional Chinese medical herbs

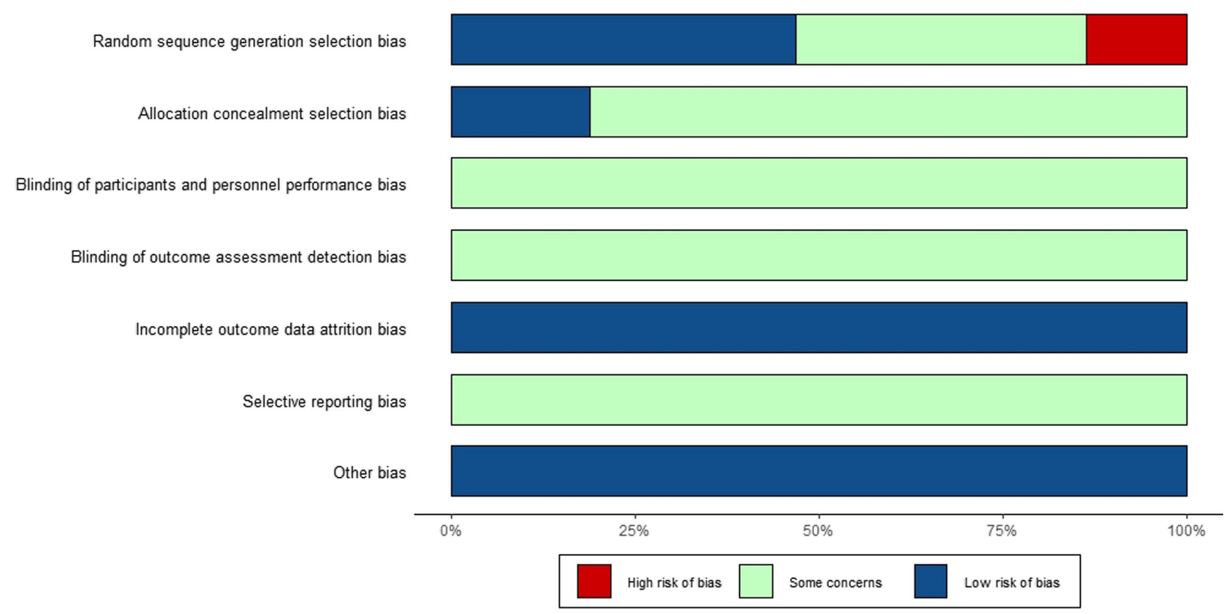

FIGURE 2
Risk of bias among included RCTs.

(SUCRA = 96.2) > acupuncture + tuina (SUCRA = 58) > acupuncture (SUCRA = 45.4) (Supplementary Figure S8).

3.6. Publication bias

In terms of the overall response rate, the heterogeneity detected in all included RCTs was low, so the fixed effects model was chosen for statistical analysis. Funnel plots were used to assess the potential role of publication bias in this review. All studies were symmetrically distributed around the X = 0 vertical line and the funnel plot was symmetric, indicating that there was no evidence of small-sample effects in the study network (Figure 5).

3.7. Adverse events

Of the 39 included studies, a total of 24 studies reported the occurrence of adverse events. A total of 50 patients had dizziness, 61 patients had nausea, 49 patients had drowsiness, 23 patients had fatigue, and eight patients had dry mouth. Among the five RCTs (acupuncture + tuina) and Western medicine, a total of four reported adverse reactions, with two cases in the experimental group and 23 cases in the control group. There were 16 cases of adverse reactions in the experimental group and 84 cases in the control group in the 17 RCTs of acupuncture + traditional Chinese medical herbs and Western medicine. In the 17 RCTs of acupuncture and Western medicine, there were 14 cases of adverse reactions reported in the experimental group and 52 cases in the control group.

3.8. GRADE classification of outcome indicators

The GRADE classification was used to grade the six outcome indicators. The results showed that the evidence of the YGTSS score, TCM syndrome score scale, and the clinical effective rate was rated as "medium" quality (Table 4).

4. Discussion

4.1. Summary of the main results

A total of 39 RCT studies on acupuncture treatment of children with tic disorders were included in this study, and strict quality evaluation and risk assessment were carried out on the included studies. From the perspective of adverse reactions, compared to conventional Western drugs such as haloperidol, thiopiride, and risperidone, ordinary acupuncture or acupuncture combined with traditional Chinese medicine or acupuncture combined with massage have a lower incidence of adverse reactions, improving clinical treatment safety, and increasing clinical patient compliance. The scores of nodding and shrugging, laryngeal vocalization, upset, and TCM main symptoms in the TCM syndrome score scale were reduced. From the perspective of adverse reactions, compared with conventional Western drugs such as haloperidol, thioperide, and risperidone, both ordinary acupuncture itself, acupuncture combined with traditional Chinese medical herbs or acupuncture combined with tuina, have a lower incidence of adverse reactions, improving the clinical safety of the treatment and increasing the compliance of clinical patients.

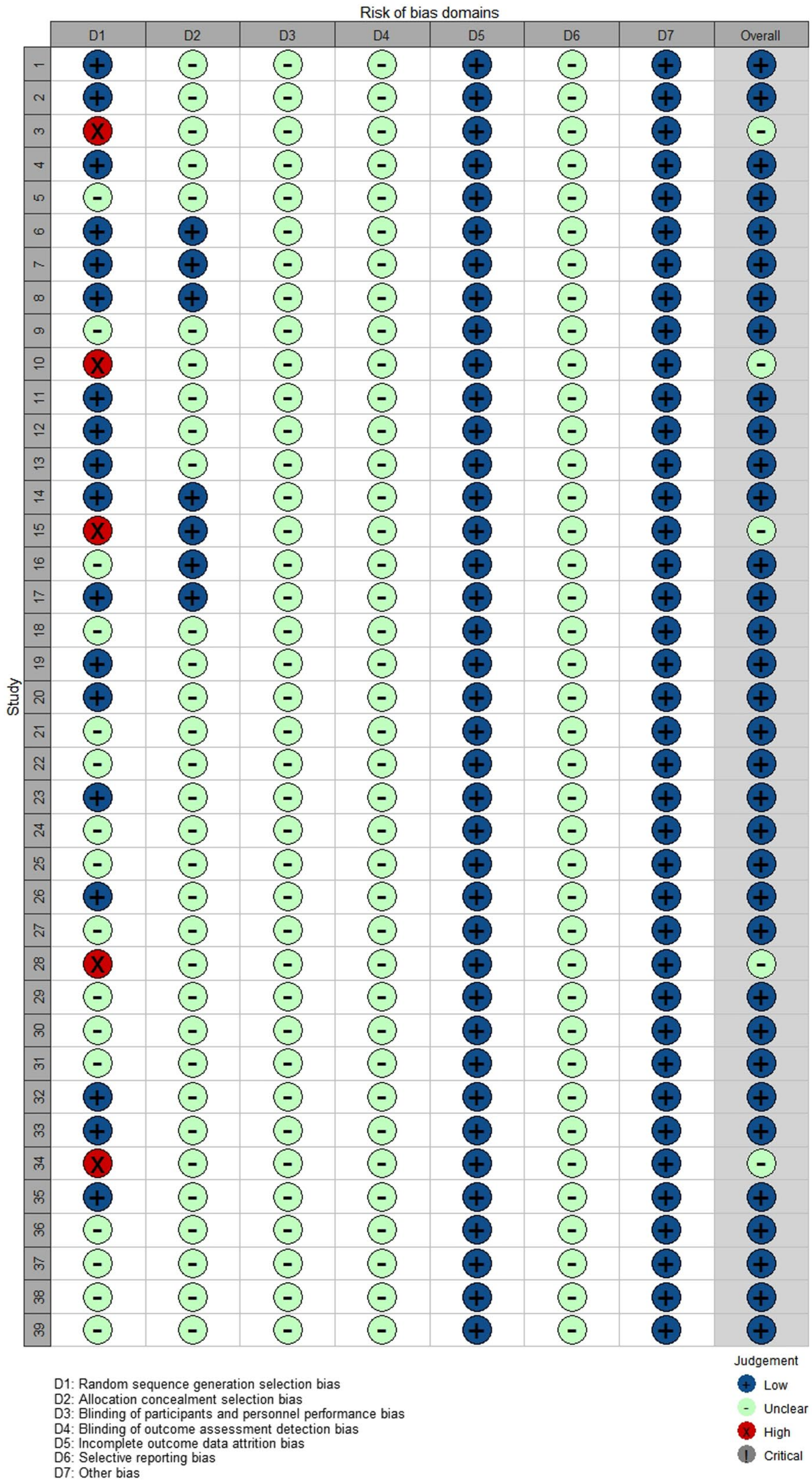

FIGURE 3
Risk of bias among included RCTs. Shuai Sun 2020 (1), Zhonghua Qin 2017 (2), Yiyi Zeng 2016 (3), Jinng Huang 2022 (4), Xueyuan Jiang 2009 (5), Lili Zhang 2021 (6), Wang Luo 2021 (7), Tao Xu 2014 (8), Xiaocheng Shi 2012 (9), Xiaowei Wei 2005 (10), Wei Wang 2019 (11), Ying Tang 2015 (12), Liping Cui 2021 (13), Lingzhi Wu 2018 (14), Wenqing Zou 2011 (15), Yan Li 2021 (16), Xiaoshu Xie 2021 (17), Qiang Li 2019 (18), Qinghua Zhu 2022 (19), Lingzhe Li 2021 (20), Kaipeng Liang 2016 (21), Zusen Guo 2014 (22), Yu Xia 2022 (23), Lanzhi Huang 2019 (24), Liping Shen 2019 (25), Yulin Chen 2016 (26), Lezhong He 2012 (27), Ning Xu 2005 (28), Aiqun Mo 2018 (29), Xijuan Zhang 2015 (30), Chaying Hu 2022 (31), Yan Zhang 2019 (32), Wei Ni 2017 (33), Dongwei Sun 2005 (34), Haisheng Wu 2021 (35), Tianhong Hu 2018 (36), Xiong Qian 2017 (37), Jianxi Wang 2013 (38), and Donglan Ye 2013 (39).

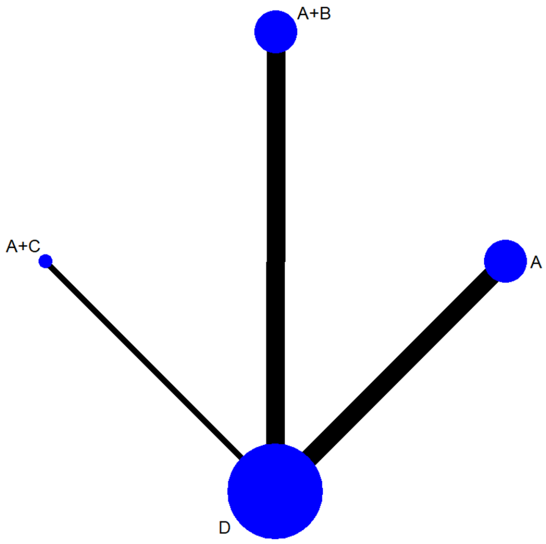

FIGURE 4
Results of pairwise meta-analyses. Acupuncture **(A)**, acupuncture+traditional Chinese medicine **(B)**, acupuncture+tuina **(C)**, western medicine **(D)**.

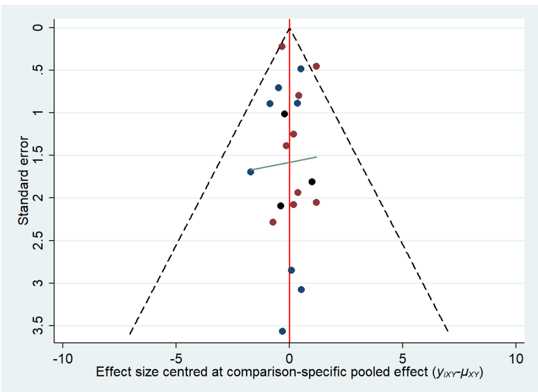

FIGURE 5
Results of publication bias.

The pathogenesis of tic disorder in children is complex, and multi-angle or multi-form combined treatment should be used in clinics. Acupuncture can effectively inhibit the occurrence of muscle twitching symptoms, and Chinese medicine can reduce the occurrence of diseases by regulating the abnormal state of the body (Guoxiang et al., 2017; Hanyuan and Zhenggang, 2017). The combination of acupuncture and traditional Chinese medical herbs has various forms, which is helpful to improve the clinical therapeutic effect, reduce the recurrence rate, and greatly improve the treatment compliance of patients and parents, thus improving the therapeutic effect.

The results of this study show that acupuncture combined with tuina, acupuncture combined with traditional Chinese medical herbs, and ordinary acupuncture can be used in the clinical treatment of children with tic disorder or as an adjunct therapy of conventional Western medicine and psychobehavioral therapy, which has a significant effect on improving the clinical efficacy of children with tic disorder and reducing the motor tic, vocal tic, and TCM syndrome score in YGTSS. Meanwhile, in terms of adverse reactions, acupuncture combined with tuina, acupuncture combined with traditional Chinese medical herbs, and ordinary acupuncture had better clinical safety than the conventional Western medicine group.

4.2. Comparison with other review studies

Previous similar meta-analyses (Ni Xinqiang et al., 2017; Runzhi et al., 2020; Lu et al., 2021; Jianrong et al., 2022) came to a conclusion similar to this systematic review that acupuncture or acupuncture combined with other therapies has better clinical efficacy than commonly used Western medicines in the treatment of tic disorders in children. However, this systematic evaluation has solved the unsolved problems of the previous systematic evaluation.

For example, previous systematic reviews only proved that acupuncture alone had better efficacy than commonly used Western medicine in the clinical treatment of tic disorders in children but did not make a horizontal comparison between acupuncture and acupuncture combined with other commonly used clinical traditional Chinese medical herbs therapies. In clinical treatment, acupuncture is not used as the only treatment means and is often supplemented by traditional Chinese medical herbs or tuina for combined treatment, so it will become very meaningful to explore the comparison of efficacy between them. In addition, GRADE was used in this system evaluation, and all the results were rated, which made this system evaluation more rigorous and the results more credible. At the same time, the time of inclusion of literature in this systematic evaluation is from the establishment date of each database to 15 November 2022, with a longer retrieval time limit.

4.3. Limitations of included studies

Although the aforementioned evidence can preliminarily indicate that acupuncture or acupuncture combined with tuina or traditional Chinese medical herbs can be used as clinically effective monotherapy or as an adjunct to conventional Western medicine and psychobehavioral therapy, we should still take a positive view of the shortcomings of the RCT articles included in this meta-analysis. (1) Most of the literature studies have random method errors or unclear descriptions, and most of the literature studies do not describe the blind method and distribution hiding, so there may be some bias in the results. (2) Due to different inclusion and exclusion criteria, the heterogeneity of some subgroup analyses in this study is large. At the same time, different RCTs may have different critical values of "recovery," "obvious effect," and "effective" for clinical effective rate, which will have a certain impact on the results of this study. (3) Fewer RCT references (acupuncture + tuina) were included than those acupuncture + traditional Chinese medical herbs and common acupuncture, which may not be sufficient to support the results of a meta-analysis. However, due to the low quality of some RCT experimental research methodologies included in this study, and the possibility of publication, selection, implementation, and other biases in some of them, a completely positive conclusion cannot be drawn. The efficacy and safety of acupuncture combined with tuina, acupuncture combined with traditional Chinese medical herbs, or

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TABLE 4 Results of GRADE classification of outcome indicators.

Comparison of acupuncture treatment for tic disorders in children			
Patient or population: pediatric tic disorder. Background: Any nationality, race or gender. Intervention: acupuncture + tuina, acupuncture + Chinese medicine, acupuncture. Control: Western medicine commonly used in clinic			
Index of outcome	Relative effect [MD (95%CI)]	Number of studies	GRADE
1. YGTSS Score Scale - Motor twitch	(Acupuncture+tuina) (MD = 3.77, 95%CI [2.12, 5.42]), (Acupuncture+Chinese medicine) (MD = 4.90, 95%CI [3.69, 6.10]), acupuncture (MD = 3.96, 95%CI [1.75, 6.17])	1,767 (24)	Middle +++○
2. YGTSS Score Scale - Vocal tics	(Acupuncture+tuina) (MD = 3.50, 95%CI [2.82, 4.18]), (acupuncture+Chinese medicine) (MD = 4.03, 95%CI [3.50, 4.55]), acupuncture (MD = 3.40, 95%CI [2.58, 4.22])	1,626 (21)	Middle +++○
3. YGTSS Score Scale - Total score	(Acupuncture+tuina) (MD = 6.25, 95%CI [5.44, 7.05]), (acupuncture+Chinese medicine) (MD = 6.98, 95%CI [6.31, 7.65]), acupuncture (MD = 5.95, 95%CI [4.23, 7.68])	1,649 (21)	Middle +++○
4. TCM Syndrome Score Scale - Nod and shrug	(Acupuncture+tuina) (MD = 1.48, 95%CI [0.93, 2.03]), (acupuncture+Chinese medicine) (MD = 1.73, 95%CI [1.16, 2.30]), acupuncture (MD = 1.70, 95%CI [1.15, 2.26])	712 (9)	Middle +++○
5. TCM Syndrome Score Scale - Vocalization in the larynx	(Acupuncture+tuina) (MD = 1.19, 95%CI [0.81, 1.57]), (acupuncture+Chinese medicine) (MD = 1.34, 95%CI [0.88, 1.80]), acupuncture (MD = 1.50, 95%CI [1.06, 1.95])	804 (10)	Middle +++○
6. TCM Syndrome Score Scale - Upset	(Acupuncture+tuina) (MD = 0.98, 95%CI [0.44, 1.52]), (acupuncture+Chinese medicine) (MD = 1.24, 95%CI [0.76, 1.72]), acupuncture (MD = 1.17, 95%CI [0.62, 1.73])	666 (10)	Middle +++○
7. TCM Syndrome Score Scale - Main disease	(Acupuncture+tuina) (MD = 2.82, 95%CI [2.14, 3.51]), (acupuncture+Chinese medicine) (MD = 4.09, 95%CI [3.45, 4.73]), acupuncture (MD = 3.17, 95%CI [2.41, 3.93])	1,174 (15)	Middle +++○
8. Clinical effectiveness	(Acupuncture+tuina) (OR = 1.97, 95%CI [1.10, 3.51]), (acupuncture+Chinese medicine) (OR = 3.06, 95%CI [2.07, 4.51]), acupuncture (OR = 1.72, 95%CI [1.29, 2.30])	3,038 (39)	Middle +++○

ordinary acupuncture in the clinical treatment of children with tic disorders still need to be confirmed by more high-quality, multi-center, and large-sample size randomized controlled trials.

In addition, in the clinical treatment of tic disorders in children, it is also necessary to pay attention to the effect of the family intervention on the reduction of symptoms and the improvement of self-confidence in children with tic disorders (Nussey et al., 2013). Family tenacity is negatively correlated with family parental pressure ($p < 0.01$), suggesting that attention should be paid to the family environment of children while focusing on the treatment of the children themselves. The clinical nursing staff can conduct corresponding interviews with the main caregivers through communication, listening, and other ways to understand their physical and mental conditions and the difficulties in the treatment process and carry out the corresponding family nursing intervention in combination with the disease situation of the children to improve family resilience. At the same time, some studies have shown that (Wang et al., 2016) probiotics have certain effects on the function of the central nervous system and can improve behaviors related to mental diseases, such as anxiety, depression, autism spectrum disorder, obsessive–compulsive disorder, and memory ability. Therefore, acupuncture sometimes can also increase beneficial bacteria by soothing the spleen and stomach, regulating neurotransmitters by upregulating the abundance of bifidobacterium and lactobacillus, and playing a role in improving neuropsychiatric disorders.

5. Conclusion

The combination of acupuncture and traditional Chinese medical herbs can effectively improve the YGTSS score and TCM syndrome score scale of children with Tourette syndrome and has higher clinical efficacy compared with acupuncture and tuina or ordinary acupuncture. Therefore, the combination of acupuncture and traditional Chinese medical herbs may be the best therapeutic combination for the clinical treatment of children with tic disorders.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

TP, HC, and WL conceived the study. TP and YL drafted the manuscript. TP, ZY, JZ, YL, and JY performed the literature search and collected the data. TP, ZY, and JZ analyzed and visualized the data. HC, JW, ZR, QS, XW, and WL helped with the final revision of this manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1156308/full#supplementary-material

References

Aiqun, M., and Guiling, L. (2018). Clinical observation on the treatment of children's tic syndrome by wheat grain moxibustion and acupuncture at cervical Jiaji point. *Guangming Tradit. Chin. Med.* 33, 834–837.

Bos-Veneman, N. G., Minderaa, R. B., and Hoekstra, P. J. (2010). The DRD4 gene and severity of tics and comorbid symptoms: main effects and interactions with delivery complications. *Mov. Disord.* 25, 1470–1476. doi: 10.1002/mds.23122

Chaimani, A., Higgins, J. P., Mavridis, D., Spyridonos, P., and Salanti, G. (2013). Graphical tools for network meta-analysis in STATA. *PLoS One* 8:e76654. doi: 10.1371/journal.pone.0076654

Chaying, H., and Hui, L. (2022). Clinical observation on the treatment of Tourette's syndrome in children with phlegm-fire disturbance by opening the four gates. *Pract. Clin. Pract. Integr. Tradit. Chin. West. Med.* 22, 17–20.

Cubo, E., Gonzalez, C., Ausin, V., Delgado, V., Saez, S., Calvo, S., et al. (2017). The association of poor academic performance with tic disorders:a longitudinal, mainstream school-based population study. *Neuroepidemiology* 48, 155–163. doi: 10.1159/000479517

Dias, S., Sutton, A. J., Ades, A. E., and Welton, N. J. (2013). Evidence synthesis for decision making 2: a generalized linear modeling framework for pairwise and network meta-analysis of randomized controlled trials. *Med. Decis. Mak.* 33, 607–617. doi: 10.1177/0272989X12458724

Donglan, Y. (2013). Clinical observation on acupuncture treatment of 82 children with tic syndrome. *China Health Ind.* 10:171+173.

Dongwei, S. (2005). Treatment of 25 children with Tourette's syndrome by scalp and body acupuncture. *Shanghai J. Acupunct. Moxibustion* 07, 15–16.

Freeman, R. D., Fast, D. K., Burd, L., Kerbeshian, J., Robertson, M. M., and Sandor, P. (2000). An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. *Dev. Med. Child Neurol.* 42, 436–447. doi: 10.1017/S0012162200000839

Guoxiang, G., Jichao, S., and Xinmin, H. (2017). Han Xinmin's experience in treating Tourette syndrome in children. *Chin. J. Pediatr. Integr. Tradit. West. Med.* 9, 545–547.

Haisheng, W., Weiting, H., and Weidong, S. (2021). Study on the efficacy of rapid needling in the treatment of multiple tic syndrome in children. *J. Clin. Acupunct. Moxibustion* 37, 49–52.

Hanyuan, G., and Zhenggang, S. (2017). Professor Shi Zhenggang's experience in the treatment of multiple tic syndrome in children. *J. Pediatr. Tradit. Chin. Med.* 13, 11–13.

Hoekstra, P. J., Dietrich, A., Edwards, M. J., Elamin, I., and Martino, D. (2013). Environmental factors in Tourette syndrome. *Neurosci. Biobehav. Rev.* 37, 1040–1049. doi: 10.1016/j.neubiorev.2012.10.010

Jianrong, L., Xian, L., Mingyue, S., Yanjun, B., and Rui, G. (2022). Netlike meta-analysis of acupuncture and moxibustion treatment for children with tic disorder. *World J. Integr. Tradit. West. Med.* 17, 1079–1084.

Jianxi, F. (2013). Acupuncture combined with haloperidol in the treatment of 30 children with tic syndrome. *J. Ext. Treat. Tradit. Chin. Med.* 22, 20–21.

Jing, H. (2022). *Observation on the Clinical Effect of Zang-Fu Acupoints on Children with Tic Disorder* Hubei University of Traditional Chinese Medicine.

Kaipeng, L. (2016). Clinical study on the treatment of multiple tic syndrome in children with acupuncture and medicine. *Asia Pac. Tradit. Med.* 12, 100–101.

Knight, T., Steeves, T., Day, L., Lowerison, M., Jette, N., and Pringsheim, T. (2012). Prevalence of tic disorders: a systematic review and meta-analysis. *Pediatr. Neurol.* 47, 77–90. doi: 10.1016/j.pediatrneurol.2012.05.002

Krause, D. L., Weidinger, E., Matz, J., Wildenauer, A., Wagner, J. K., Obermeier, M., et al. (2012). Infectious agents are associated with psychiatric diseases. *Ment. Illn.* 4:e10. doi: 10.4081/mi.2012.e10

Lanzhi, H. (2019). *Clinical Observation on the Therapeutic Effect of Acupuncture For regulating the Mind and Calming the Liver on Children with Tic Disorder* Fujian University of Traditional Chinese Medicine.

Lezhong, H., Keqin, H., Junwei, L., and Lala, Q. (2012). Acupuncture combined with auricular point sticking and pressing for the treatment of 29 children with multiple tic syndrome. *Zhejiang J. Tradit. Chin. Med.* 47:907.

Li Liang, P., and Xiang, W. X. (2012). Clinical observation on acupuncture treatment of 32 children with tic disorder. *J. Changchun Univ. Tradit. Chin. Med.* 28, 131–132. doi: 10.13463/j.cnki.cczyy.2012.01.037

Li Lingzhe, L., Yuexian, S. F., Haiqiong, H., Bihong, Y., Fang, F., and Ying, Z. (2021). Clinical observation of acupuncture combined with Shenzhe Zhenqi decoction in treating 40 cases of children with tic disorder of spleen deficiency and phlegm accumulation. *J. Pediatr. Tradit. Chin. Med.* 17, 73–78. doi: 10.16840/j.issn1673-4297.2021.04.20

Lili, Z., Shun, W., and Yan, L. (2021). Treatment of 30 cases of Tourette's syndrome in children (liver wind hyperactivity syndrome) with the combination of acupuncture and medicine for invigorating the mood. *Jiangxi Tradit. Chin. Med.* 52, 36–38.

Liping, S. (2019). *Evaluation of the Effect of Acupuncture and Moxibustion on Children with tic Disorder* Chengdu University of Traditional Chinese Medicine.

Liping, C., and Ningning, L. (2021). The efficacy and safety of Jiuwei Xifeng granules combined with acupuncture in the treatment of multiple tic syndrome in children. *J. Henan Med. Coll.* 33, 76–79.

Lu, C., Wu, L. Q., Hao, H., Kimberly Leow, X., Xu, F. W., Li, P. P., et al. (2021). Clinical efficacy and safety of acupuncture treatment of TIC disorder in children: a systematic review and meta-analysis of 22 randomized controlled trials. *Complement. Ther. Med.* 59:102734. doi: 10.1016/j.ctim.2021.102734

Maier, R., Greter, S. E., and Maier, N. (2004). Effects of pulsed electromagnetic fields on cognitive processes - a pilot study on pulsed field interference with cognitive regeneration. *Acta Neurol. Scand.* 110, 46–52. doi: 10.1111/j.1600-0404.2004.00260.x

McHugh, R. K., Whitton, S. W., Peckham, A. D., Welge, J. A., and Otto, M. W. (2013). Patient preference for psychological vs pharmacologic treatment of psychiatric disorders. *J. Clin. Psychiatry* 74, 595–602. doi: 10.4088/JCP.12r07757

Mogwitz, S., Buse, J., Ehrlich, S., and Roessner, V. (2013). Clinical pharmacology of dopamine-modulating agents in Tourette's syndrome. *Int. Rev. Neurobiol.* 112, 281–349. doi: 10.1016/B978-0-12-411546-0.00010-X

Müller-Vahl, K. R., Buddensiek, N., Geomelas, M., and Emrich, H. M. (2008). The influence of different food and drink on tics in Tourette syndrome. *Acta Paediatr.* 97, 442–446. doi: 10.1111/j.1651-2227.2008.00675.x

Ni Xinqiang, W., Zhengzhi, Q. J., Limin, L., and Yinghong, L. (2017). Meta-analysis of randomized controlled trial on acupuncture treatment of children with tic disorder. *Chin. J. Tradit. Chin. Med.* 35, 2608–2614.

Ning, X., and Haizhu, L. (2005). Observation on the therapeutic effect of acupuncture plus drugs on 55 children with multiple tic syndrome. *New Chin. Med.* 10, 68–69.

Nussey, C., Pistrang, N., and Murphy, T. (2013). How does psycho-education help? A review of the effects of providing information about Tourette syndrome and attention-deficit/hyperactivity disorder. *Child Care Health Dev.* 39, 617–627. doi: 10.1111/cch.12039

Pasamanick, B., and Kawi, A. (1956). A study of the association of prenatal and paranatal factors with the development of tics in children; a preliminary investigation. *J. Pediatr.* 48, 596–601. doi: 10.1016/S0022-3476(56)80095-4

Qiang, L. (2019). Observation on the clinical efficacy of Xiehuang powder combined with acupuncture in the treatment of multiple tic syndrome in children. *Worlds Latest Med. Inf. Abstr.* 19, 198–199. doi: 10.19613/j.cnki.1671-3141.2019.15.139

Qinghua, Z., Weiguang, Y., and Chengguo, L. (2022). Clinical observation on the treatment of multiple tic syndrome in children with spleen deficiency and hyperactivity of liver by traditional Chinese medicine combined with invigorating the spleen, soothing the liver and calming the wind acupuncture. *Zhejiang J. Tradit. Chin. Med.* 57, 587–588. doi: 10.13633/j.cnki.zjtcm.2022.08.023

Robertson, M. M., Eapen, V., and Cavanna, A. E. (2009). The international prevalence, epidemiology, and clinical phenomenology of Tourette syndrome: a cross-cultural perspective. *J. Psychosom. Res.* 67, 475–483. doi: 10.1016/j.jpsychores.2009.07.010

Runzhi, Z., Yuan, X., Wenyu, W., Wenqin, W., and Kexing, S. (2020). Meta-analysis of clinical efficacy of acupuncture in the treatment of tic disorders. *Shanghai J. Acupunct. Moxibustion* 39, 244–252.

Salanti, G., Ades, A., and Ioannidis, J. P. (2011). Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. *J. Clin. Epidemiol.* 64, 163–171. doi: 10.1016/j.jclinepi.2010.03.016

Scharf, J. M., Miller, L. L., Mathews, C. A., and Ben-Shlomo, Y. (2012). Prevalence of Tourette syndrome and chronic tics in the population-based Avon longitudinal study of parents and children cohort. *J. Am. Acad. Child Adolesc. Psychiatry* 51, 192–201.e5. doi: 10.1016/j.jaac.2011.11.004

Shuai, S. (2020). *Based on the Theory of "Supporting the Earth and Restraining the Wood", Observe the Clinical Effect of Liu's Child Massage Combined with Acupuncture on Tourette's Syndrome in Children* Hunan University of Traditional Chinese Medicine.

Tao, X., and Jingdong, L. (2014). Clinical observation on 70 cases of Tourette's syndrome in children treated with acupuncture and medicine. *J. Pediatrics Tradit. Chin. Med.* 10, 55–58.

Tianhong, H. (2018). Clinical observation on the treatment of 40 children with Tourette syndrome by burr acupuncture. *Zhejiang J. Tradit. Chin. Med.* 53:686.

Wang, L. (2021). *Clinical Observation on the Therapeutic Effect of Traditional Chinese Medicine Combined with Acupuncture on Children with Tic Disorder of Spleen Deficiency and Hyperactivity of Liver* Jiangxi University of Traditional Chinese Medicine.

Wang, H., Lee, I. S., Braun, C., and Enck, P. (2016). Effect of probiotics on central nervous system functions in animals and humans: a systematic review. *J Neurogastroenterol Motil* 22, 589–605. doi: 10.5056/jnm16018

Wei, N. (2016). *Evaluation of the Therapeutic Effect of the Method of Regulating the Liver and Calming the Wind on Multiple tic Syndrome in Children with Yin Deficiency and Wind Movement* Nanjing University of Traditional Chinese Medicine.

Wei, W., and Fei, G. (2019). Observation on the therapeutic effect of acupuncture and medicine combined with ear point sticking and pressing on children with multiple tic syndrome. *J. Mod. Integr. Tradit. Chin. West. Med.* 28, 1901–1904.

Weisman, H., Qureshi, I. A., Leckman, J. F., Scahill, L., and Bloch, M. H. (2013). Systematic review: pharmacological treatment of tic disorders – efficacy of antipsychotic and alpha-2 adrenergic agonist agents. *Neurosci. Biobehav. Rev.* 37, 1162–1171. doi: 10.1016/j.neubiorev.2012.09.008

Wenqing, Z. (2011). Observation on the therapeutic effect of acupuncture and medicine combined treatment on children with multiple tic syndrome. *J. Tradit. Chin. Med.* 26, 1018–1019. doi: 10.16368/j.issn.1674-8999.2011.08.031

Wu Lingzhi, H., Yichao, J. T., and Jing, Z. (2018). Observation on the efficacy of self-prepared Jianpi Xifeng decoction combined with acupuncture in the treatment of multiple tic syndrome in children. *J. Guangzhou Univ. Tradit. Chin. Med.* 35, 827–832. doi: 10.13359/j.cnki.gzxbtcm.2018.05.013

Xia, Y., and Hongnan, W. (2022). Clinical observation on the treatment of 31 children with multiple tic disorder by acupuncture with the method of clearing the heart and calming the liver. *J. Pediatr. Tradit. Chin. Med.* 18, 89–93. doi: 10.16840/j.issn1673-4297.2022.05.21

Xiaocheng, S., Zhengxin, W., Liping, J., and Zhaozhao, G. (2012). Observation on the efficacy of acupuncture and drug combination in the treatment of Tourette syndrome in children. *Shanghai J. Acupunct. Moxibustion* 31, 512–514.

Xiaoshu, X., Qingxia, Z., Xianhua, Y., Xin, L., Shengka, L., and Huijie, L. (2021). Clinical study of Xiaoyao powder combined with acupuncture in treating children with tic syndrome. *New Chin. Med.* 53, 43–45. doi: 10.13457/j.cnki.jncm.2021.16.012

Xiaowei, W., Rong, M., Xilian, Z., Yaping, L., Ping, R., and Changren, S. (2005). Clinical observation of acupuncture combined with traditional Chinese medicine in the treatment of 60 children with multiple tic syndrome. *Chin. J. Basic Med. Tradit. Chin. Med.* 4, 302–303.

Xijuan, Z., Xilin, W., Yilan, W., and Yizhong, W. (2015). Clinical study on the treatment of multiple tic syndrome by moving and retaining acupuncture. *Shandong J. Tradit. Chin. Med.* 34, 266–267.

Xiong, Q., Yinmin, Z., Zongqi, L., Qiuhai, L., and Yanru, X. (2017). 37 cases of children's transient tic disorder treated by acupuncture with the method of reducing the south and reinforcing the north. *Zhejiang J. Tradit. Chin. Med.* 52:608.

Xueyuan, J. (2009). Acupuncture and massage combined with treatment of multiple tic syndrome in children. *Sichuan Tradit. Chin. Med.* 27, 115–116.

Yan, Z. (2019). Clinical observation of acupuncture combined with haloperidol in the treatment of 35 children with Tourette's syndrome. *J. Pediatr. Tradit. Chin. Med.* 15, 74–77.

Yan, L., Chen, P., and Xinmin, H. (2021). Clinical observation on the treatment of 32 children with Tourette syndrome by combination of acupuncture and medicine. *Hunan J. Tradit. Chin. Med.* 37, 97–98+111.

Yang, C., Zhang, L., Zhu, P., Zhu, C., and Guo, Q. (2016). The prevalence of tic disorders for children in China:a systematic review and meta-analysis. *Medicine* 95:e4354. doi: 10.1097/MD.0000000000004354

Ying, T., Qing, S., Wentao, L., and Shifen, X. (2015). Clinical control study on the treatment of Tourette syndrome in children with acupuncture and medicine. *China Acupunct. Moxibustion* 35, 141–144. doi: 10.13703/j.0255-2930.2015.02.010

Yiyi, Z., Zhonghua, Q., and Yiyong, S. (2016). Clinical analysis of the treatment of children with tic disorder by adjusting atlantoaxial dislocation with external treatment. *J. Ext. Treat. Tradit. Chin. Med.* 25, 42–43.

Yulin, C. (2016). Clinical observation on acupuncture treatment of children with tic disorder. *Hubei J. Tradit. Chin. Med.* 38, 6–8.

Zhang, C., Yan, J., and Sun, F. (2014). Differentiation and handling of homogeneity in network meta-analysis. *Chin. J. Evid. Based Med.* 14, 884–888. doi: 10.1186/s12918-018-0538-1

Zhonghua, Q., Huang Renxiu, H., Yijia, Z. Y., and Yiyong, S. (2017). Observation on the therapeutic effect of acupuncture combined with massage on children with tic disorder combined with atlantoaxial dislocation. *Sichuan Tradit. Chin. Med.* 35, 206–209.

Zinner, S. H., Conelea, C. A., Glew, G. M., Woods, D. W., and Budman, C. L. (2012). Peer victimization in youth with Tourette syndrome and other chronic tic disorders. *Child Psychiatry Hum. Dev.* 43, 124–136. doi: 10.1007/s10578-011-0249-y

Zusen, G., Wenting, T., Zhen, L., and Yongwei, D. (2014). Tiaogan Xifeng decoction for the treatment of 60 children with tic disorder caused by liver wind and phlegm. *Mod. Dist. Educ. Chin. Tradit. Med.* 12, 15–17.

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The increased functional connectivity between the locus coeruleus and supramarginal gyrus in insomnia disorder with acupuncture modulation

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Background: Insomnia disorder (ID) seriously affects the quality of people's daily life, and acupuncture is an effective therapy for it. As an essential component of the upward activation system, the locus coeruleus (LC) plays a crucial role in sleep–wake regulation, its aberrant functional connectivity (FC) is found to be involved in ID. The purpose of this study was to explore the modulation effect of acupuncture on the resting state FC of LC in ID patients.

Methods: 60 ID patients were recruited and randomly assigned to real acupuncture (RA) or sham acupuncture (SA) treatment. Resting-state functional magnetic resonance imaging (fMRI) data were collected before and after the treatment. With LC as the region of interest, the FC method was adopted to examine acupuncture-related modulation of intrinsic connectivity in ID patients. The Pittsburgh Sleep Quality Index (PSQI), Hyperarousal Scale (HAS), and actigraphy were used to assess sleep quality and cortical hyperarousal states. Associations between clinical outcomes and FC features were calculated using Pearson's correlation analysis.

Results: The improvement in sleep quality and hyperarousal in the RA group was greater than that in the SA group. After treatment, the FC between the LC and left inferior frontal gyrus (IFG) decreased in the RA group. The FC between the LC and left insula and supramarginal gyrus (SMG) was higher in the RA group. The change of LC FC values with the SMG was negatively associated with the change in PSQI scores.

Conclusion: Acupuncture can modulate FC between the LC and IFG, insular gyrus, and SMG. This may imply the potential mechanism of acupuncture treatment for insomnia.

KEYWORDS

insomnia disorder, acupuncture, locus coeruleus, resting-state functional connectivity, fMRI, neuroimaging

1. Introduction

Insomnia disorder (ID) is the inability to initiate and maintain sleep despite adequate opportunity for sleep, accompanied by significant daytime dysfunction (Battle, 2013). With a prevalence rate of 30–35% and an annual incidence rate of 7–15% (Morin et al., 2015), ID affects our mental and physical health widely (Pigeon et al., 2017; Khan and Aouad, 2022).

As a kind of complementary therapy, acupuncture is widely recognized as a potentially effective treatment method for ID. It has shown a positive effect on improving sleep quality (Zhang et al., 2020; Zhao et al., 2021) and alleviating the symptoms of anxiety and depression brought on by insufficient sleep (Liu C. et al., 2021). While the potential mechanisms remain to be elucidated.

The hyperarousal theory, which proposes increased activation in specific brain regions in insomniacs, is largely recognized as an explanation for the origin of insomnia (Bonnet and Arand, 1997). The locus coeruleus (LC), a crucial part of the ascending activation system, is a major norepinephrine (NE) producer and projects a large number of neurons to the spinal cord and cortex (Benarroch, 2018). LC-NE neurons remain active while awake, and at a resting state during sleep, especially during the rapid eye movement phase (Eschenko and Sara, 2008; Eschenko et al., 2012). Therefore, LC was regarded as an important contributor to promoting wakefulness and maintaining sleep.

Studies using fMRI have shown that LC plays an important role in insomnia. The functional connectivity (FC) between the LC and the posterior cingulate, thalamus, and caudate nucleus alters with the awakening state (Song et al., 2017). In insomniacs, the FC of LC increased in areas of the sensory cortex and default mode network (DMN) while decreased in the prefrontal cortex (Gong et al., 2021). Acupuncture has been shown to regulate the electrophysiology of LC in rat models of insomnia (Lee and Kim, 2017). We hypothesize that regulating the functional activity of LC may be one of the mechanisms for acupuncture to improve sleep. This study aims to investigate the modulation effect of acupuncture on the FC of the LC in ID.

2. Methods

2.1. Study design and ethical approval

This is a single-center, randomized, sham-controlled trial. The trial was registered on the Chinese Clinical Trial Registry (ChiCTR1800015282, http://www.chictr.org.cn/index.aspx) and was approved by the Medical Ethical Committee of the Beijing Hospital of Traditional Chinese Medicine (2018BL-002-02). Prior to the beginning of the trial, each participant was required to sign an informed consent form.

2.2. Participants

Sixty right-handed subjects who scored a total of 8 or more in the Pittsburgh Sleep Quality Index (PSQI) and had a diagnosis of insomnia disorder according to the Diagnostic and Statistical Manual of Mental Disorders (5th edition) were recruited for the trial. Those with an unstable medical condition, a diagnosis of anxiety, depression, or other sleep disorders, such as sleep apnea and restless leg syndrome, a contraindication for MRI, an addiction to drugs or alcohol, or a history of acupuncture therapy within 1 month were excluded. All eligible participants were randomly assigned to either the real acupuncture (RA) or sham acupuncture (SA) group.

Out of 60 subjects recruited for the study, ten patients dropped out due to COVID-19 or scheduling conflicts. Fifty patients (26 in the RA group and 24 in the SA group) who completed the two fMRI scans were included for data analysis.

2.3. Interventions

Patients in the RA group received acupuncture at Baihui (GV-20), Shenting (GV-24), Benshen (GB13), Sishencong (EX-HN1), bilateral Sanyinjiao (SP-6), bilateral Neiguan (PC-6), and bilateral Shenmen (HT-7). The selection of these acupuncture points was based on our previous clinical study, which found that these points improved sleep quality and daytime function in patients with insomnia (Guo et al., 2013). Needles were entered 10 mm horizontally for GV-20, GV-24, GB13, and EX-HN1, 5 mm vertically for HT7 and PC6, and 10 mm vertically for SP6, all while twisting the needle to induce a Deqi sensation, a sensation that includes numbness, soreness, and distension.

In the SA group, acupoints not related to insomnia treatment were selected, such as Binao (LI-14), Shousanli (LI-10), Yangchi (TE-4), Waiguan (TE-5), Fengshi (GB-31), Liangqiu (ST-34), and Futu (ST-32) (all bilateral). These points are often used to treat local tissue ailments and have little therapeutic effect on insomnia according to literature search. Needles were inserted superficially without manual stimulation.

All subjects received three 30-min acupuncture sessions per week for 4 weeks.

The acupoint positions in both groups are depicted in Figure 1.

2.4. Randomization and blinding

Randomization was conducted with a block size of six. Random numbers generated by the SAS statistical analysis system were sealed in opaque envelopes. A research assistant who was not involved in the intervention or assessment of the trial was in charge of the randomization. Owing to the characteristics of acupuncture, acupuncturists were not blinded to the assignments. The non-effective acupoints acupuncture design can however guarantee a good blinding effect for the participants. Assessors and statisticians involved in data collection and analysis were blinded to the assignments.

2.5. Assessment scale

The PSQI consists of 19 self-assessed and five other-assessed items. It is used to assess the sleep quality of the subjects over the last month. The higher the PSQI score, the worse the sleep quality (Buysse et al., 1989). The Hyperarousal Scale (HAS) consists of 26 arousal-related items and is used to assess cortical hyperarousal states, with higher scores indicating higher levels of arousal (Pavlova et al., 2001). The PSQI and HAS scales were assessed before and after treatment.

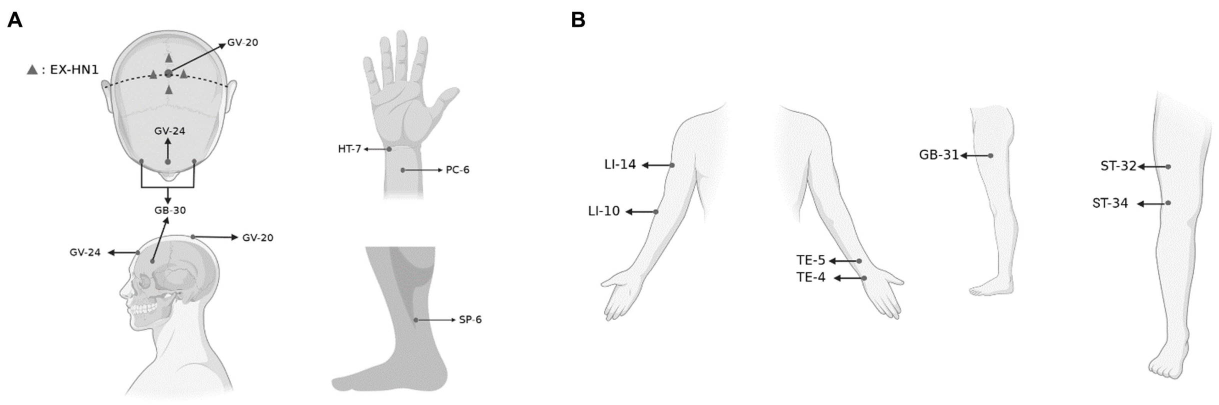

FIGURE 1
The acupoints selected in the trial. **(A)** The acupoints selected in the RA group and **(B)** the acupoints selected in the SA group. Created with BioRender.com.

2.6. Actigraphy

Participants were required to wear an actigraphy (MTI Health Services Company, Pensacola, FL, United States) on their non-dominant wrist to obtain objective data on sleep such as total sleep time (TST), the time of wake after sleep onset (WASO), and sleep efficiency (EFFICIENCY). The device was worn for a week before and after treatment.

2.7. Imaging acquisition

MRI scans were performed on a 3.0-Tesla MRI scanner (MAGNETOM, Trio, Siemens, Germany) at the Beijing Hospital of Traditional Chinese Medicine. High-resolution whole-brain structural images were recorded using a T1-weighted isotropic multi-echo magnetization-prepared rapid acquisition gradient-echo (MPRAGE) pulse sequence. Resting-state functional images were obtained by blood oxygen level dependent (BOLD) echo-planar imaging (EPI) sequences. To correct for the effects of magnetic field inhomogeneity, a field map sequence was scanned before EPI (Jezzard and Balaban, 1995). Further details are provided in Supplementary Table S1. During scanning, subjects were required to stay awake and avoid thinking about anything.

2.8. Image processing and analysis of fMRI data

Image pre-processing and FC analysis were conducted by Data Processing & Analysis of Brain Imaging (DPABI, http://rfmri.org/dpabi) (Yan et al., 2016), a toolbox in MATLAB 2021b (The MathWorks, Inc.). After removing the first 10 image points of each participant to eliminate the effect of uneven magnetic fields at the beginning or participant discomfort with the scan, the main steps of pre-processing included: slice-timing, realignment, normalization to the Montreal Neurological Institute (MNI) coordinate space with $3 \times 3 \times 3\,mm^3$, smooth with a $6 \times 6 \times 6\,mm^3$ full-width at half maximum Gaussian kernel, linear detrending; band-pass filtering (0.01–0.1 Hz), and nuisance signals were regressed out, including the Friston 24 head motion parameters (Friston et al., 1996), global signal, white matter signal, and cerebrospinal fluid signal.

After pre-processing, the LC, derived from automated anatomical labeling 3 (AAL3) (Rolls et al., 2020) was selected as the region of interest (ROI) for FC analysis. First-level correlation maps were produced by extracting the BOLD time course from the ROI and computing Pearson's correlation coefficients between that time course and the time courses of all other voxels in the brain. Then correlation coefficients were Fisher transformed into z scores to increase normality.

Whole-brain second-level group analysis was applied using paired t-tests for within-group comparisons before and after treatment and sample t-tests for between-group comparisons. Age, gender, and head movement parameters were included as covariates of non-interest. A Gaussian random field (GRF) correction at a threshold of p-voxel < 0.001 and p-cluster < 0.05 was applied. Brain regions with differences were visualized with the BrainNet Viewer[1] (Xia et al., 2013).

2.9. Statistical analysis

The trial data were analyzed according to the per-protocol principle, and patients who completed the two fMRI scans were included in the data analysis. Normality was assessed by the Shapiro–Wilk test. Categorical variables are represented as counts and percentages, and continuous variables as means with standard deviations (SD) or medians with interquartile ranges (IQR). Differences in continuous variables were compared with the use of a Student's t-test or Mann–Whitney U test. Categorical outcomes were compared with the use of a chi-square test. A two-sided p value of less than 0.05 was considered to indicate statistical significance. Pearson's correlation analysis was used to investigate correlations between clinical outcome changes (post- minus pre-) and the ΔFC z-score (post- minus pre-). The Bonferroni method was used for multiple comparison correction, $p < 0.01$ (0.05/5) was considered to be statistically significant. Analyses were performed with the use of SPSS software, version26.0 (International Business Machines Corporation).

1 http://www.nitrc.org/projects/bnv/

3. Results

Detailed baseline demographic and clinical characteristics are shown in Table 1. No significant difference between groups was observed (all $p > 0.05$).

3.1. Clinical data

After 4 weeks of acupuncture, the ΔPSQI score was −4.73 ± 3.47 in the RA group and − 1.25 ± 2.56 in the SA group, with a between-group difference of −3.48 ± 0.86 ($p < 0.001$); the median of the ΔHAS score was −10.00 in the RA group and − 1.00 in the SA group, with a between-group difference ($Z = 0.381$, $p < 0.001$). Similarly, the sleep efficiency in the RA group after treatment was significantly improved compared with the baseline period and the SA group (all $p < 0.05$). The specific data is shown in Tables 2, 3.

3.2. fMRI data

No brain regions were found different between groups at baseline at the threshold we set.

Compared with baseline, the LC FC with the left inferior frontal gyrus (IFG) was decreased in RA after treatment (Table 4; Figure 2), and no FC changes in any brain region in the SA groups were found at the threshold we set.

In the post-treatment between-group comparison, the LC FC of the left insula and supramarginal gyrus (SMG) was higher than that in the SA group (Table 5; Figure 3). Intergroup comparisons revealed that ΔFC values of LC-SMG were statistically different in brain regions ($p < 0.05$) (Table 6).

The results of the correlation analysis results showed that ΔPSQI was negatively associated with the ΔFC value of the LC- left SMG in both RA ($r = 0.432$, $p = 0.028$) and all subjects ($r = -0.377$, $p = 0.007$), and was negatively associated with the ΔFC value of the LC-left insula ($r = -0.279$, $p = 0.049$). After Bonferroni correction, only the correlation between ΔPSQI and ΔFC value of the LC-left SMG in all subjects was statistically significant ($p < 0.01$) (Figure 4). No correlation was found between ΔFC and clinical outcome changes (all $p > 0.01$) (Supplementary Table S2).

4. Discussion

This study aims to explore the effect of acupuncture on the FC of LC in ID patients. The clinical data suggested that acupuncture could modulate the state of arousal and improve sleep quality in insomniacs. Sleep quality was improved in both groups, but more significantly in the RA group. The doctor-patient interaction during acupuncture

TABLE 1 Demographic and clinical characteristics of ID patients.

Demographic characteristics		RA (N=26)	SA (N=24)	p-value
Age[a]		31.50 (18)	40.00 (20)	0.240
Sex[b]	Male	8 (30.77%)	10 (41.67%)	0.423
	Female	18 (69.23%)	14(58.33%)	
Educational levels[b]	Below bachelor's degree	5 (20.7%)	10 (40.0%)	0.084
	Bachelor's degree or above	21 (79.3%)	14 (60.0%)	
PSQI		12.46 ± 2.27	12.50 ± 2.36	0.953
HAS		43.88 ± 7.76	42.88 ± 7.62	0.245
Actigraphy	TST (min)	390.23 ± 48.27	387.00 ± 66.20	0.854
	WASO (min)[a]	83.00 (55)	65.67 (53)	0.697
	EFFICIENCY (%)[a]	78.63 (10)	83.98 (10)	0.119

Unless otherwise indicated, data are represented as mean ± SD, and the *p*-values were obtained by using a *t*-test. PSQI, Pittsburgh Sleep Quality Index; HAS, Hyperarousal Scale; TST, total sleep time; WASO, wake after sleep onset. [a]Data are represented as median and IQR, and the value of ps were obtained by using a Mann–Whitney *U* test.
[b]Data are represented as counts and percentages, and the value of ps were obtained by using a Chi-square test.

TABLE 2 Results of statistical analysis of clinical outcome after treatment (intra- and inter-group).

Clinical outcome	RA	SA	p_1	p_2	p_3
PSQI	7.73 ± 3.24	11.25 ± 2.25	<0.001***	0.025*	<0.001***
HAS	33.42 ± 10.21	41.38 ± 9.23	<0.001***	0.167	0.006**
TST (min)	404.70 ± 55.96	380.14 ± 52.76	0.328	0.476	0.148
WASO (min)[a]	50 (49)	58.92 (48)	0.085	0.445	0.601
EFFICIENCY (%)	87.70 ± 4.10	83.67 ± 7.04	<0.001***	0.185	0.032*

Unless otherwise indicated, data are represented as mean ± SD, and the *p*-values were obtained by using a *t*-test. PSQI, Pittsburgh Sleep Quality Index; HAS, Hyperarousal Scale; TST, total sleep time; WASO, wake after sleep onset. *p*1: compared with baseline in RA group; *p*2: compared with baseline in SA group; *p*3: compared between RA and SA groups. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. [a]Data are represented as median and IQR, and the value of ps were obtained by using a Mann–Whitney *U* test.

TABLE 3 Results of statistical analysis of clinical outcome changes (post-minus pre-) between groups.

Clinical outcome changes	RA	SA	*p*
ΔPSQI	−4.73 ± 3.47	−1.25 ± 2.56	<0.001***
ΔHAS[a]	−10.00 (9)	−1.00 (7)	<0.001***
ΔTST (min)	14.47 ± 69.40	4.96 ± 48.67	0.611
ΔWASO (min)[a]	−14.50 (46.83)	−8.00 (27.46)	0.436
ΔEFFICIENCY (%)[a]	7.53 (9.15)	2.73 (6.60)	0.006**

Unless otherwise indicated, data are represented as mean ± SD, and the value of ps were obtained by using a *t*-test. PSQI, Pittsburgh Sleep Quality Index; HAS, Hyperarousal Scale; TST, total sleep time; WASO, wake after sleep onset. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. [a]Data are represented as median and IQR, and the *p*-values were obtained by using a Mann–Whitney *U* test.

TABLE 4 Brain regions showing decreased FC in RA after treatment compared with baseline.

Clusters	MNI coordinates			Voxels	AAL Brain Region	Peak *t* value
	X	Y	Z			
Left inferior frontal gyrus	−45	18	30	34	Frontal_Inf_Tri_L	−6.21

GRF correction, P-voxel < 0.001, P-cluster < 0.05, cluster size > 21. AAL, Anatomical Automatic Labeling.

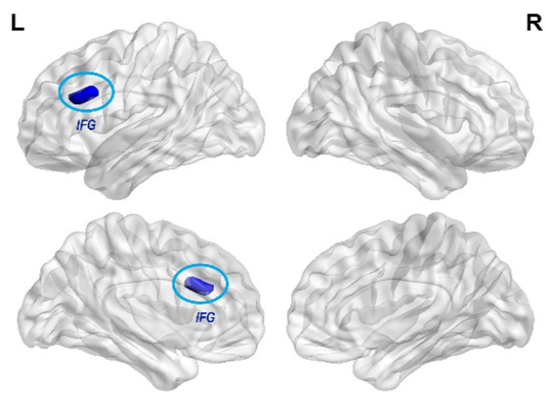

FIGURE 2
Changes of LC FC in the RA group compared with baseline. The blue color indicates the LC -left IFG FC is decreased in RA after treatment. (GRF correction, *p*-voxel<0.001, *p*-cluster<0.05, cluster size>21).

treatment may relieve the patients' negative emotions in both groups (Liu, 2007). The fMRI analysis revealed that the FC between the LC and the IFG, insular, and SMG were modulated following acupuncture treatment.

The IFG is considered a key brain region for sleep. Individuals with lower gray matter density in the left IFG are more likely to develop insomnia and wake early (Stoffers et al., 2012). Studies have shown that the activity of the IFG decreases during sleep while increases during sleep deprivation (Vartanian et al., 2014). Li et al. discovered that the amplitude of the low-frequency fluctuation value of the left IFG was reduced in insomnia patients and negatively correlated with the duration of insomnia (Li et al., 2016). Yan et al. discovered that the degree centrality value of the left IFG decreased proportionally to the PSQI score in insomniacs (Yan et al., 2018). The IFG is a fundamental component of the cognitive control network (CCN), which is involved in executive function and cognitive control (MacDonald et al., 2000). In a previous study, we discovered that patients with insomnia had higher LC FC in the middle frontal gyrus than healthy people (Chen et al., 2022). Both the middle frontal gyrus and IFG belong to CNN, demonstrating the importance of CNN in sleep regulation. Abnormal CCN function was found in ID patients, which may account for the heightened sensitivity to external stimulation (Nofzinger, 2004). Our study found that the FC between the LC and IFG was downregulated after acupuncture, which is consistent with the previous findings; thus, acupuncture may inhibit the overactivation of the IFG.

The insula is part of the salience network (SN) and is crucial for decision-making, cognition, emotion regulation, and attention control (Uddin, 2015). Patients with ID exhibited abnormal insular activity during both task execution and resting states. ID patients showed decreased FC between the insula, amygdala, striatum, and thalamus compared to normal sleepers (Martin et al., 2015; Lu et al., 2017). The co-activation of the insula with the SN is increased when ID patients fall asleep (Chen et al., 2014). Prior studies found that the FC value between the bilateral insula in ID patients decreased (Huang et al., 2017; Liu et al., 2018). The anatomical connection between the LC and insula is related to the processing of unexpected events (Dayan and Yu, 2006; Eschenko et al., 2012), and the changes in the FC of the LC-insula may be associated with the modulation of perceptual awareness skills and salience event processing (Liu J. et al., 2021). Our study found the consistent results, real acupuncture could increase the FC of the LC-left insular. But we were unable to establish a significant correlation between this finding and clinical assessments, which may be related to the sample size. Based on the above studies, we speculated that acupuncture may be beneficial for interoceptive and emotional processing in ID by modulating the abnormal functional of the insula.

The ΔFC value of SMG showed differences between groups. And ΔPSQI was negatively associated with the ΔFC value of LC-left SMG. The SMG belongs to the inferior parietal lobule, which is part of the DMN (Husbani et al., 2021) and plays an important role in the adjustment of consciousness (Marques et al., 2018). Kay et al. discovered an inverse correlation between the rate of glucose metabolism in SMG and sleep efficiency (Kay et al., 2016). A voxel-based morphometry study showed that the white matter volume of SMG was negatively associated with the PSQI score (Bai et al., 2022), and the higher volume of SMG was correlated with the longer sleep duration (Cheng et al., 2021). Studies found that the increasement in parietal lobe activity could compensate for the decreased cognition caused by insomnia (Chee and Choo, 2004; Li Y. et al., 2022). Based on these findings, we believe that acupuncture may relieve cognitive decline caused by insomnia by modulating LC-SMG FC.

An earlier study found decreased LC-IFG FC and elevated LC-SMG FC in ID patients (Gong et al., 2021), which appears inconsistent with our findings. This difference may be related to the compensatory mechanism described above and, on the other hand, to the variation in depression and anxiety in the included population. Anxiety and depression were found to affect the FC of IFG and brain regions in DMN (Fang et al., 2016; Yan et al., 2019; Yu et al., 2021). Therefore, it is difficult to determine whether insomnia or anxiety and depression had a greater impact

TABLE 5 Brain regions showing stronger FC in RA compared with SA.

Clusters	MNI coordinates			Voxels	AAL Brain Region	Peak *t* value
	X	Y	Z			
Left insular	−39	18	−3	32	Insula_L	4.772
Left supramarginal gyrus	−60	−42	33	30	SupraMarginal_L	4.857

GRF correction, P-voxel < 0.001, P-cluster < 0.05, cluster size > 26. AAL, Anatomical Automatic Labeling.

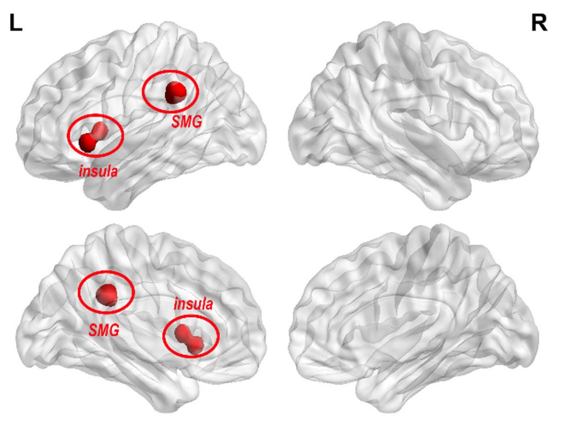

FIGURE 3
Changes of LC FC in the RA group compared with the SA group after treatment. The red color in the figure indicates the brain areas with LC FC increases in the RA group compared with the SA group (GRF correction, *p*-voxel<0.001, *p*-cluster<0.05, cluster size>26).

TABLE 6 Intergroup comparisons of ΔFC values in brain regions with differences.

	RA	SA	*t* value	*p*-value
ΔFC of insula	−0.01 ± 0.20	−0.10 ± 0.15	1.87	0.067
ΔFC of SMG	0.01 ± 0.21	−0.11 ± 0.21	2.15	0.037*

SMG, supramarginal gyrus. *: $p < 0.05$.

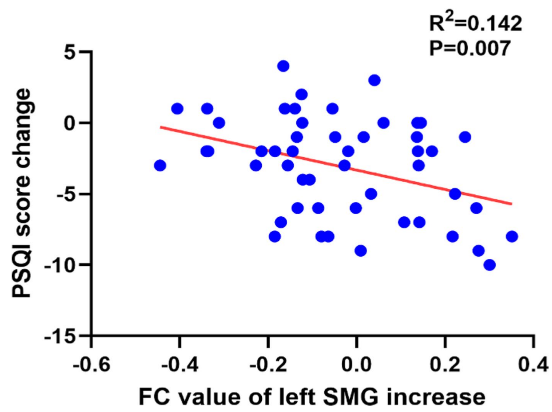

FIGURE 4
Analysis of the correlation between the FC changes and PSQI improvement in all subjects.

on the FC of LC-IFG and LC-SMG in that study. The FC of LC is also influenced by age, differences in demographic characteristics can also contribute to inconsistent results (Zhang et al., 2016; Song et al., 2021). In addition, the difference in the method of LC localization may also play roles. There are relatively few studies on the FC of LC in insomnia patients, and the results lack uniformity (Gong et al., 2021; Li C. et al., 2022). To obtain more accurate results, a big-scale study focused on ID patients without emotional problems is needed. As for the brain regions that showed differences in the between-group comparisons but did not appear in the within-group comparisons (insula and SMG), it may be related to the non-specific effect of the sham acupuncture, which needs to be further explored. Furthermore, we discovered that the brain regions with FC differences are in the left hemisphere, which is intriguing. We suspect this is because the insomnia patients we recruited were all right-handed. Activation in the left hemisphere of the brain was found to be more dominant than the right in right-handed people (Gut et al., 2007). We will include left-handed patients in our future study to test our hypothesis.

Acupuncture has been found to regulate heart rate variability, indicating that acupuncture can inhibit the sympathetic nerve and activate the vagus nerve (Wang et al., 2002; da Silva and Dorsher, 2014). Acupuncture's regulation mechanism on the LC FC may be related to afferent vagus nerve stimulation. The nucleus of the solitary tract (NTS) is primarily innervated by vagal afferents, and the NTS projects to the locus coeruleus. The peripheral vagus nerve can be stimulated to further regulate the functional activities of the LC. Future research should be combined with heart rate variability and other indicators to evaluate vagal nerve activity in order to validate this hypothesis.

There are some limitations in our research. First of all, the LC is the main source of NE, but this study did not involve the measurement of NE, and future studies should be combined with relevant examinations. Second, actigraphy relies on exercise to identify the awake period, although it is relatively objective but not accurate enough, polysomnography should be adopted. Thirdly, the LC has only 45,000–50,000 cells, making its precise location difficult. Even though the LC region has been added to AAL3, a more precise map of the Chinese brain is required. Additionally, the sample size was relatively small in this study. Additional studies should be conducted with larger sample sizes to confirm our findings. Lastly, there was no follow-up in this study, in the future, a long-term observation is required.

5. Conclusion

To our knowledge, this is the first study to investigate the mechanism of acupuncture treatment of insomnia utilizing the FC of LC. We found that the behavioural results of the insomniacs improved after acupuncture, and the imaging results showed that the FC between the LC and IFG, insular and SMG were modulated. The LC, which connects the SN, CCN, and DMN, may be an important target for acupuncture to improve insomnia.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethical Committee of the Beijing Hospital of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZC participated in the conception and design of this study, data collection, organization, and drafting of the paper, and revised the content of the paper. TJ and XY participated in the design, organization, and analysis of this study. ZT participated in the fMRI experimental design. JG and BL participated in the design and implementation of the experiment, participated in the analysis and interpretation of the data, provided guidance on the experiment, provided administrative, technical, and material support, and made key revisions to the academic content of the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1131916/full#supplementary-material

References

Bai, Y., Zhang, L., Liu, C., Cui, X., Li, D., and Yin, H. (2022). Association of white matter volume with sleep quality: a voxel-based morphometry study. *Brain Imaging Behav.* 16, 1163–1175. doi: 10.1007/s11682-021-00569-7

Battle, D. E. (2013). Diagnostic and statistical manual of mental disorders (DSM). *Codas* 25, 190–191. doi: 10.1590/s2317-17822013000200017

Benarroch, E. E. (2018). Locus coeruleus. *Cell Tissue Res.* 373, 221–232. doi: 10.1007/s00441-017-2649-1

Bonnet, M. H., and Arand, D. L. (1997). Hyperarousal and insomnia. *Sleep Med. Rev.* 1, 97–108. doi: 10.1016/s1087-0792(97)90012-5

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., and Kupfer, D. J. (1989). The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213. doi: 10.1016/0165-1781(89)90047-4

Chee, M. W. L., and Choo, W. C. (2004). Functional imaging of working memory after 24 Hr of Total sleep deprivation. *J. Neurosci.* 24, 4560–4567. doi: 10.1523/JNEUROSCI.0007-04.2004

Chen, M. C., Chang, C., Glover, G. H., and Gotlib, I. H. (2014). Increased insula coactivation with salience networks in insomnia. *Biol. Psychol.* 97, 1–8. doi: 10.1016/j.biopsycho.2013.12.016

Chen, Z., Yin, X., Jiang, T., Song, Z., Wang, G., and Guo, J. (2022). Exploring the central mechanism of insomnia through the functional connectivity of locus coeruleus: a resting-state functional magnetic resonance imaging study. *Chinese J. Magn. Reson. Imaging* 13, 48–52. doi: 10.12015/issn.1674-8034.2022.11.009. (Chinese)

Cheng, W., Rolls, E., Gong, W., Du, J., Zhang, J., Zhang, X.-Y., et al. (2021). Sleep duration, brain structure, and psychiatric and cognitive problems in children. *Mol. Psychiatry* 26, 3992–4003. doi: 10.1038/s41380-020-0663-2

da Silva, M. A. H., and Dorsher, P. T. (2014). Neuroanatomic and clinical correspondences: acupuncture and Vagus nerve stimulation. *J. Altern. Complement. Med.* 20, 233–240. doi: 10.1089/acm.2012.1022

Dayan, P., and Yu, A. J. (2006). Phasic norepinephrine: a neural interrupt signal for unexpected events. *Netw. Comput. Neural Syst.* 17, 335–350. doi: 10.1080/09548980601004024

Eschenko, O., Magri, C., Panzeri, S., and Sara, S. J. (2012). Noradrenergic neurons of the locus coeruleus are phase locked to cortical up-down states during sleep. *Cereb. Cortex* 22, 426–435. doi: 10.1093/cercor/bhr121

Eschenko, O., and Sara, S. J. (2008). Learning-dependent, transient increase of activity in noradrenergic neurons of locus coeruleus during slow wave sleep in the rat: brain stem-cortex interplay for memory consolidation? *Cereb. Cortex* 18, 2596–2603. doi: 10.1093/cercor/bhn020

Fang, J., Rong, P., Hong, Y., Fan, Y., Liu, J., Wang, H., et al. (2016). Transcutaneous Vagus nerve stimulation modulates default mode network in major depressive disorder. *Biol. Psychiatry* 79, 266–273. doi: 10.1016/j.biopsych.2015.03.025

Friston, K. J., Williams, S., Howard, R., Frackowiak, R. S., and Turner, R. (1996). Movement-related effects in fMRI time-series. *Magn. Reson. Med.* 35, 346–355. doi: 10.1002/mrm.1910350312

Gong, L., Shi, M., Wang, J., Xu, R., Yu, S., Liu, D., et al. (2021). The abnormal functional connectivity in the locus Coeruleus-norepinephrine system associated with anxiety symptom in chronic insomnia disorder. *Front. Neurosci.* 15:678465. doi: 10.3389/fnins.2021.678465

Guo, J., Wang, L.-P., Liu, C.-Z., Zhang, J., Wang, G.-L., Yi, J.-H., et al. (2013). Efficacy of acupuncture for primary insomnia: a randomized controlled clinical trial. *Evid. Based Complement. Alternat. Med.* 2013:e163850, 1–10. doi: 10.1155/2013/163850

Gut, M., Urbanik, A., Forsberg, L., Binder, M., Rymarczyk, K., Sobiecka, B., et al. (2007). Brain correlates of right-handedness. *Acta Neurobiol. Exp. (Wars)* 67, 43–51.

Huang, S., Zhou, F., Jiang, J., Huang, M., Zeng, X., Ding, S., et al. (2017). Regional impairment of intrinsic functional connectivity strength in patients with chronic primary insomnia. *Neuropsychiatr. Dis. Treat.* 13, 1449–1462. doi: 10.2147/NDT.S137292

Husbani, M. A. R., Shuhada, J. M., Hamid, A. I. A., Suardi, K. P. S., Abdullah, M. S., Latif, A. Z. A., et al. (2021). Effective connectivity between precuneus and supramarginal gyrus in healthy subjects and temporal lobe epileptic patients. *Med J Malaysia* 76, 360–368.

Jezzard, P., and Balaban, R. S. (1995). Correction for geometric distortion in echo planar images from B0 field variations. *Magn. Reson. Med.* 34, 65–73. doi: 10.1002/mrm.1910340111

Kay, D. B., Karim, H. T., Soehner, A. M., Hasler, B. P., Wilckens, K. A., James, J. A., et al. (2016). Sleep-wake differences in relative regional cerebral metabolic rate for glucose among patients with insomnia compared with good sleepers. *Sleep* 39, 1779–1794. doi: 10.5665/sleep.6154

Khan, M. S., and Aouad, R. (2022). The effects of insomnia and sleep loss on cardiovascular disease. *Sleep Med. Clin.* 17, 193–203. doi: 10.1016/j.jsmc.2022.02.008

Lee, G., and Kim, W. (2017). The modulatory effect of acupuncture on the activity of locus Coeruleus neuronal cells: a review. *Evid. Based Complement. Alternat. Med.* 2017, 9785345–9785348. doi: 10.1155/2017/9785345

Li, C., Liu, Y., Yang, N., Lan, Z., Huang, S., Wu, Y., et al. (2022). Functional connectivity disturbances of the locus Coeruleus in chronic insomnia disorder. *Nat Sci Sleep* 14, 1341–1350. doi: 10.2147/NSS.S366234

Li, C., Ma, X., Dong, M., Yin, Y., Hua, K., Li, M., et al. (2016). Abnormal spontaneous regional brain activity in primary insomnia: a resting-state functional magnetic resonance imaging study. *Neuropsychiatr. Dis. Treat.* 12, 1371–1378. doi: 10.2147/NDT.S109633

Li, Y., Ma, M., Shao, Y., and Wang, W. (2022). Enhanced effective connectivity from the middle frontal gyrus to the parietal lobe is associated with impaired mental rotation after total sleep deprivation: an electroencephalogram study. *Front. Neurosci.* 16:910618. doi: 10.3389/fnins.2022.910618

Liu, T. (2007). Role of acupuncturists in acupuncture treatment. *Evid. Based Complement. Alternat. Med.* 4, 3–6. doi: 10.1093/ecam/nel061

Liu, J., Tao, J., Xia, R., Li, M., Huang, M., Li, S., et al. (2021). Mind-body exercise modulates locus Coeruleus and ventral tegmental area functional connectivity in individuals with mild cognitive impairment. *Front. Aging Neurosci.* 13:646807. doi: 10.3389/fnagi.2021.646807

Liu, C., Zhao, Y., Qin, S., Wang, X., Jiang, Y., and Wu, W. (2021). Randomized controlled trial of acupuncture for anxiety and depression in patients with chronic insomnia. *Ann Transl Med* 9:1426. doi: 10.21037/atm-21-3845

Liu, X., Zheng, J., Liu, B.-X., and Dai, X.-J. (2018). Altered connection properties of important network hubs may be neural risk factors for individuals with primary insomnia. *Sci. Rep.* 8:5891. doi: 10.1038/s41598-018-23699-3

Lu, F.-M., Liu, C.-H., Lu, S.-L., Tang, L.-R., Tie, C.-L., Zhang, J., et al. (2017). Disrupted topology of Frontostriatal circuits is linked to the severity of insomnia. *Front. Neurosci.* 11:214. doi: 10.3389/fnins.2017.00214

MacDonald, A. W., Cohen, J. D., Stenger, V. A., and Carter, C. S. (2000). Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science* 288, 1835–1838. doi: 10.1126/science.288.5472.1835

Marques, D. R., Gomes, A. A., Caetano, G., and Castelo-Branco, M. (2018). Insomnia disorder and Brain's default-mode network. *Curr. Neurol. Neurosci. Rep.* 18:45. doi: 10.1007/s11910-018-0861-3

Martin, L. E., Pollack, L., McCune, A., Schulte, E., Savage, C. R., and Lundgren, J. D. (2015). Comparison of obese adults with poor versus good sleep quality during a functional neuroimaging delay discounting task: a pilot study. *Psychiatry Res.* 234, 90–95. doi: 10.1016/j.pscychresns.2015.08.011

Morin, C. M., Drake, C. L., Harvey, A. G., Krystal, A. D., Manber, R., Riemann, D., et al. (2015). Insomnia disorder. *Nat. Rev. Dis. Primers* 1:15026. doi: 10.1038/nrdp.2015.26

Nofzinger, E. A. (2004). What can neuroimaging findings tell us about sleep disorders? *Sleep Med.* 5, S16–S22. doi: 10.1016/s1389-9457(04)90003-2

Pavlova, M., Berg, O., Gleason, R., Walker, F., Roberts, S., and Regestein, Q. (2001). Self-reported hyperarousal traits among insomnia patients. *J. Psychosom. Res.* 51, 435–441. doi: 10.1016/s0022-3999(01)00189-1

Pigeon, W. R., Bishop, T. M., and Krueger, K. M. (2017). Insomnia as a precipitating factor in new onset mental illness: a systematic review of recent findings. *Curr. Psychiatry Rep.* 19:44. doi: 10.1007/s11920-017-0802-x

Rolls, E. T., Huang, C.-C., Lin, C.-P., Feng, J., and Joliot, M. (2020). Automated anatomical labelling atlas 3. *NeuroImage* 206:116189. doi: 10.1016/j.neuroimage.2019.116189

Song, A. H., Kucyi, A., Napadow, V., Brown, E. N., Loggia, M. L., and Akeju, O. (2017). Pharmacological modulation of noradrenergic arousal circuitry disrupts functional connectivity of the locus Ceruleus in humans. *J. Neurosci.* 37, 6938–6945. doi: 10.1523/JNEUROSCI.0446-17.2017

Song, I., Neal, J., and Lee, T.-H. (2021). Age-related intrinsic functional connectivity changes of locus Coeruleus from childhood to older adults. *Brain Sci.* 11:1485. doi: 10.3390/brainsci11111485

Stoffers, D., Moens, S., Benjamins, J., van Tol, M.-J., Penninx, B. W. J. H., Veltman, D. J., et al. (2012). Orbitofrontal gray matter relates to early morning awakening: a neural correlate of insomnia complaints? *Front. Neurol.* 3:105. doi: 10.3389/fneur.2012.00105

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nat. Rev. Neurosci.* 16, 55–61. doi: 10.1038/nrn3857

Vartanian, O., Bouak, F., Caldwell, J. L., Cheung, B., Cupchik, G., Jobidon, M.-E., et al. (2014). The effects of a single night of sleep deprivation on fluency and prefrontal cortex function during divergent thinking. *Front. Hum. Neurosci.* 8:214. doi: 10.3389/fnhum.2014.00214

Wang, J. D., Kuo, T. B. J., and Yang, C. C. H. (2002). An alternative method to enhance vagal activities and suppress sympathetic activities in humans. *Auton. Neurosci.* 100, 90–95. doi: 10.1016/S1566-0702(02)00150-9

Xia, M., Wang, J., and He, Y. (2013). BrainNet viewer: a network visualization tool for human brain connectomics. *PLoS One* 8:e68910. doi: 10.1371/journal.pone.0068910

Yan, R., Tao, S., Liu, H., Chen, Y., Shi, J., Yang, Y., et al. (2019). Abnormal alterations of regional spontaneous neuronal activity in inferior frontal orbital gyrus and corresponding brain circuit alterations: a resting-state fMRI study in somatic depression. *Front. Psych.* 10:267. doi: 10.3389/fpsyt.2019.00267

Yan, C. Q., Wang, X., Huo, J.-W., Zhou, P., Li, J.-L., Wang, Z.-Y., et al. (2018). Abnormal global brain functional connectivity in primary insomnia patients: a resting-state functional MRI study. *Front. Neurol.* 9:856. doi: 10.3389/fneur.2018.00856

Yan, C. G., Wang, X. D., Zuo, X.-N., and Zang, Y.-F. (2016). DPABI: Data Processing & Analysis for (resting-state) brain imaging. *Neuroinformatics* 14, 339–351. doi: 10.1007/s12021-016-9299-4

Yu, H., Li, M. L., Meng, Y., Li, X.-J., Wei, W., Li, Y.-F., et al. (2021). Inferior frontal gyrus seed-based resting-state functional connectivity and sustained attention across manic/hypomanic, euthymic and depressive phases of bipolar disorder. *J. Affect. Disord.* 282, 930–938. doi: 10.1016/j.jad.2020.12.199

Zhang, J., He, Y., Huang, X., Liu, Y., and Yu, H. (2020). The effects of acupuncture versus sham/placebo acupuncture for insomnia: a systematic review and meta-analysis of randomized controlled trials. *Complement. Ther. Clin. Pract.* 41:101253. doi: 10.1016/j.ctcp.2020.101253

Zhang, S., Hu, S., Chao, H. H., and Li, C.-S. R. (2016). Resting-state functional connectivity of the locus Coeruleus in humans: in comparison with the ventral tegmental area/substantia Nigra pars compacta and the effects of age. *Cereb. Cortex* 26, 3413–3427. doi: 10.1093/cercor/bhv172

Zhao, F.-Y., Fu, Q.-Q., Kennedy, G. A., Conduit, R., Zhang, W.-J., Wu, W.-Z., et al. (2021). Can acupuncture improve objective sleep indices in patients with primary insomnia? A systematic review and meta-analysis. *Sleep Med.* 80, 244–259. doi: 10.1016/j.sleep.2021.01.053

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Qualitative and quantitative meta-analysis of acupuncture effects on the motor function of Parkinson's disease patients

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Objective: To explore the association between acupuncture sessions and its effects on the motor function of Parkinson's Disease (PD).

Methods: Eight databases and two clinical trials registries were searched from inception to August 2022. Randomized controlled trials (RCTs) that compared acupuncture with sham acupuncture, or antiparkinsonian drugs, were included. After qualitative meta-analysis, a non-linear meta regression approach with restricted cubic spline was used to investigate the dose-response relationship between acupuncture sessions and their efficacy on the Unified Parkinson's Disease Rating Scale Part III (UPDRS-III) score. Subgroup meta-analysis was performed of the included studies according to the weekly acupuncture frequency. And finally, the included studies containing the determination of intermediate efficacy were compared.

Results: Of the 268 citations screened, 16 studies (462 patients of PD) were included. The qualitative meta-analysis showed that the acupuncture group had better effect on UPDRS-III scores than the control group. And the quantitative meta-analysis suggested that acupuncture dose was correlated with the reduction of UPDRS-III score in PD patients with motor symptoms. In subgroup analysis, on the one hand, when the frequency of acupuncture was no more than 3 times a week, with the increase of acupuncture session, the changes of UPDRS-III score decreased and then increased ($P = 0.000$). On the other hand, when acupuncture for more than 3 times a week and the dose of acupuncture treatment was <60 times, the changes of UPDRS-III score increased with the increase of acupuncture dose, but the score stopped to decrease if the dose continued to increase ($P = 0.020$). The comparative analysis of two quantitative RCTs found that the score improvement was more significant at the higher weekly acupuncture frequency.

Interpretation: This study found that when treating PD patients with motor symptoms, acupuncture treatment may need to reach a certain dose to obtain better therapeutic effect and excessive acupuncture stimulation may cause the body to develop a certain tolerance. However, the above results still need to be verified by more high-quality clinical studies. The protocol was registered on PROSPERO International Prospective Register of Systematic Reviews (CRD42022351428).

KEYWORDS

acupuncture, Parkinson's disease, dose-response, meta-analysis, motor function

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder primarily characterized by the deterioration of motor activities, including tremor at rest, bradykinesia, rigidity and postural instability (Pajares et al., 2020). The incidence of PD has increased rapidly in the past two decades. In 2016, the number of PD patients worldwide has reached 61 million (Dorsey et al., 2018). Although many treatments have been carried out to treat PD, none of them, alone or in combination, are capable of halting the disease progression in the long run (Sola et al., 2022).

In traditional Chinese medicine (TCM), PD symptom was first described as shaking palsy by Huangdi Neijing (ca. 100 A.D.), and TCM has played an indispensable role in medical care of PD patients for thousands of years. Many doctors and patients worldwide now use acupuncture, a technique of TCM that originated 2000 years ago (Ma et al., 2016), as a treatment for PD to alleviate its motor and non-motor symptoms. However, the international recognition of acupuncture treatment of PD is not high enough, for evidence-based medical literature related to acupuncture treatment of PD is mainly published in Chinese journals, and their quantity and quality are not high. In recent years, more and more randomized controlled trials (RCTs) have focused on acupuncture therapy for PD and many evidences have confirmed that acupuncture can alleviate motor symptoms and non-motor symptoms in patients with PD (Xia et al., 2012; de Amorim Aroxa et al., 2017; Wu et al., 2021). Since there are many different non-motor symptoms in PD and the number of RCTs related to each symptom is small, it is not advisable to directly combine related RCTs, so this study focused on motor symptoms. A randomized controlled crossover study found that a single acupuncture treatment can significantly improve the motor symptoms of PD, including gait speed, gait cadence, support base width, medio-lateral oscillation, left-right step length, and the like (Pereira et al., 2021). Several recently published qualitative meta-analyses suggested that acupuncture-related therapies combined with conventional medication showed a moderate or large effect on movement function in patients with PD (Lee and Lim, 2017; Kwon et al., 2021; Wen et al., 2021), and compared with using conventional medication alone, the combination of acupuncture in the treatment of PD, to some extent, can also improve clinical safety (Liu et al., 2017).

Exploring the relationship between different exposure levels and the development of disease is a research hotspot in epidemiology (Bauer et al., 2020). As early as more than 10 years ago, some researchers has recognized the "dose-response" in acupuncture, and they proposed the definition of dose should include the physical procedures applied in each session, using one or more needles, taking account of the patient's resulting perception (sensory, affective and cognitive) and other responses (including motor) (White et al., 2008). Research examining an adequate dose of acupuncture therapy with optimal intervention parameters and time table has also long been neglected and is now urgent (Ma, 2020). Recently, a dose-response meta-analysis on major depressive disorder have shown that acupuncture sessions were strongly correlated with its efficacy (Xu et al., 2022). On acupuncture for PD, to date, there is no systematic review of the dose-response relationship between acupuncture sessions and its efficacy on PD, but some RCTs have confirmed that the increase of acupuncture sessions may be related to the degree of remission in motor symptoms (Lei et al., 2016). In order to provide reference for the dose-response relationship and the optimal dose of acupuncture in treating motor symptoms of Parkinson's disease, we systematically collected relevant clinical RCTs literature, and made a qualitative and quantitative meta-analysis on acupuncture treatment for motor symptoms of PD patients.

2. Subjects/materials and methods

2.1. Methods

The protocol was registered on PROSPERO International Prospective Register of Systematic Reviews (CRD42022351428). This dose-response meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statements (Moher et al., 2009).

2.1.1. Search strategy

Eight databases (Pubmed, medline (*via* Embase), Cochrane, Embase, China National Knowledge Infrastructure (CNKI), China Biomedical Literature Database (CBM), VIP Database (VIP) and WanFangData) and two clinical trial registries (ClinicalTrials.gov and Chinese Clinical Trial Registry) were searched for RCTs published from the database inception to August 2022. Various combinations of Medical Subject Headings (MeSH) and non-MeSH terms were used, including "Parkinson's disease", "Parkinson disease", "paralysis tremor", "Parkinsonian", "acupuncture", "acupuncture", "warm acupuncture", "electric acupuncture", "head acupuncture", "body acupuncture" "Abdominal acupuncture", "acupuncture", "acupuncture", "moxibustion", "motor symptoms", "random", "RCT". Language, study population, or country restrictions were not applied. The specific search strategy is provided in Table 1.

2.1.2. Inclusion and exclusion criteria

All RCTs with eligible intervention(s) and outcome(s) for motor symptoms of PD published in Chinese or English languages were included. The included RCTs should strictly follow the principle of randomization, but because acupuncture therapy is difficult to strictly follow blinding and placebo control, there were no strict requirements. The included subjects should meet the diagnostic criteria for PD (Litvan et al., 2012; Yu et al., 2017).

Studies with more interventions, such as rehabilitation therapy or traditional Chinese medicine, were excluded. The included patients did not change their medication regimen 1 month before or during the intervention. There were no distinctions on manipulation methods, acupoint selection, needle retaining time and follow-up period. Moreover, no restrictions were set for comparators.

The primary outcome was the Unified Parkinson's Disease Rating Scale Part III (UPDRS-III), version 3.0. The UPDRS-III scores were rated before and after acupuncture. The improvement rate was defined as the percentage change in UPDRS-III score compared to the baseline to assess the effectiveness of acupuncture.

TABLE 1 Search strategy for PubMed.

Number	Search terms
#1	"Acupuncture"[Mesh] OR "Acupuncture Therapy"[Mesh]
#2	"Pharmacopuncture"[Title/Abstract] OR "Therapy, Acupuncture"[Title/Abstract] OR "Pharmacoacupuncture Therapy"[Title/Abstract] OR "Therapy, Pharmacoacupuncture"[Title/Abstract] OR "Acupuncture Treatment"[Title/Abstract] OR "Acupuncture Treatments"[Title/Abstract] OR "Treatment, Acupuncture"[Title/Abstract] OR "Pharmacoacupuncture Treatment"[Title/Abstract] OR "Treatment, Pharmacoacupuncture[Title/Abstract] OR "Acupotomy"[Title/Abstract] OR "Acupotomies"[Title/Abstract]
#3	#1 or #2
#4	"Parkinson Disease"[Mesh]
#5	"Lewy Body Parkinson's Disease"[Title/Abstract] OR "Parkinson's Disease, Idiopathic"[Title/Abstract] OR "Parkinson's Disease, Lewy Body"[Title/Abstract] OR "Parkinson Disease, Idiopathic"[Title/Abstract] OR "Parkinson's Disease"[Title/Abstract] OR "Idiopathic Parkinson Disease"[Title/Abstract] OR "Lewy Body Parkinson Disease"[Title/Abstract] OR "Primary Parkinsonism"[Title/Abstract] OR "Parkinsonism, Primary"[Title/Abstract] OR "Paralysis Agitans"[Title/Abstract]
#6	#4 or #5
#7	"motor symptom"[Title/Abstract]
#8	"randomized controlled trial"[Publication Type] OR "randomized"[Title/Abstract] OR "placebo"[Title/Abstract]
#9	#3 and #6 and #7 and #8

2.2. Studies selection process

Figure 1 illustrates the process of studies selection. Firstly, the retrieved literatures were imported into NoteExpress 3.6.0.9220, and its automatic review function was used to remove duplicate articles. Secondly, two independent investigators (LSY and XXY) sifted out unrecognized duplicates, including duplications from different publications and multilingual publications, as well as reports on different aspects of the same research. These two investigators then screened the titles and abstracts of the articles to select eligible studies based on the type of research, interventions/comparators and outcomes. Thirdly, a fulltext assessment was performed by two investigators (LX and FJQ) to exclude articles according to the exclusion criteria. Any discrepancies were resolved by consensus or consulting a fifth investigator (ZLX).

2.3. Risk of bias assessment

The methodological quality of eligible trials was measured *via* the Cochrane scoring system (Higgins et al., 2011). Each included study was evaluated by two independent researchers (LSY and XXY) based on seven items, including the method of random sequence generation, concealment of treatment allocation; blinding (participants, healthcare providers, data collectors, outcome assessors, and data analysts); infrequent missing outcome data and free from selective outcome reporting. The evaluation was graded into one of the three categories: low risk, high risk or unclear risk of bias. Any dissent occurs in the procedures were judged by a third investigator (FJQ) after the cross-checking of the study assessment.

2.4. Data extraction

Data were independently extracted from eligible studies by two researchers (LSY and XXY), and a chief investigator (FJQ) made a final assessment on any inconsistencies to reach a consensus. An electronic data-extraction form was designed, including study characteristics (author information, publication year, title, study design, etc.); participant details (age, gender, duration, diagnosis, etc.); method of intervention/control (number of treatments, frequency, etc.) and outcomes (types of primary and secondary outcome measures, mean and standard deviation (SD) of UPDRS-III scores, adverse event, etc.).

2.5. Data synthesis and statistical analysis

Qualitative meta-analysis was performed using Review Manager 5.4 and if the therapeutic effect of acupuncture group is significantly better than that of control group, quantitative meta-analysis will be conducted. The dose-response relationship between acupuncture sessions and reduction in UPDRS-III scores were established in Stata 16.0 software (Stata Corp., College Station, TX, USA), using the robust-error meta-regression (REMR) method (Xu and Doi, 2018), Each studies was treated as a cluster, and the mean changes in UPDRS-III scores were used as effect estimations while the acupuncture session as "dose" in the meta-regression analysis. The effects of different acupuncture doses on the difference of UPDRS-III score before and after the experiment were explored. Meta-regression models were fitted to the data according to the "one-stage" framework of REMR method. The non-linear relationship was approximated using restricted cubic spline (RCS). Three knots were set to place splines inserting values for the mean changes of UPDRS-III score, to ensure that the cubic spline was restricted to be linear at the tails of the function. Modeling of potential non-linear relationships was tested by restricting the regression coefficient to zero and a P-value < 0.1 (Wold, 1974).

Previous studies have shown that the efficacy of acupuncture can be affected by many factors (Lin, 2016; Zhang et al., 2021). Among these factors that may influence the efficacy of acupuncture, weekly acupuncture frequency was selected for study. Moreover, a comparative analysis was performed among the studies with documented intermediate efficacy in the included studies and line plots were drawn by SigmaPlot 12.0. Theoretically, there were methodological blind spots for regression publication bias analysis, because the dose-response relationship in this study was essentially based on non-linear regression theory (Xuchang et al., 2015). Due to the lack of valid measurement methods for one-stage dose-response meta-analysis, the heterogeneity was roughly estimated by I-R^2. Two-sided p-values < 0.05 were statistically significant.

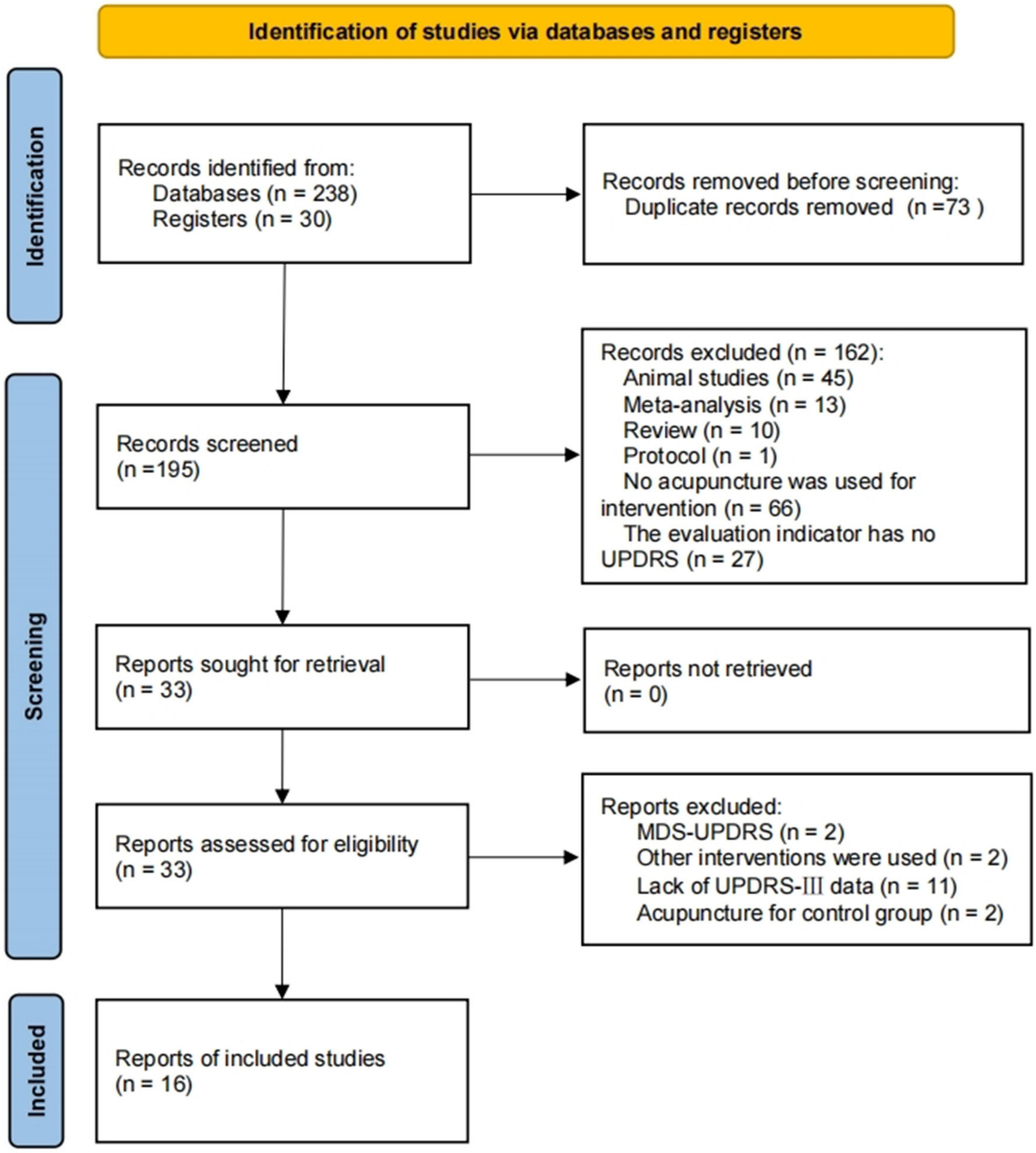

FIGURE 1
Flowchart of study selection for inclusion.

3. Results

3.1. Study selection

As shown in Figure 1, the electronic search yielded 268 unique records. Among these, 73 studies were excluded due to duplication. After that, 162 studies were excluded due to the following reasons: animal studies ($n = 45$), meta-analysis ($n = 13$), review studies ($n = 10$), protocol ($n = 1$), no acupuncture was used for intervention ($n = 66$) and the evaluation indicator has no UPDRS ($n = 27$). Consequently, 33 relevant studies remained for retrieval and all the articles were retrieved. After assessing for eligibility, 17 studies were removed due to lack of required information, including 2 studies (Toosizadeh et al., 2015; Jia et al., 2022) used MDS-UPDRS (Goetz et al., 2008) in evaluating motor function in PD. Finally, 16 studies were included in the current study.

3.2. Characteristics of the included studies

The features of the included studies were shown in Table 2. There were 16 trials with a total of 462 participants. The studies were conducted from 2006 to 2022 and their sample size varied from 14(Li, 2016) to 50 (Li, 2015) participants, with age ranged from 49.33 (Wu, 2006) to 69.48 (Li, 2015). Three studies lacked of the mean duration of patients (Wu, 2006; Li, 2015; Nazarova et al.,

TABLE 2 Characteristics of included studies.

Study ID	Sample size	Frequency (week)	Session	Sex (man)	Age (year)	Duration (year)	UPDRS-III_baseline
Li, 2015	50	5	60	30	69.48 ± 5.42	2.18*	20.28 ± 2.93
Li, 2016	14	2	24	8	62.85 ± 5.00	5.03 ± 4.73	26.00 ± 15.07
Wu, 2006	35	4	30	17	49.33 ± 13.71	2.58 ± 0.71	24.17 ± 11.01
Yang, 2009	31	5	80	19	62.21 ± 4.48	4.98 ± 2.86	25.25 ± 11.14
Zhang, 2020	25	5	20	15	67.68 ± 5.105	2.07*	33.08 ± 6.21
Zhuang and Zhuang, 2012	31	5	40	19	61.21 ± 4.48	5.28 ± 3.44	25.25 ± 11.14
Chen et al., 2012	30	6	36	19	61.93 ± 3.67	6.40 ± 2.15	34.88 ± 8.49
Qiu, 2021	26	6	48	15	61.23 ± 6.51	2.72*	23.38 ± 10.52
Jiang et al., 2006	15	5	30	8	56.80 ± 10.93	6.9 ± 2.64	43.40 ± 11.9
Nazarova et al., 2022	15	2	16	9	66.90 ± 7.80	–	17.47 ± 7.51
Kong et al., 2018	19	2	10	6	62.90 ± 9.70	–	27.10 ± 13.7
Xu et al., 2020	33	4	32	15	61.95 ± 9.77	3.26 ± 2.32	35.61 ± 10.02
Han et al., 2022	48	5	80	30	61.00 ± 3.00	4.40 ± 2.60	36.04 ± 6.12
Wu, 2006	31	2	6	16	62.03 ± 10.23	2.88*	27.81 ± 7.04
Xu, 2021	29	2	6	12	69.28 ± 6.48	6.09*	30.59 ± 12.91
Zhang et al., 2018	30	3	24	–	–	–	27.53 ± 9.13

*Missing standard errors.

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2022) and one study not identified its mean age (Nazarova et al., 2022). At the baseline, the UPDRS-III score was ranged from 17.47 (Nazarova et al., 2022) to 43.40 (Jiang et al., 2006).

3.3. Risk of bias

Figure 2 presented the risk of bias in each study. One major limitation was the low levels of reported blinding for participants, investigators, and outcome assessors. Among the included studies, only 1 study (Nazarova et al., 2022) did not specify the randomization methods; 3 studies (Toosizadeh et al., 2015; Li, 2016; Xu et al., 2020) mentioned assignment concealment; 4 studies (Jiang et al., 2006; Kong et al., 2018; Xu et al., 2020; Yang et al., 2020) specified assessor blinding and 2 studies (Kong et al., 2018; Xu et al., 2020) blinded patients in addition to assessor blinding; 9 studies (Jiang et al., 2006; Li, 2015, 2016; Kong et al., 2018; Xu et al., 2020; Yang et al., 2020; Qiu, 2021; Xu, 2021; Nazarova et al., 2022) reported dropout, loss to follow-up; 2 studies (Kong et al., 2018; Xu et al., 2020) has been registered, and one of the studies (Xu et al., 2020) did not report the registered primary outcome indicators such as Berg Balance Scale, PDQ-39, so that the selective reporting outcome was high risk. 2 studies (Kong et al., 2018; Xu et al., 2020) identified no conflict of interest, 1 study (Nazarova et al., 2022) lacked of baseline data and diagnostic criteria.

3.4. Side effects

Among the 16 included literatures, only 2 studies (Zhang, 2020; Hong et al., 2022) reported halo needle. 3 studies (Jiang et al., 2006; Chen et al., 2012; Han et al., 2022) reported that the incidence of side effects like dizziness, vomiting and insomnia in acupuncture combined with conventional medicines were lower than those in conventional medicines alone.

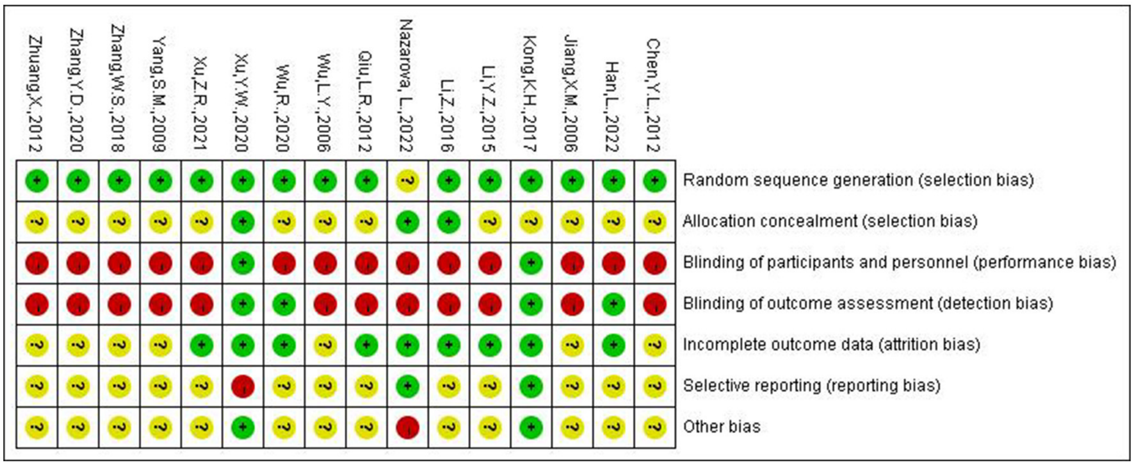

FIGURE 2
Risk of bias summary graph.

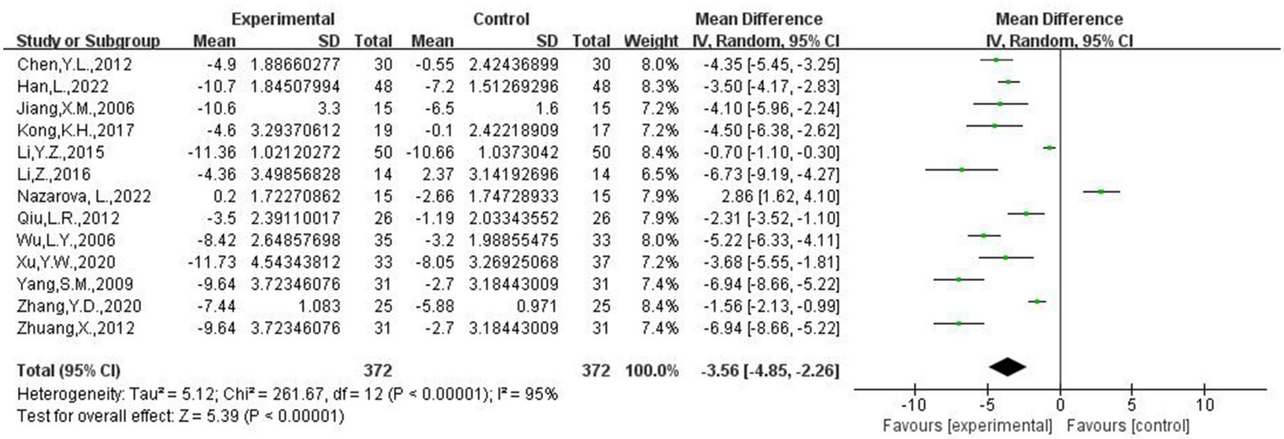

FIGURE 3
Meta-analysis of the difference between acupuncture group and control group before and after treatment.

3.5. Qualitative meta analysis

A total of 13 studies (Jiang et al., 2006; Wu, 2006; Yang, 2009; Chen et al., 2012; Zhuang and Zhuang, 2012; Li, 2015, 2016; Kong et al., 2018; Xu et al., 2020; Zhang, 2020; Qiu, 2021; Han et al., 2022; Nazarova et al., 2022) were included after the removal of 3 studies (Zhang et al., 2018; Yang et al., 2020; Xu, 2021) in which acupuncture was used in both the experimental group and the control group. Figure 3 showed significant heterogeneity between the acupuncture group and the control group (I^2 = 95%, P < 0.00001), so the random effects model was used, and the results showed that the acupuncture group had a better effect on the UPDRS-III score of PD patients than the control group [MD = −3.56, 95% CI (−4.85, −2.26), P < 0.00001].

3.6. Dose-response meta analysis

3.6.1. Relationship between acupuncture session and the change of UPDRS-III score

16 literatures were included (Jiang et al., 2006; Wu, 2006; Yang, 2009; Chen et al., 2012; Zhuang and Zhuang, 2012; Li, 2015, 2016; Kong et al., 2018; Zhang et al., 2018; Xu et al., 2020; Yang et al., 2020; Zhang, 2020; Qiu, 2021; Xu, 2021; Han et al., 2022; Nazarova et al., 2022) to illustrate the dose-response relationship between the number of acupuncture sessions and the change of the UPDRS-III score. The results in Figure 4 showed that there was a non-linear dose-response relationship between acupuncture session and changes of UPDRS-III score. The change of UPDRS-III score decreased and then increased with increasing acupuncture

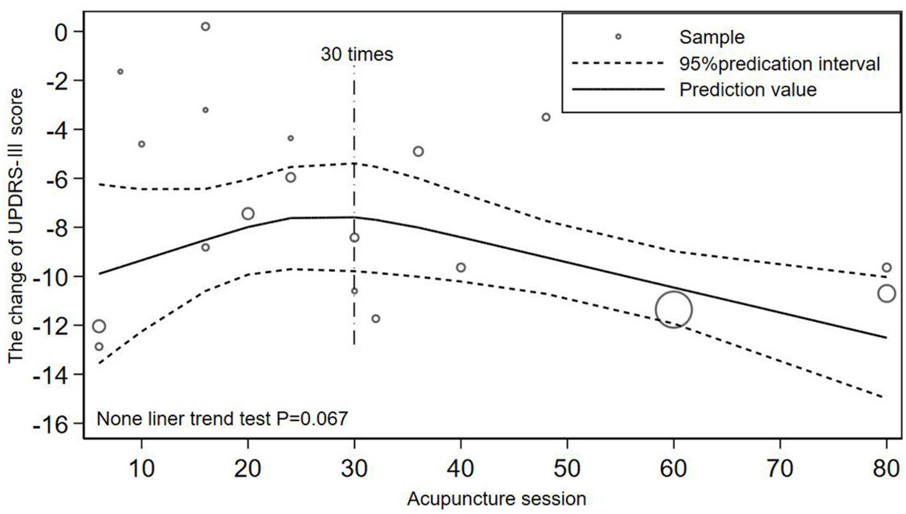

FIGURE 4
Non-linear relationship between acupuncture session and changes of UPDRS-III score.

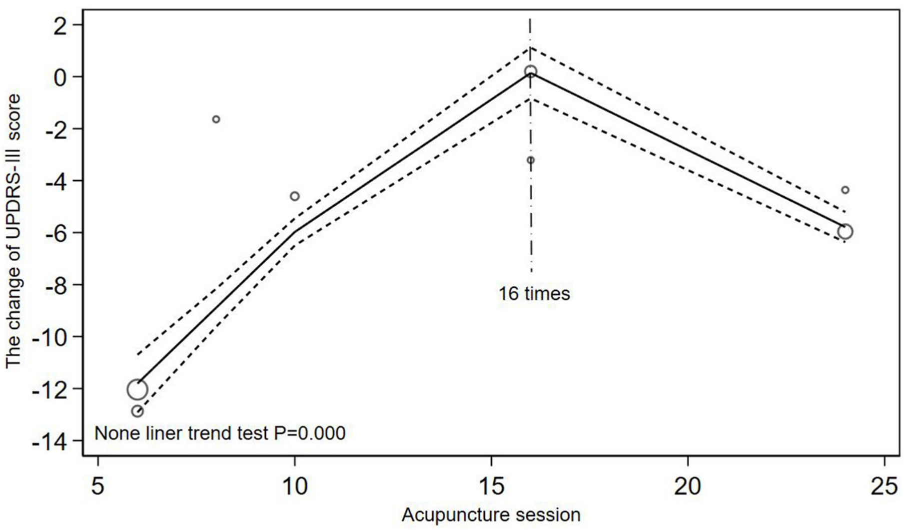

FIGURE 5
Inverted V-shaped relationship between acupuncture dose and the changes of UPDRS-III score when acupuncture for <3 times a week.

session ($P = 0.067$; Figure 4). Dose of 30 times was the inflection point of UPDRS-III score changes.

3.6.2. Subgroup analysis of the weekly acupuncture frequency

The included studies were categorized into two groups with acupuncture <3 times a week (Toosizadeh et al., 2015; Li, 2016; Kong et al., 2018; Zhang et al., 2018; Yang et al., 2020; Xu, 2021; Jia et al., 2022; Nazarova et al., 2022) and acupuncture more than 3 times a week (Jiang et al., 2006; Wu, 2006; Yang, 2009; Chen et al., 2012; Zhuang and Zhuang, 2012; Li, 2015; Xu et al., 2020; Zhang, 2020; Qiu, 2021; Han et al., 2022). Figures 5, 6 illustrated a non-linear dose-response relationship of the two subgroups, respectively. Figure 5 showed the subgroup with acupuncture <3 times a week. With the increase of acupuncture session, the changes of UPDRS-III score decreased and then increased and the inflection point appeared at 16 times of acupuncture session ($P = 0.000$). The non-linear dose-response relationship of subgroup with acupuncture more than 3 times a week was demonstrated in Figure 6 ($P = 0.020$). When the dose of acupuncture treatment was <60 times, the changes of UPDRS-III score increased with the increase of acupuncture session, but the score stopped to decrease if the dose continued to increase.

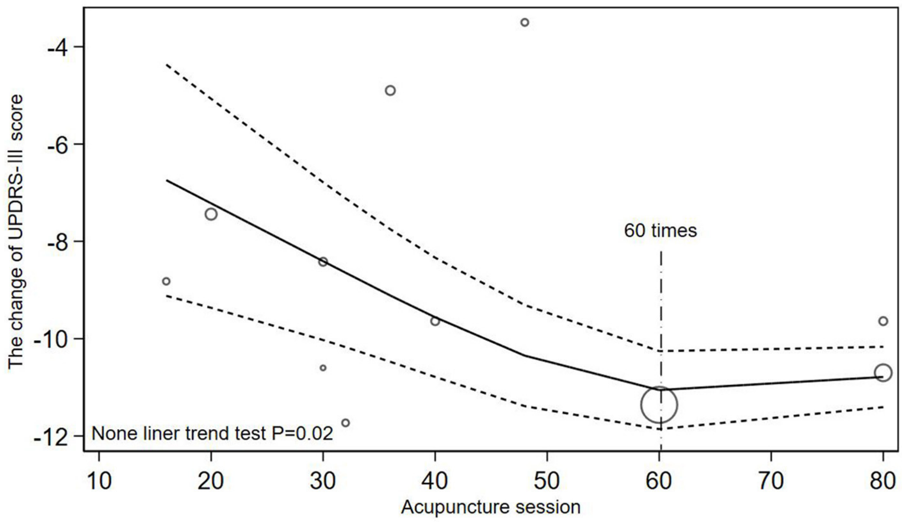

FIGURE 6
Non-linear relationship between acupuncture dose and the changes of UPDRS-III score when acupuncture for more than 3 times a week.

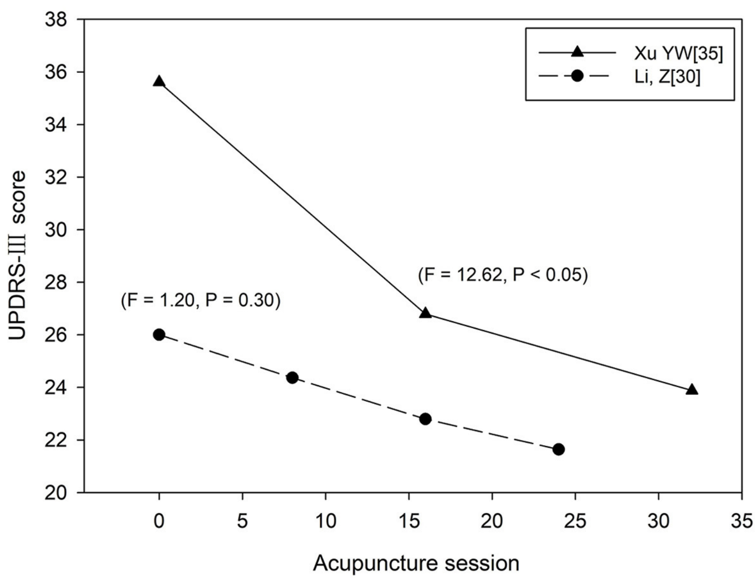

FIGURE 7
Comparative analysis of two quantitative RCTs.

3.6.3. Comparative analysis of two quantitative RCTs

Two of the included studies recorded the scores at different stages of the treatment process (Li, 2016; Xu et al., 2020). As shown in Figure 7 (Li, 2016), by treating acupuncture twice a week and comparing the UPDRS-III score after 0, 8, 16 and 24 acupuncture sessions, Li found no significant difference within the group before and after treatment ($P = 0.30$). The multicentre RCT of acupuncture for PD patients by Xu showed that UPDRS-III score varied significantly at session of 0,16, and 32 when acupuncture treatment applied by four times a week ($P < 0.05$).

4. Discussion

4.1. Main findings

Acupuncture has been used to treat PD patients since ancient times (Ma et al., 2016). Madopar or other dopaminergic drugs combined with acupuncture have been used to treat PD patients by physicians aiming to reduce side effects and increase therapeutic effectiveness (Xia et al., 2012; de Amorim Aroxa et al., 2017; Wu et al., 2021). Many systematic reviews have found that acupuncture showed a moderate or large effect with moderate or high certainty evidence in many diseases or conditions, including neurological diseases (Lee and Lim, 2017; Kwon et al., 2021; Wen et al., 2021; Lu et al., 2022). Recently, a quantitative meta-analysis on major depressive disorder found that the number of acupuncture sessions was correlated with a reduction in HAMD score in patients with MDD (Xu et al., 2022). On acupuncture for PD patients, to date, there is no systematic review of the dose-response relationship between acupuncture sessions and its efficacy on PD, but some RCTs have confirmed that the increase of acupuncture sessions may be related to the degree of remission in motor symptoms (Lei et al., 2016). Therefore, it is of certain value to further explore the optimal dose of acupuncture therapy.

In this research, due to the small number of relevant studies, the conclusions drawn are only preliminary analysis, but the effect of acupuncture dose is an important issue that deserves further study. The results of this qualitative and quantitative dose-response meta-analysis found that the combination of acupuncture could significantly improve UPDRS-III scores in PD patients compared with the simple application of medicine and the number of acupuncture sessions was correlated with the reduction of UPDRS-III score. On the one hand, acupuncture session needs to reach a certain dose to obtain better efficacy. In terms of the weekly acupuncture frequency, acupuncture 4 times a week could obtain more obvious improvement in score than acupuncture 2 times a week; the total number of acupuncture session also needs to reach a certain dose to bring about significant improvement in efficacy. On the other hand, acupuncture stimulation may cause the body tolerance. When the number of acupuncture was more than 3 times a week, the improvement of UPDRS-III score significantly increased from 16 to 60 acupuncture sessions, but the score stopped to decrease if the dose continued to increase; the single clinical study of Xu also found that after more than 15 acupuncture session, the improvement on UPDRS-III score was less than before.

This research also found that acupuncture therapy has many characteristics, few side effects, relatively safe, and in the treatment of PD, it has a certain antagonistic effect on the adverse reactions caused by madopar or other dopaminergic drugs, such as nausea and vomiting, which are typical symptoms of the gastrointestinal adverse reactions. Previous study also found that compared with using conventional medication alone, the combination of acupuncture in the treatment of PD, to some extent, can improve clinical safety (Liu et al., 2017). Therefore, it is recommended to use acupuncture in combination with modern medicine when treating PD patients.

4.2. Acupuncture session may need to reach a certain dose to obtain better efficacy

Based on the quantitative meta-analysis and the comparative analysis of the two included quantitative RCTs, it can be speculated that both the total acupuncture dose and the weekly frequency of acupuncture may need to reach a certain value to obtain better efficacy. A recent quantitative meta-analysis found no significant improvement in efficacy when the total number of acupuncture treatments was less than 18 times (Xu et al., 2022). According to Figure 4 the clinical effectiveness decreased until 30 acupuncture sessions were reached, which indicated that acupuncture treatment needs to reach a certain dose to obtain better therapeutic effect. So in clinical practice, acupuncture dose should be >30 times to achieve better clinical therapeutic effect. The conclusion in Figure 5 was exactly in line with this, but due to the large dispersion and small number of related studies with acupuncture frequency less than 3 times a week and the quantitative RCT of Li related to low-frequency acupuncture did not find such a large reversal of efficacy, more high-quality quantitative RCTs are needed to verify this result. In addition, comparing the results of Li and Xu, the UPDRS-III score varied significantly before and after high-frequency acupuncture treatment, and no significant difference before and after low-frequency acupuncture treatment, which meant that the improvement of motor symptoms in PD patients was more obvious at the higher weekly frequency of acupuncture. As there were some differences in the baseline between the two studies, more quantitative studies with better homogeneity will be needed to confirm this conclusion. Animal experiments have proved that there is a certain scientific basis for acupuncture to improve motor function in PD, such as reducing neuronal apoptosis of the striatum (Lu et al., 2012), normalizing the brain functional connectivity (Oh et al., 2022), inhibiting the level of lipid peroxides in dopaminergic neurons and protect neurons from oxidative damage (Zuo et al., 2022). However, to the best of our knowledge, there are no multi-session animal experiments to explore the dose-response mechanism of acupuncture in the treatment of PD, so high-quality scientific evidence is still needed.

4.3. Acupuncture stimulation may cause the body tolerance

Various acupuncture treatment sessions (twice, three times, four times and so on per week) have been used in acupuncture clinical trials for a total length (periods) of 4, 5, and 8 weeks

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(Kong et al., 2018; Xu et al., 2020; Hong et al., 2022). This study found that excessive acupuncture stimulation may cause the body to develop a certain tolerance. Acupuncture therapy achieves the purpose of treating diseases by stimulating acupoints on the body surface and mobilizing the inherent regulating function of the body. That means, acupuncture stimulation on acupoints is the start of acupuncture effect, and the generation of acupuncture tolerance may be closely related to it (Xi, 2009). Previous study suggested that the tolerance to endogenous 5-HT may serve as one of the possible mechanisms underlying the development of electro-acupuncture tolerance (Li et al., 1982).

Firstly, in Figure 4, due to the lack of related studies with acupuncture dose more than 80 times and the influence of more small acupuncture dose sample, we cannot speculate that the efficacy will still increase after 80 times. After subgroup analysis, the influence of low acupuncture dose on fitting effect was reduced and Figure 6 ($P = 0.020$) showed a higher goodness of fit than Figure 4 ($P = 0.067$). As shown in Figure 6, when the frequency of acupuncture was at least once every 2 days and the total number of acupuncture exceeded 60 times, the score stopped to decrease, which meant that the clinical efficacy may not be improved as before. It can also be seen that in Figure 6, when acupuncture dose approached 48 times, the efficacy was not as good as before, but it may be the effect of a discrete sample with 48 acupuncture sessions. Secondly, the quantitative clinical study of Xu also found that when acupuncture was applied for 4 times a week, the improvement effect after 15 times of acupuncture treatment was not as good as before. Thirdly, previous quantitative meta-analysis also found that the improvement rate gradually decreased after >36 acupuncture sessions (Xu et al., 2022). Therefore, in clinical practice, considering the economic burden of PD patients, the optimal dose of acupuncture is from 48 to 60 times when the frequency of acupuncture treatment is more than 3 times a week. After the patient have accepted acupuncture treatment for more than 48 times, they can stop acupuncture for a period of time to avoid the body tolerance. Studying the acupuncture frequency with the best clinical efficacy can enable patients to obtain the best clinical efficacy without substantial loss of economic benefit, rather than blindly pursuing high-frequency and long-term treatment. However, there still needs more clinical studies with large samples to explore the impact of high-dose acupuncture treatment on clinical effectiveness.

4.4. Feasibility and necessity of this study

Many systematic reviews found that acupuncture therapy showed a moderate or large effect in PD with motor symptoms (Zhou et al., 2020; Kwon et al., 2021). A dose-response meta-analysis on major depressive disorder has found that acupuncture session was strongly correlated with its efficacy (Xu et al., 2022). The published RCT suggests that more acupuncture sessions lead to greater clinical efficacy in motor function of PD (Li et al., 2022). However, to the best of our knowledge, there is currently no published systematic review comparing the effect of the number of acupuncture sessions on the efficacy of PD with motor functions. All indicate that this study has great feasibility and necessity.

4.5. Limitations

It should be pointed out that our study also has limitations. Firstly, the definition of dose should include the physical procedures applied in each session, using one or more needles, taking account of the patient's resulting perception (sensory, affective and cognitive) and other responses (including motor) (White et al., 2008). Among them, only the number of acupuncture sessions was compared. Secondly, this study could not account for the individual efficacy of the selected acupuncture points and the results of this study are based on the assumption that different acupuncture points have the same efficacy. Thirdly, more studies with high acupuncture doses are needed to further demonstrate the impact of acupuncture on clinical efficacy. Finally, due to methodological limitations, this study did not examine sources of heterogeneity and publication bias, which may decrease the robustness of the findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis: LZ. Concept and design: SL, XL, and JF. Drafting of the manuscript: SL and XX. Critical revision of the manuscript for important intellectual content: SL, XL, JF, and LZ. Statistical analysis: SL. Obtained funding: LZ and JF. Acquisition, analysis, or interpretation of data: All authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Bauer, M., McDonald, J. L., and Saunders, N. (2020). Is acupuncture dose dependent? Ramifications of acupuncture treatment dose within clinical practice and trials. *Integr Med Res*. 9, 21–27. doi: 10.1016/j.imr.2020.01.003

Chen, Y. L., Feng, W. J., and Zhang, X. L. (2012). Parkinson's disease combined with overactive bladder syndrome treated with acupuncture and medication. *Chin. Acupuncture Moxibustion* 32, 215–218. doi: 10.13703/j.0255-2930.2012.03.008

de Amorim Aroxa, F. H., de Oliveira Gondim, I. T. G., Santos, E. L. W., de Sales, M. D. G. W., Asano, A. G. C., and Asano, N. M. J. (2017). Acupuncture as adjuvant therapy for sleep disorders in Parkinson's disease. *J. Acupunct. Meridian Stud.* 10, 33–38. doi: 10.1016/j.jams.2016.12.007

Dorsey, E. R., Elbaz, A., Nichols, E., Abbasi, N., Abd-Allah, F., Abdelalim, A., et al. (2018). Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the global burden of disease study 2016. *Lancet Neurol.* 17, 939–953. doi: 10.1016/S1474-4422(18)30295-3

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin, P., et al. (2008). Movement disorder society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* 23, 2129–2170. doi: 10.1002/mds.22340

Han, L., Su, X. Z., Zhang, Z. Y., Liu, Y., Wei, Z. X., Zhang, Q. L., et al. (2022). Effect of panlong needling at Jiaji (EX-B 2) on motor dysfunction in patients with Parkinson's disease of liver and kidney deficiency: a randomized controlled trial. *Zhongguo Zhen Jiu*. 42, 493–497.

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, d5928. doi: 10.1136/bmj.d5928

Hong, Z. M., Qiu, J. F., Zhang, S. Q., Wang, Y. B., He, K. L., Ma, R. J., et al. (2022). Jiao's scalp acupuncture combined with virtual reality rehabilitation training for motor dysfunction in patients with Parkinson's disease: a randomized controlled trial. *Zhongguo Zhen Jiu* 42, 726–730.

Jia, Y. B., Wang, X. J., Liu, X. P., Peng, W. A., Chao, D. E., Li, Z. Z., et al. (2022). Effect of combined scalp and body acupuncture on Parkinson's disease: a randomized clinical trial. *World J. Acupuncture Moxibustion.* 32, 342–50. doi: 10.1016/j.wjam.2022.07.011

Jiang, X.-M., Huang, Y., Zhuo, Y., Gao, Y.-P. (2006). Therapeutic effect of scalp electroacupuncture on Parkinson disease. Nan Fang yi ke da xue xue bao. *J. Southern Med. Univ.* 26, 114–116.

Kong, K. H., Ng, H. L., Li, W., Ng, D. W., Tan, S. I., Tay, K. Y., et al. (2018). Acupuncture in the treatment of fatigue in Parkinson's disease: a pilot, randomized, controlled, study. *Brain Behav.* 8, e00897. doi: 10.1002/brb3.897

Kwon, M., Cheong, M. J., Leem, J., and Kim, T-. H. (2021). Effect of acupuncture on movement function in patients with Parkinson's disease: network meta-analysis of randomized controlled trials. *Healthcare.* 9:1502. doi: 10.3390/healthcare9111502

Lee, S-. H., and Lim, S. (2017). Clinical effectiveness of acupuncture on Parkinson disease: a PRISMA-compliant systematic review and meta-analysis. *Medicine* 96, e5836. doi: 10.1097/MD.0000000000005836

Lei, H., Toosizadeh, N., Schwenk, M., Sherman, S., Karp, S., Sternberg, E., et al. (2016). A pilot clinical trial to objectively assess the efficacy of electroacupuncture on gait in patients with parkinson's disease using body worn sensors. *PLoS ONE* 11, e0155613. doi: 10.1371/journal.pone.0155613

Li, L., Jin, X., Cong, W., Du, T., and Zhang, W. (2022). Acupuncture in the treatment of Parkinson's disease with sleep disorders and dose response. *BioMed Res. Int.* 2022, 1–7. doi: 10.1155/2022/7403627

Li, S. J., Tang, J., and Han, J. S. (1982). Tolerance to 5-HT and its implication in electro-acupuncture tolerance and morphine tolerance. *Zhongguo Yao Li Xue Bao* 3, 159–163.

Li, Y. Z. (2015). *Study of Scalp Acupuncture on Parkinson's Disease with the Chorea Trembling Control Area*. Guangzhou: Guangzhou University of Chinese Medicine.

Li, Z. (2016). *Clinical Study of Acupuncture Paratherapy for Parkinson's Disease Tremor and its Underlying Neuromechanism Based on Functional Magnetic Resonance Imaging.* Guangzhou: Guangzhou University of Chinese Medicine.

Lin, Y. F. (2016). Analysis on the influencing factors of acupuncture efficacy. *Asia-Pacific Traditional Med.* 12, 100–102.

Litvan, I., Goldman, J. G., Tröster, A. I., Schmand, B. A., Weintraub, D., Petersen, R. C., et al. (2012). Diagnostic criteria for mild cognitive impairment in Parkinson's disease: movement disorder society task force guidelines. *Mov. Disord.* 27, 349–56. doi: 10.1002/mds.24893

Liu, H., Chen, L., Zhang, Z., Geng, G., Chen, W., Dong, H., et al. (2017). Effectiveness and safety of acupuncture combined with Madopar for Parkinson's disease: a systematic review with meta-analysis. *Acupunct. Med.* 35, 404–412. doi: 10.1136/acupmed-2016-011342

Lu, L., Zhang, Y., Tang, X., Ge, S., Wen, H., Zeng, J., et al. (2022). Evidence on acupuncture therapies is underused in clinical practice and health policy. *BMJ.* 376, e067475. doi: 10.1136/bmj-2021-067475

Lu, Z-. Y., Zhao, H., Wang, T., Chen, J., and Zong, L. (2012). Effects of acupuncture on behavior and striatal apoptosis in mice with Parkinson disease. *Zhen Ci Yan Jiu* 37, 186–190.

Ma, S. X. (2020). Establishing an adequate dose of acupuncture is essential for clinical trial studies. *Clin. Res. Trials* 6. doi: 10.15761/CRT.1000295

Ma, Y., Dong, M., Zhou, K., Mita, C., Liu, J., Wayne, P. M., et al. (2016). Publication trends in acupuncture research: a 20-year bibliometric analysis based on pubmed. *PLoS ONE* 11, e0168123. doi: 10.1371/journal.pone.0168123

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., and PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 339, b2535. doi: 10.1136/bmj.b2535

Nazarova, L., Liu, H., Xie, H., Wang, L., Ding, H., An, H., et al. (2022). Targeting gut-brain axis through scalp-abdominal electroacupuncture in Parkinson's disease. *Brain Res.* 1790, 147956. doi: 10.1016/j.brainres.2022.147956

Oh, J-. Y., Lee, Y-. S., Hwang, T-. Y., Cho, S-. J., Jang, J-. H., Ryu, Y., et al. (2022). Acupuncture regulates symptoms of Parkinson's disease *via* brain neural activity and functional connectivity in mice, front aging. *Neuroscience* 14, 885396. doi: 10.3389/fnagi.2022.885396

Pajares, M., Rojo, A. I., Manda, G., Boscá, L., and Cuadrado, A. (2020). Inflammation in Parkinson's disease: mechanisms and therapeutic implications. *Cells* 9, 1687. doi: 10.3390/cells9071687

Pereira, C. R., Criado, M. B., Machado, J., Pereira, C. T., and Santos, M. J. (2021). Acute effects of acupuncture in balance and gait of Parkinson disease patients—a preliminary study. *Complement. Ther. Clin. Pract.* 45, 101479. doi: 10.1016/j.ctcp.2021.101479

Qiu, L. R. (2021). *Clinical Study on Bushen Yangsui Acupuncture Therapy in Treatment of Parkinson's Disease with Mild Cognitive Impairment of Suihai Buzu Type.* Jinan: Shandong University of Traditional Chinese Medicine.

Sola, P., Krishnamurthy, P. T., Kumari, M., Byran, G., Gangadharappa, H. V., Garikapati, K. K., et al. (2022). Neuroprotective approaches to halt Parkinson's disease progression. *Neurochem. Int.* 158, 105380. doi: 10.1016/j.neuint.2022.105380

Toosizadeh, N., Lei, H., Schwenk, M., Sherman, S. J., Sternberg, E., Mohler, J., et al. (2015). Does integrative medicine enhance balance in aging adults? Proof of concept for the benefit of electroacupuncture therapy in Parkinson's disease. *Gerontology* 61, 3–14. doi: 10.1159/000363442

Wen, X., Li, K., Wen, H., Wang, Q., Wu, Z., Yao, X., et al. (2021). Acupuncture-related therapies for Parkinson's disease: a meta-analysis and qualitative review. *Front. Aging Neurosci.* 13, 676827. doi: 10.3389/fnagi.2021.676827

White, A., Cummings, M., Barlas, P., Cardini, F., Filshie, J., Foster, N. E., et al. (2008). Defining an adequate dose of acupuncture using a neurophysiological approach—a narrative review of the literature. *Acupunct. Med.* 26, 111–20. doi: 10.1136/aim.26.2.111

Wold, S. (1974). Spline functions in data analysis. *Technometrics* 16, 1–11. doi: 10.1080/00401706.1974.10489142

Wu, L. Y. (2006). *Clinical and Experimental Study on Treating Parkinson's Disease Mainly with the Therapy of Trembling-Three Needles.* Guangzhou: Guangzhou University of Chinese Medicine.

Wu, M-. X., Wang, L-. G., Li, H-. P., and Zeng, X. (2021). Acupuncture adjuvant treatment for dysphagia in patients with Parkinson's disease: a randomized controlled trial. *Zhongguo Zhen Jiu* 41, 485–8.

Xi, Q. et al. (2009). Acupuncture tolerance. *Clin. J. Acupuncture Moxibustion* 25, 43–44.

Xia, Y., Wang, H. D., Ding, Y., Kang, B., Liu, W. G., et al. (2012). Parkinson's disease combined with depression treated with electroacupuncture and medication and its effect on serum BDNF. *Zhongguo Zhen Jiu* 32, 1071–1074. doi: 10.13703/j.0255-2930.2012.12.007

Xu, C., and Doi, S. (2018). The robust error meta-regression method for dose-response meta-analysis. *Int. J. Evid. Based Healthc.* 16, 138–144. doi: 10.1097/XEB.0000000000000132

Xu, G., Lei, H., Huang, L., Xiao, Q., Huang, B., Zhou, Z., et al. (2022). The dose-effect association between acupuncture sessions and its effects on major depressive disorder: a meta-regression of randomized controlled trials. *J. Affect. Disord.* 310, 318–327. doi: 10.1016/j.jad.2022.04.155

Xu, Y., Cai, X., Qu, S., Zhang, J., Zhang, Z., Yao, Z., et al. (2020). Madopar combined with acupuncture improves motor and non-motor symptoms in Parkinson's disease patients: a multicenter randomized controlled trial. *Eur. J. Integr. Med.* 34, 101049. doi: 10.1016/j.eujim.2019.101049

Xu, Z. R. (2021). *The Observation of Qihuang Acupuncture Therapy in the Treatment of Parkinson's Disease: A Randomization Clinical Trial.* Guangzhou: Guangzhou University of Chinese Medicine.

Xuchang, Z. G., Hanfang-fang, N. M., and Kuangxin-ying, Z. (2015). How to perform dose-response meta-analysis: a brief introduction of methodology. *Chin. J. Evidence-Based Med.* 15, 1236–1239.

Yang, L. S., Li, Y. M., Zhou, D. F., Zhao, B. M., Zheng, S. Z., Chen, Z. H. (2020). *The Observation of Qihuang Acupuncture Therapy in the Treatment of Parkinson' s Disease with Dyskinesia: A Randomization Clinical Trial.* Guangzhou: Guangzhou University of Chinese Medicine.

Yang, S. M. (2009). *The Clinical Study of Using Tremor Three Needles in the Treatment of Parkinson's Disease.* Guangzhou: Guangzhou University of Chinese Medicine.

Yu, R. L., Wu, R. M., Chan, A. Y., Mok, V., Wu, Y. R., Tilley, B. C. (2017). Chinese society of Parkinson's disease and movement disorders, Parkinson's disease and movement disorder section of neurologist branch of chinese medical doctor association, Chinese guidelines for the treatment of Parkinson′s disease (fourth edition). *Chin. J. Neurol.* 53, 973–986.

Zhang, L., Xue, X., Mu, D., Yan, H., and Han, L. (2021). Research progress on the relationship between acupuncture curative effect and acupuncture retention and interval time. *J. Tianjin Univ. Traditional Chinese Med.* 40, 666–673.

Zhang, W. S., Zong, L., and Gu, K. (2018). Clinical observation of ordinary acupuncture plus Zhigou (TE6) and Zhaohai (KI6) for constipation due to yin deficiency in Parkinson's disease. *Shanghai J. Acupuncture Moxibustion* 37, 165–169.

Zhang, Y. D. (2020). *Analysis of Rules of Acupoint Prescription for Acupuncture Treatment and Effect of Acupuncture of Zishui Hanmu Method plus Medoba on Parkinson's Disease.* Shenyang: Liaoning University of Traditional Chinese Medicine.

Zhou, J., Li, J., Li, J., Yang, J., and Wang, C. (2020). Meta analysis of acupuncture in the treatment of pathogenic motor symptoms of Parkinson' s dis-ease. *Global Traditional Chin. Med.* 13, 326–333.

Zhuang X., and Zhuang L. (2012). Clinical research on the treatment of Parkinson's disease with trembling three-needle. *Chin. Med. Clinic. J.* 24, 1162–1163. doi: 10.16448/j.cjtcm.2012.12.007

Zuo, T., Xie, M., Yan, M., Zhang, Z., Tian, T., Zhu, Y., et al. (2022). *In situ* analysis of acupuncture protecting dopaminergic neurons from lipid peroxidative damage in mice of Parkinson's disease. *Cell Prolif.* 55, e13213. doi: 10.1111/cpr.13213

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Neuroimaging mechanisms of acupuncture on functional reorganization for post-stroke motor improvement: a machine learning-based functional magnetic resonance imaging study

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Objective: Motor recovery is crucial in stroke rehabilitation, and acupuncture can influence recovery. Neuroimaging and machine learning approaches provide new research directions to explore the brain functional reorganization and acupuncture mechanisms after stroke. We applied machine learning to predict the classification of the minimal clinically important differences (MCID) for motor improvement and identify the neuroimaging features, in order to explore brain functional reorganization and acupuncture mechanisms for motor recovery after stroke.

Methods: In this study, 49 patients with unilateral motor pathway injury (basal ganglia and/or corona radiata) after ischemic stroke were included and evaluated the motor function by Fugl–Meyer Assessment scores (FMA) at baseline and at 2-week follow-up sessions. Patients were divided by the difference between the twice FMA scores into one group showing minimal clinically important difference (MCID group, n=28) and the other group with no minimal clinically important difference (N-MCID, n=21). Machine learning was performed by PRoNTo software to predict the classification of the patients and identify the feature brain regions of interest (ROIs). In addition, a matched group of healthy controls (HC, n=26) was enrolled. Patients and HC underwent magnetic resonance imaging examination in the resting state and in the acupuncture state (acupuncture at the Yanglingquan point on one side) to compare the differences in brain functional connectivity (FC) and acupuncture effects.

Results: Through machine learning, we obtained a balance accuracy rate of 75.51% and eight feature ROIs. Compared to HC, we found that the stroke patients with lower FC between these feature ROIs with other brain regions, while patients in the MCID group exhibited a wider range of lower FC. When acupuncture was applied to Yanglingquan (GB 34), the abnormal FC of patients was decreased, with different targets of effects in different groups.

Conclusion: Feature ROIs identified by machine learning can predict the classification of stroke patients with different motor improvements, and the FC between these ROIs with other brain regions is decreased. Acupuncture can modulate the bilateral cerebral hemispheres to restore abnormal FC *via* different targets, thereby promoting motor recovery after stroke.

Clinical trial registration: https://www.chictr.org.cn/showproj.html?proj=37359, ChiCTR1900022220.

KEYWORDS

stroke, motor recovery, minimal clinically important difference (MCID), acupuncture, machine learning, fMRI

1. Introduction

Stroke is the third-leading cause of death and disability worldwide, with ischemic stroke accounting for 62.4% (Collaborators, G. B. D. Stroke, 2021). Motor impairment is one of the main disabilities associated with stroke, causing a substantial social and psychological burden, and the concern about motor function recovery after stroke is growing (Stinear et al., 2020). The Fugl–Meyer Upper and Lower Extremity scales are recommended to be used as primary indicators of motor deficits and outcomes in stroke populations (Bushnell et al., 2015). A minimal clinically important difference (MCID) is the smallest improvement in an outcome measure that would be noticed as beneficial to a patient and be of clinical relevance, which is important to patient-centered care and evidence-based research (Embry and Piccirillo, 2020). The MCID may also be useful in advancing personalized medicine by characterizing those who are most likely to benefit from a treatment (Malec and Ketchum, 2020). Thus, the MCID for the Fugl–Meyer Assessment (FMA) is perceived as a meaningful recovery of motor function by post-stroke patients, deserving increasing interest and importance in medical practice and research.

Plasticity changes and functional reorganization occur spontaneously in post-stroke brains, and these alternations may contribute to the restoration of motor function following stroke (Murphy and Corbett, 2009; Wang et al., 2010; Dimyan and Cohen, 2011). Recent developments in functional magnetic resonance imaging (fMRI) have enabled the visualization of functional abnormalities and reorganization between brain regions or networks (Cramer et al., 2011). Degree centrality (DC) is a voxel-based analysis that can identify neural hubs associated with functional reorganization, reflecting the centrality or functional importance of the voxel or brain regions in whole-brain networks (Zuo et al., 2012; Zhang et al., 2017). One study found that DC was correlated with motor recovery after cerebral infarction (Liu et al., 2019). Moreover, functional connectivity (FC) is a seed-based analysis that demonstrates the temporal correlation across regions of interest (ROIs) (Carter et al., 2010) and is commonly used in neuroimaging studies. The joint application of DC and FC can better identify functional hubs associated with motor recovery and reveal local and global neurological remodeling after stroke. Both the recovery of motor function and neurological remodeling after stroke are crucial for the patient's rehabilitation and may help to explore potential neural biomarkers. Clinical assessment of motor impairment combined with neuroimaging biomarkers of motor function can help to predict both motor recovery and motor outcomes, and stratify patients in clinical trials after stroke (Stinear, 2017). Thus, machine learning (ML) can help to implement and increasingly be used for diagnosis, prognosis prediction, and biomarkers selection for diseases (Zeng et al., 2018; Tu et al., 2021; Lian et al., 2022). Compared to *a priori* empirical or statistical comparisons, applying ML to select brain regions associated with motor recovery after stroke is more characteristic, personalized, and predictive.

Acupuncture is one of the traditional Chinese medical therapies that provides a positive effect in improving post-stroke symptoms and stroke rehabilitation (Wu et al., 2010). Studies have shown that acupuncture can improve motor dysfunction after stroke (Birch and Robinson, 2022) and has a certain safety profile (Zhang et al., 2005). However, the mechanisms of acupuncture remain elusive. Neuroimaging may be able to provide some evidence for the central nervous system effects of acupuncture in the treatment of stroke (Qi et al., 2014; Wu et al., 2016). Our previous studies have shown that acupuncture was able to modulate the disrupted patterns of the whole-brain network following the subcortical ischemic stroke (Han et al., 2020). Acupuncture triggered unique responses in the sensorimotor cortex in post-stroke hemiplegia patients, related to the neurological functional damage and the stage of stroke (Wang et al., 2022). Nowadays, ML studies of neuroimaging biomarkers exploration are increasing, especially in stroke recovery and acupuncture.

In the present study, we included stroke patients with impaired unilateral motor pathways, grouped them according to their MCID of the FMA over 2 weeks, and analyzed the DC of the whole brain. The L1-multi kernel learning machine (L1-MKL) in PRoNTo was used to select feature brain regions that could distinguish between these two groups of patients. Our primary aim of the present work was to search for differential ROIs between stroke patients with different manifestations of motor function recovery. Then we analyzed the FC between these selected ROIs and the whole brain, comparing abnormal functional connectivity between patients and healthy controls in the resting and acupuncture states. We hypothesized that (1) functional hubs with prediction-related features exist between patients with different motor recovery profiles, (2) these feature brain regions have different responses to the whole brain after stroke, and (3) acupuncture has its unique neuroimaging mechanisms.

2. Materials and methods

2.1. Participants

The present study included 69 stroke hemiplegia patients due to unilateral motor pathway (basal ganglia and/or corona radiata) injury. Patients were evaluated for motor function on the day of enrolment and 2 weeks later, and grouped according to the changes in motor function. In addition, a total of 26 healthy subjects were recruited as healthy controls (HC). Participants were enrolled at Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine and received clinical routine treatment during the follow-up period. All participants underwent MRI scans on the day of enrollment. The study was approved by Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine Institutional Review Boards (NO: DZMEC-KY-2018-58). All participants provided written informed consent. Figure 1A shows the study protocol.

The included patients met the following criteria: (1) patients with cerebral infarction whose course of the disease is 3 months and conform to the diagnostic criteria of cerebral infarction; (2) right-handed patients; (3) between 40 and 80 years of age, both men and women are eligible; (4) patients in whom the infarct was located in the unilateral basal ganglia and/or the radiation coronal region; (5) the patient with unconscious disorder and the condition is relatively stable; (6) The patient himself or his immediate family members sign the informed consent.

The exclusion criteria of patients were as following: (1) the patient had ever taken psychotropic drugs in the past months; (2) the patient being pregnant or lactation; (3) the patient had a history of neurologic or psychiatric disorders; (4) the patient had any other health problems or poor physical conditions that may influence participation; (5) the patient had any other brain structure damage or abnormalities identified by MRI examinations; (6) the patient had any history of alcohol or drug dependency; (7) the patient had any MRI contraindications.

The included healthy controls met the following criteria: (1) right-handed people; (2) between 40 and 80 years of age, both men and women are eligible; (3) people proved to be healthy by a medical examination; (4) people signed the informed consent and volunteered to participate in the experiment.

The exclusion criteria of healthy controls were as following: (1) the person had ever taken psychotropic drugs in the past months or had a family genetic history of the mental and nervous system; (2) the person being pregnant or lactation; (3) the person had a history of neurologic or psychiatric disorders; (4) the person had any other health problems or poor physical conditions that may influence participation; (5) the person had any other brain structure damage or abnormalities identified by MRI examinations; (6) the person had any history of alcohol or drug dependency; (7) the person had any MRI contraindications; (8) the person was conducted to other similar research.

2.2. Clinical evaluation and grouping

We evaluated the motor impairment of patients by the Fugl–Meyer Assessment (FMA). FMA includes assessments of the upper extremity (FMA-UE) and lower extremity (FMA-LE) (Sullivan et al., 2011). Two professional neurologists evaluated their motor function at baseline and 2-week follow-up. We grouped patients according to the changes of FMA scores ($\Delta FMA = FMA_{follow\text{-}up} - FMA_{baseline}$). We divided patients with an ΔFMA-UE ≥ 5 or ΔFMA-LE ≥ 6 (Bushnell et al., 2015; Pandian et al., 2016) into the MCID group ($n = 28$, 17 male), and those with an ΔFMA-UE < 5 or ΔFMA-LE < 6 (Page et al., 2012) into the N-MCID (Non-MCID) group ($n = 21$, 15 male). To avoid the ceiling effects, patients with less severe motor impairment (FMA-UE > 61 or FMA-LE > 28) were not included in the analysis (Gladstone et al., 2002).

2.3. Image data acquisition

MRI data were acquired using a 3.0 Tesla Siemens scanner (MAGNETOM Verio Siemens Medical Systems, Erlangen, Germany) with a 32-channel head coil. For resting-state and acupuncture-state fMRI scans, participants were instructed to keep their eyes closed and stay awake without performing any cognitive tasks. The imaging parameters of the EPI sequence were as follows: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, slice number = 31, thickness = 3.5 mm, flip angle = 90°, and matrix size = 64 × 64. High-resolution structural images (T1) were acquired through a magnetization-prepared rapid acquisition with gradient-echo (MPRAGE) sequence with the following parameters: TR/TE = 1900/2.53 ms, field of view (FOV) = 250 × 250 mm^2, matrix size = 256 × 256, flip angle = 9°, slice number = 176, and slice thickness = 1 mm.

2.4. Details of acupuncture operations

The acupuncture operations were on the affected side in patients, or the left side in HC during the acupuncture state fMRI scans. The needles were disposable sterile silver needles (specification parameter: φ0.40 × 40 mm, purchased from Beijing Zhongyantaihe Medical Instrument Co., LTD., manufactured by Suzhou Shenlong Medical Instrument Co., LTD). Yanglingquan (GB 34) is located on the outside of the lower leg, in the middle of the concavity of the anterior and inferior parts of the fibula head. Acupoint selection is performed according to the National standard GB/T 12346–2006 Name and Location of Acupoints. The position of GB 34 is shown in Figure 1B. After the routine skin disinfection, the needle was vertically inserted for 1–1.5 Cun (about 15–25 mm depending on the height and weight of a participant) at GB 34. There was a 10 s post-onset phase of the resting state with the needle inserted, followed by a 1-min manual stimulation phase by using the mild reinforcing-reducing method at the frequency of 1 Hz. Then, an 8-min post-stimulation phase occurred with the needle remaining inside the leg. The needle was removed and disposed of after the acupuncture scanning.

2.5. Preprocessing of fMRI data

The structural and functional MRI images were preprocessed using Data Processing & Analysis for Brain Imaging (DPABI[1]) (Yan

1 http://rfmri.org/DPABI

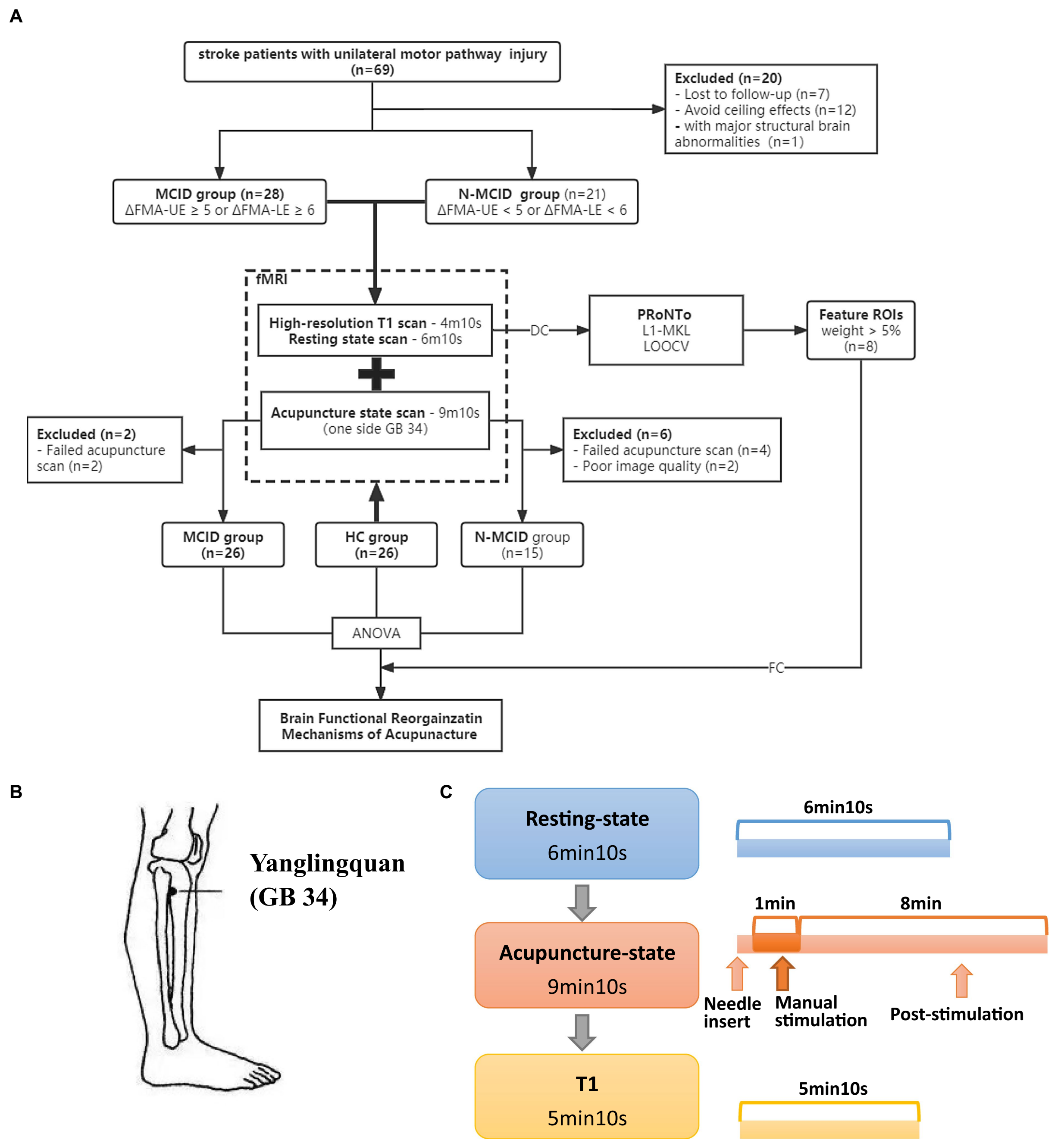

FIGURE 1
Study design. **(A)** The flowchart of the study protocol. **(B)** The location of Yanglingquan (GB 34). **(C)** The scanning protocol. FMA-UE, Fugl–Meyer assessment upper extremity scores; FMA-LE, Fugl–Meyer assessment lower extremity scores; HC, healthy controls; MCID, minimal clinically important difference; N-MCID, non-minimal clinically important difference; DC, degree centrality; FC, functional connectivity; ROI, regions of interest; L1-MKL, L1-multi kernel learning machine; LOOCV, leave-one-out cross-validation.

et al., 2016), which is based on Statistical Parametric Mapping (SPM 12[2]). These toolboxes were based on Matlab 2017a (Mathworks, Sherborn, MA).

All images were flipped to constrain the lesion's location to the right brain hemisphere (i.e., all patients' lesions were constrained to be on the positive MNI x-coordinates by simply inverting the signal of the voxels along the x-axis when the lesion was located on the left hemisphere).

2.5.1. Preprocessing of resting state fMRI data

The first 10 volumes of each participant were discarded as usual. Slice timing and head motion correction were conducted for the remaining time points. Participant data were excluded if they met the head motion criteria, which included head motion >3 mm translation

2 http://www.fil.ion.ucl.ac.uk/spm

or a 3° rotation in any direction. In order to achieve better registration, functional and anatomical images were manually reoriented to the anterior commissure. A linear transformation was used to co-register anatomical images to the functional images for each subject. Subsequently, the transformed anatomical images were segmented into gray matter, white matter, and cerebrospinal fluid by using the new segmentation tool in SPM 12 (Ashburner and Friston, 2005). The transformation from individual space to Montreal Neurological Institute (MNI) space was computed and resampled at a resolution of 3 mm × 3 mm × 3 mm voxels. Next, a Friston 24-parameter model was used to regress out the effects of head motion (Friston et al., 1996). Other nuisance variables, including white matter signal, cerebrospinal fluid signal, and global signal were regressed out from the time series of all voxels *via* linear regression. Then, the images were smoothed using a 6 mm full-width-at-half-maximum Gaussian kernel. After, a temporal filter (0.01–0.08 Hz) was applied to reduce physiological noise at other frequency bands. Finally, we manually checked each subject's structural and functional images to promise the quality of data.

2.5.2. Preprocessing of acupuncture state fMRI data

The first 45 volumes of each participant were discarded (during this period of time, we inserted the needle and stimulated). The other steps of preprocessing were the same to resting state fMRI data, and we also checked the data manually.

2.6. Degree centrality measurement

Weighted DC measures were calculated using DPABI. To obtain each subject's graph, we computed the Pearson correlation coefficients between any pairs of voxels. Each voxel acted as a node in the graph, and each significant Pearson correlation between any pair of voxels represented an edge. An $n \times n$ matrix of Pearson correlation coefficients between any pair of voxels was obtained for each subject by thresholding each correlation at $r > 0.2$ to eliminate possible spurious connectivity. Then, the weighted DC strength of a voxel was computed as the sum of the connectivity between a given brain voxel and all other voxels. Finally, the individual-level voxel-wise DC value for each subject was converted into a Z-score map by the Fisher-Z transformation to improve normality.

2.7. PRoNTo analyses

To classify two groups, we used L1-MKL from the PRoNTo toolbox[3] (Schrouff et al., 2013). Classification based on L1-MKL is viewed as a supervised learning algorithm because it facilitates learning a model from training data whose class label was previously defined and assigns class labels to test data. Support Vector Machines (SVM) transformed low dimensional data into a higher dimension and generated support vector classifiers that separated higher dimensional data into two groups *via* kernel functions (Lanckriet et al., 2004; You et al., 2021).

3 http://www.mlnl.cs.ucl.ac.uk/pronto/

2.7.1. Regions of interest-based machine kernel learning

Degree centrality maps were served as inputs to classify two groups in machine learning. The ROIs are defined on the basis of Craddock's work, which generated an ROI atlas by parcellating whole brain resting-state fMRI data into spatially coherent regions of homogeneous FC (Craddock et al., 2012). For each participant, 200 features were extracted from 200 ROIs as an machine kernel learning (MKL) source (ROI-MKL). Features were selected to form a kernel matrix through a multi-kernel strategy. A nested cross-validation (CV) scheme was used to obtain unbiased estimates of classification performance.

2.7.2. Performance evaluation of classification methods

Machine learning applications apply a leave-one-out cross-validation (LOOCV) strategy with an optimized nested hyper-parameter meter range of 2.^[−5:5] to evaluate the generalizability of classifiers (Wen et al., 2017). Overall classification accuracy, sensitivity (i.e., the proportion of MCID patients correctly classified), and specificity (i.e., the proportion of N-MCID patients correctly classified) can be defined from CV results quantifying the performance of classifiers. *Value of ps* were calculated using permutation tests (1,000 permutations).

2.7.3. Weights map

Next, anatomical atlas weights were computed to visualize the relative importance of each region in the multivariate pattern analysis decision function displaying regional patterns of the DC maps. The weight of each feature in ROIs can also be obtained because the coefficient is learned as a single optimization problem in equations and weights relevant to each kernel. In this study, each kernel was known as an "ROI-weight" that reflected "voxel-weight." Higher absolute indicator weight values discriminated corresponding features. Because there is no conventional threshold for the optimal number of ROIs to be retained, in this exploratory study we presented the ROIs that weight vector value more than 5%, called feature ROIs, number of eight.

2.8. Functional connectivity measurement

The voxel-wise functional connectivity analyses between each ROI (the feature ROIs) and each voxel in the brain areas were performed to generate seed-based FC maps at baseline and at 2 weeks after stroke. For group analyses, the correlation coefficients were transformed to Z values using Fisher's Z-transformation to improve the normality of the correlation coefficient.

2.9. Statistical analysis

All data were analyzed using the statistical program SPSS 25.0 for intergroup comparisons of demographic data and FMA scores, and the Shapiro–Wilk test was used to verify the normality of the data. Subject characteristics were compared among three groups using ANOVA or the Mann–Whitney U test depending on their distributions, and two groups using a two-sample t-test or the

Mann–Whitney U test depending on their distributions. The proportions of sex and lesion side were examined using the chi-square test. $p < 0.05$ indicated statistical significance.

The statistical analysis was conducted by DPABI software. The mean framewise displacement (i.e., Mean FD_Jenkinson) was taken as the covariate to control the impact of unnecessary head motion in the statistical analysis. One-way ANOVA ($p < 0.05$, Bonferroni corrected) in the statistical analysis module of DPABI software was performed to compare variables among the three groups (HC, MCID, and N-MCID). A mask was built according to the results of ANOVA. Based on the mask, the inter-group differences were obtained by using the *post hoc* *t*-test. Two-tailed Gaussian random field (GRF) correction (voxel threshold of $p < 0.01$ and cluster threshold of $p < 0.05$) was performed during the two-sample *t*-test (the *post hoc* test).

3. Results

3.1. Demographic and clinical data

A total of 69 patients were registered for this study period. After excluding 7 patients with unavailable 2-week FMA scores, 12 patients with ceiling effects, and 1 patient with major structural brain abnormalities, 49 patients were finally included. The drop-out rate was 10.14%. The included patients were divided into the MCID group ($n = 28$, 17 male) and the N-MCID group ($n = 21$, 15 male). There was no significant difference between the MCID and N-MCID in sex ($\chi^2 = 0.608$, $p = 0.436$), age ($Z = -0.101$, $p = 0.919$), lesion side ($\chi^2 = 1.612$, $p = 0.204$), course of disease ($Z = -0.192$, $p = 0.848$), and FMA scores at baseline. Table 1 and Supplementary Table S1 show the demographic and clinical information of both groups of patients. Figure 2 shows the distributions of motor function between the two groups of patients at baseline.

In the acupuncture state scanning, 7 patients failed to complete this fMRI state scanning and 2 patients were excluded for poor image quality. Finally, 26 patients in the MCID group, 15 patients in the N-MCID group, and 26 age ($\chi^2 = 0.131$, $p = 0.937$) and sex ($\chi^2 = 1.363$, $p = 0.506$) matched healthy controls (16 male) were included. Table 2 shows the demographic and clinical information of all the participants who completed the acupuncture-state scanning.

3.2. Results of feature ROIs identified by machine learning classification

The ML analysis was able to classify the MCID and N-MCID groups with 75.51% balanced accuracy (BA, $p = 0.018$ during 1,000 permutation testing), based on DC of whole brain functional regions maps. Specifically, class accuracy was 82.14% (23/28) for the MCID group and 66.67% (14/21) for the N-MCID group. In addition, the class predictive value was 76.67% for the MCID group and 73.68% for the N-MCID group. The AUC was 0.800. Figures 3A,B illustrate the performance of machine learning classification. The PRoNTo identified several functional regions with weights used by the decision function of the machine to predict group classification. Figure 3C shows the weight maps of all the regions with prediction weights.

We remained 8 feature ROIs with relatively high predictive weights for the group classification, including the bilateral supplementary motor areas (SMA), precentral gyrus (PreCG), postcentral gyrus (PoCG), paracentral lobule (PCL), etc.; the contralesional caudate nucleus (CAU), putamen (PUT), middle occipital gyrus (MOG), superior occipital gyrus (SOG), angular gyrus (ANG), etc.; and the ipsilesional dorsolateral superior frontal gyrus (SFG), inferior frontal gyrus (IFG), orbital gyrus, middle cingulate and paracingulate gyri (MCC), precuneus (PCUN), etc. Table 3 lists the regions with weights >5% and the intergroup comparisons of DC. Moreover, we compared DCs of these feature ROIs between groups. Compared to N-MCID, MCID exhibited significantly higher DC in ROI 1 (i.e., the left caudate nucleus and the left putamen, $t = -2.016$, $p = 0.049$) and ROI 2 (i.e., the right SMA and the right SFG, $t = -3.042$, $p = 0.004$), indicating that MCID had stronger nodal centralities in these brain regions. However, there were no significant differences between the other ROIs, indicating that group comparisons of DC cannot classify two groups.

3.3. Functional connectivity and acupuncture effects of feature ROIs

Eight feature ROIs were used as seed-points to analyze the FC with the whole brain and the immediate effects of acupuncture

TABLE 1 Demographic and clinical data.

Characteristics	Group		χ^2/Z	Value of *p*
	MCID (*n*=28)	N-MCID (*n*=21)		
Sex (male/female)	17/11	15/6	0.608	0.436[a]
Age (years)	62.00(57.00–67.75)	62.00(53.50–69.00)	−0.101	0.919[b]
Lesion side (left/right)	13/15	6/15	1.612	0.204[a]
Course of disease (days)	18.50(9.50–30.75)	21.00(6.50–31.00)	−0.192	0.848[b]
FMA-UE	22.00(7.00–54.75)	33.00(11.00–53.50)	−0.354	0.723[b]
FMA-LE	27.00(21.00–32.00)	20.00(13.00–30.00)	−1.600	0.110[b]
FMA-total	48.00(27.00–84.50)	55.00(25.00–79.50)	−0.455	0.649[b]

The data are presented as the median (interquartile range) for non-normally distributed data. [a]The value of *p* was obtained by a chi-square test; [b]The value of *p* was obtained by a two-sample nonparametric test. MCID, minimal clinically important difference; N-MCID, non-minimal clinically important difference; FMA-UE, Fugl–Meyer assessment upper extremity scores; FMA-LE, Fugl–Meyer assessment lower extremity scores; FMA-Total, Fugl–Meyer assessment total scores.

among MCID, N-MCID, and HC. Table 4; Figure 4; Supplementary Figure S1 show the results of FC from ANOVA among three groups in the resting state and acupuncture state (*GRF* correction, voxel-$p < 0.01$, cluster-$p < 0.05$). In the resting state, the MCID group exhibited significantly lower FCs between almost all the ROIs (no significant difference in ROI 6) with other brain regions compared to HC (e.g., ROI 1 with the bilateral cerebellum, bilateral MCC, bilateral PCL, and right SMA), but higher FC only between ROI 8 (i.e., the right MCC, right precuneus, right PCL, and right SMA) with the left middle temporal gyrus (MTG) and left inferior temporal gyrus (ITG). Compared to HC, the N-MCID group similarly exhibited significantly lower FCs between the ROIs (i.e., ROI 1–3, ROI 5, and ROI 7–8) with other brain regions (e.g., ROI 2 with the bilateral anterior cingulate, left supramarginal gyrus, and left superior temporal gyrus), but higher FC between ROI 4 (i.e., the left MOG, left SOG, and left ANG) with the bilateral posterior cingulate gyrus (PCC). The FC between ROI 5 (i.e., the right IFG opercular part, right PreCG, right middle frontal gyrus, and right IFG triangular part) with the left cerebellar, left lingual gyrus (LING), left inferior occipital (IOG), and left MOG were lower in the MCID group than in the N-MCID group. There were no significant differences in FC between the other ROIs in the

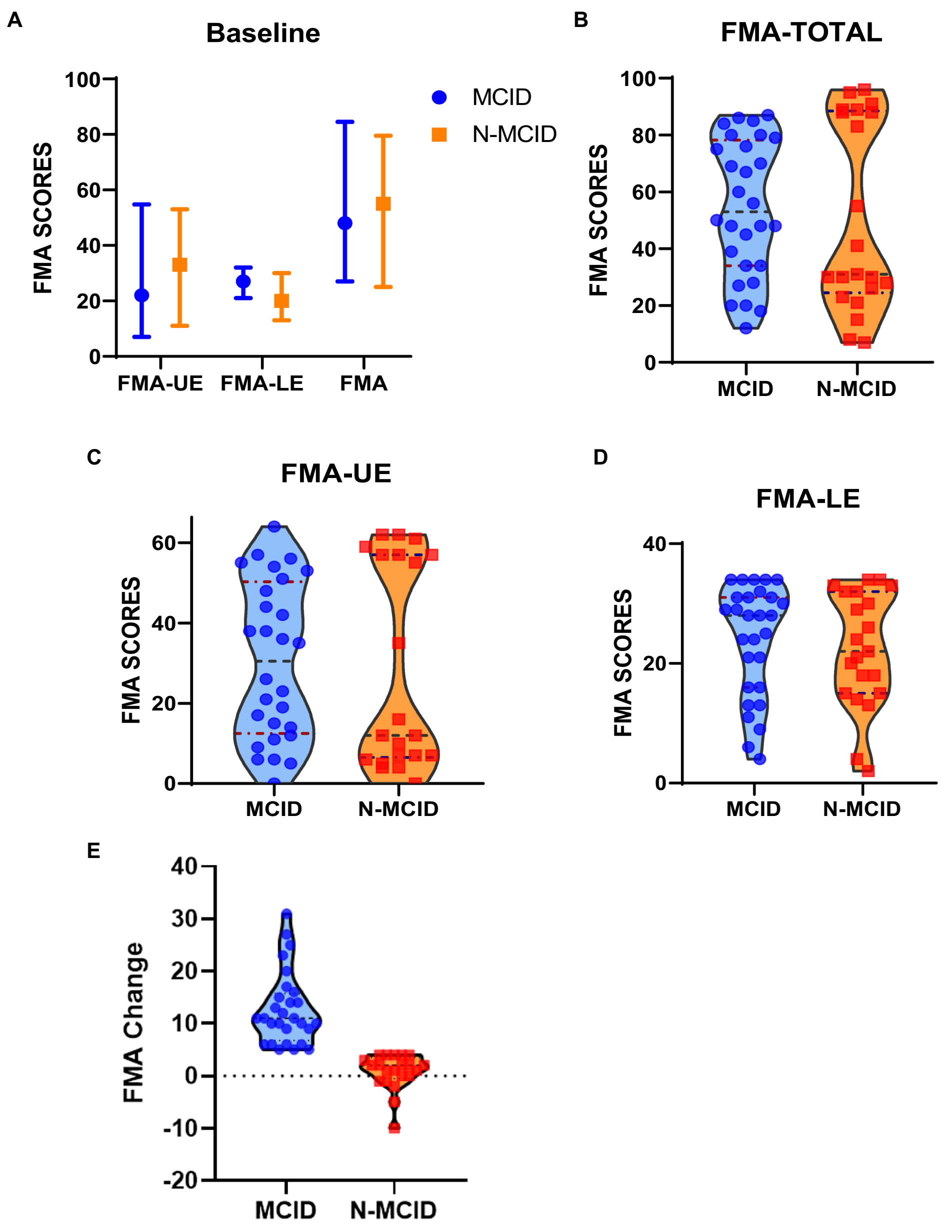

FIGURE 2
Fugl–Meyer assessment (FMA) scores at baseline for each group of stroke patients. **(A)** The median, upper and lower quartile distribution of FMA at baseline for each group. **(B)** The distribution of FMA-TOTAL for the two groups. **(C)** The distribution of FMA-UE for the two groups. **(D)** The distribution of FMA-LE for the two groups. **(E)** The distribution of FMA-Change for the two groups. The blue dots represent patients in the MCID group; the orange squares represent patients in the N-MCID group. FMA-UE, Fugl–Meyer assessment upper extremity scores; FMA-LE, Fugl–Meyer assessment lower extremity scores; patients. FMA-TOTAL, Fugl–Meyer assessment total extremity scores; MCID, minimal clinically important difference; N-MCID, non-minimal clinically important difference.

TABLE 2 Demographic and clinical data of acupuncture state.

Characteristics	Group				
	MCID (*n*=26)	N-MCID (*n*=15)	HC (*n*=26)	χ^2/Z	Value of *p*
Sex (male/female)	16/10	10/5	16/10	0.131	0.937[a]
Age (years)	62.00(56.50–66.25)	61.00(50.00–66.00)	59.50(53.75–62.25)	1.363	0.506[b]
Lesion side (left/right)	13/13	4/11		2.134	0.195[a]
Course of disease (days)	20.50(12.50–35.00)	28.00(18.00–42.00)		−1.300	0.194[b]
Motor assessment					
FMA-UE	35.50(13.50–51.25)	12.00(5.00–55.00)		−1.070	0.285[b]
FMA-LE	28.00(17.00–31.00)	21.00(15.00–32.00)		−0.976	0.329[b]
FMA-Total	54.00(34.00–79.00)	30.00(21.00–87.00)		−0.921	0.357[b]

The data are presented as the median (interquartile range) for non-normally distributed data. [a]The value of *p* was obtained by a chi-square test; [b]The value of *p* was obtained by a nonparametric test. MCID, minimal clinically important difference; N-MCID, non-minimal clinically important difference; HC, healthy controls; FMA-UE, Fugl–Meyer assessment upper extremity scores; FMA-LE, Fugl–Meyer assessment lower extremity scores; FMA-Total, Fugl–Meyer assessment total scores.

MCID group and the N-MCID group, but MCID revealed a wider range of lower FC, implying more generalized abnormal FC.

In the acupuncture state, the MCID group generally exhibited significantly lower FC between the feature ROIs and other brain regions compared to HC (e.g., ROI 1 with the bilateral cerebellum, left MOG, left MTG, and left ANG), but only a small proportion of higher FC between ROI 7 (i.e., the right PoCG, right PreCG, right rolandic operculum, and right IFG opercular part) with the right supramarginal gyrus (SMG), and right ANG; ROI 8 with the left ANG, left MTG and left MOG. There were two ROIs that exhibited stronger FC with other brain regions in the N-MCID group than in HC, mainly including ROI 5 with the left precuneus, bilateral LING, left cerebellum, left PCC, bilateral MFG orbital part, and ROI 8 with the left ANG, left MTG, left MOG, and left inferior parietal gyrus (IPG). There were no significant differences in FC between the other ROIs and the whole brain regions compared to HC. ROI 1, ROI 4, ROI 5, and ROI 6 presented lower FC with other brain regions in the MCID group than the N-MCID group. There were no significant differences in all FCs between the three groups of participants in the resting and acupuncture states, but patients exhibited fewer brain regions with abnormal FC in the acupuncture state than in the resting state.

4. Discussion

In this study, we applied a machine learning analysis method to screen functional brain regions that can classify clinical differences of motor recovery in patients with unilateral motor pathway injury (basal ganglia and/or corona radiata) after ischemic stroke. We identified eight regions with predicted weights>5% as regions of interest (ROIs) and found that these ROIs were located bilaterally in the cerebral hemispheres (Figure 3C). The weights of the contralesional brain regions accounted for approximately 35.16% (ROI 1, ROI 3, and ROI 4; Table 3) and the ipsilesional brain regions accounted for approximately 40.75% (ROI 2, and ROI 5–8; Table 3), suggesting that motor recovery after unilateral motor pathway injury is closely related to the regulation of bilateral brain regions. This was consistent with the point that the pattern of bilateral actions may contribute to engaging ipsilateral motor pathways in a motor behavior (Tazoe and Perez, 2014).

Motor rehabilitation can be affected by many factors. Because of this, we chose the MCID as a basis for our grouping, since it is the smallest improvement that patients can perceive as beneficial and focuses on the patient's self-perception. The feature brain regions we obtained, mainly including the contralesional CAU (Graff-Radford et al., 2017), the bilateral SMA (Liu et al., 2022), PreCG (Park et al., 2011), PoCG (Ward et al., 2003), and PCL (Kang and Kim, 2008), were observed to be associated with motor behavior and outcomes in previous studies. There is bilateral interaction effect between the cerebral hemispheres. The balance between the bilateral hemispheres is disrupted after stroke and there is spontaneous functional regulation between the bilateral hemispheres to facilitate recovery. We found that the feature ROIs mainly involved the basal ganglia area (i.e., the location of the lesion corresponding to the contralesional hemisphere) and distal motor-related regions (e.g., SMA, PreCG, etc.) in the constralesional hemisphere, whereas mainly involved the distal motor-related regions in the ipsilesional hemisphere. This indicates that: (1) the distal regions of the ipsilesional hemisphere is important in predicting recovery after stroke, performed the intra-hemisphere adjustment; (2) the basal ganglia area of the contralesional hemisphere may compensate for the ipsilesional hemisphere, which may perform the compensate ability between two hemispheres; (3) functional synergistic changes in the distal motion-related regions of the bilateral hemispheres affected motor recovery through bilateral modulation, which may perform the interaction between two hemispheres.

Beside of these, we also found some brain regions with predictive weights related to mental and psychological aspects, such as the contralesional putamen (Klingbeil et al., 2022), the contralesional occipital lobe (Park et al., 2011), the ipsilesional frontal lobe (Stangeland et al., 2018), the ipsilesional orbital gyrus (Pan et al., 2022), etc. Some studies have also reported that psychosocial factors and non-motor brain regions have an impact on stroke rehabilitation (Ward et al., 2003; Qian et al., 2019). As can be seen, the ROIs we extracted involved motor and mental brain regions, and could be used as features to more comprehensively predict the classification of motor recovery after stroke.

Furthermore, we also extracted and compared the DC values of the ROIs, and found a statistically significant difference in DC values for only two ROIs (i.e., ROI 1, ROI 2) between the two groups. It is illustrated that machine learning can identify unexpected informative

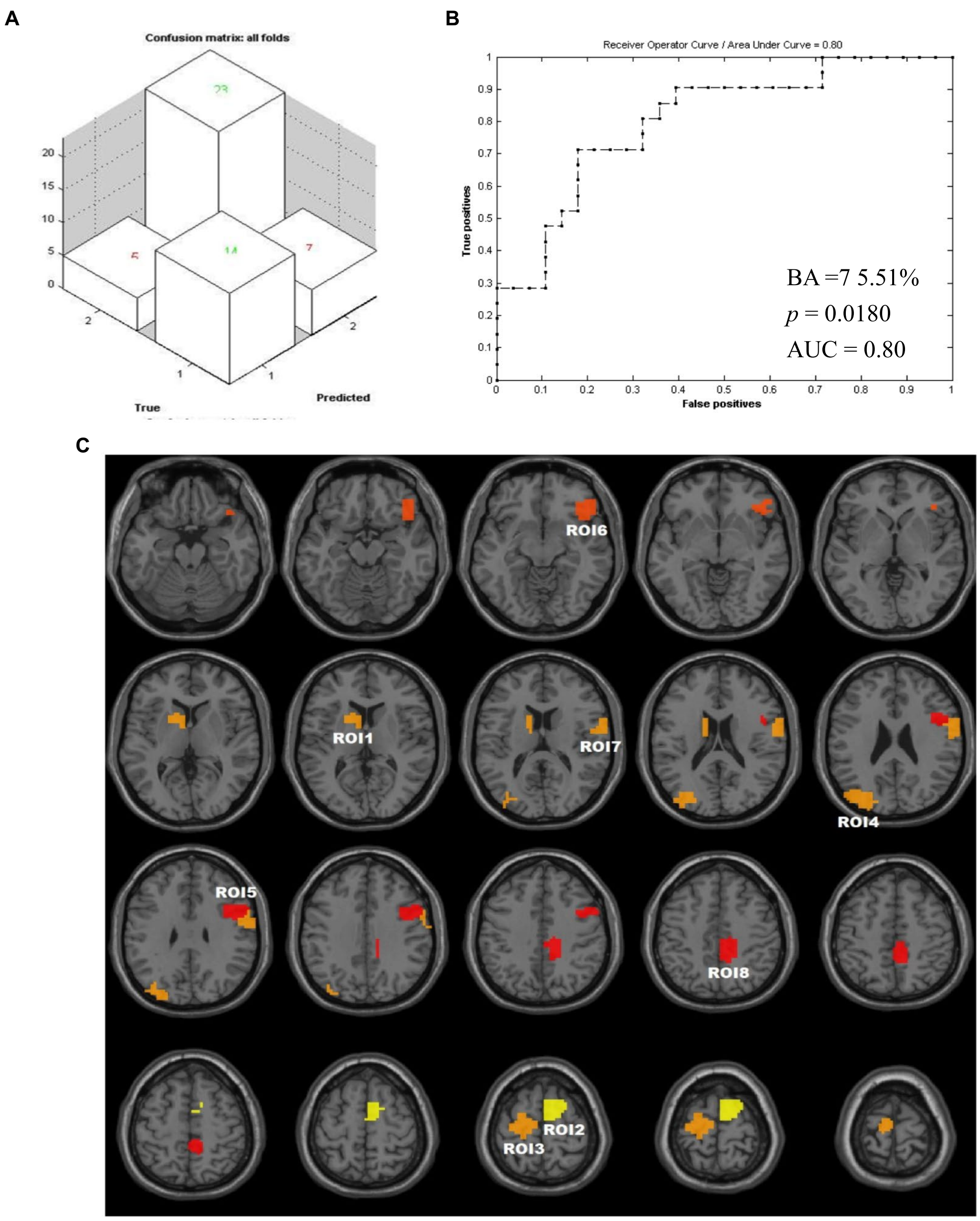

FIGURE 3
Results of machine learning. **(A)** The confusion matrix output by this classification. (1) i.e., N-MCID; (2) i.e., MCID. **(B)** Receiver operating characteristic curve and area under curve for the classification developed with two groups as inputs. The value of *p* was obtained during 1,000 permutation testing. **(C)** Weights map of all feature ROIs. The color of the clusters from red to yellow represents that the weight becomes larger. AUC, area under curve; BA, balanced accuracy; ROI, regions of interest.

variables by traditional statistics and capture new potential features (Deo, 2015). Herein, the ML classifier achieved a balance accuracy of 75.51% and an AUC of 80.00%, indicating a relatively good performance. Therefore, we applied machine learning algorithms to classify patients with clinical differences in motor outcomes, identifying more personalized features of predicting motor outcomes in order to provide novel and referable neuroimaging evidence toward precision medicine for motor recovery after stroke (Deo, 2015; Handelman et al., 2018).

Subsequently, we compared the differences in FC among MCID, N-MCID, and HC in the resting and acupuncture states, respectively, to discover the brain functional effects of motor impairment and recovery after stroke. We demonstrated the response patterns of these characteristic ROIs in motor recovery and under acupuncture intervention and explored possible central neural mechanisms of acupuncture (Table 4; Figure 4; Supplementary Figure S1). In the resting state, compared to HC, abnormal FCs were found in patients and most of them exhibited lower FC, indicating that the synergy of different brain regions was reduced after stroke. Previous studies reported decreased functional connectivity between hemispheric brain regions in the early stages

TABLE 3 Weights of feature ROIs and comparison of DC.

Feature ROIs	ROI weight (%)	Brain regions in AAL	Hemi-sphere	DC	
				t	Value of *p*
ROI 1	17.67	Caudate nucleus (CAU)	L	−2.016	0.049
		Lenticular nucleus, putamen (PUT)			
ROI 2	12.02	Supplementary motor area (SMA)	R	−3.042	0.004
		Superior frontal gyrus, dorsolateral (SFG)			
ROI 3	8.86	Precentral gyrus (PreCG)	L	1.972	0.054
		Paracentral lobule (PCL)			
		Supplementary motor area (SMA)			
		Superior frontal gyrus, dorsolateral (SFG)			
		Postcentral gyrus (PoCG)			
ROI 4	8.63	Middle occipital gyrus (MOG)	L	2.337	0.024
		Superior occipital gyrus (SOG)			
		Angular gyrus (ANG)			
ROI 5	8.42	Inferior frontal gyrus, opercular part (IFGoperc)	R	0.742	0.462
		Precentral gyrus (PreCG)			
		Middle frontal gyrus (MFG)			
		Inferior frontal gyrus, triangular part (IFGtriang)			
ROI 6	7.53	Inferior frontal gyrus, orbital part (IFGorb)	R	1.823	0.075
		Posterior orbital gyrus (OFCpost)			
		Lateral orbital gyrus (OFClat)			
		Insula (INS)			
		Temporal pole: superior temporal gyrus (TPOsup)			
		Inferior frontal gyrus, triangular part (IFGtriang)			
		Anterior orbital gyrus (OFCant)			
ROI 7	6.75	Postcentral gyrus (PoCG)	R	0.575	0.568
		Precentral gyrus (PreCG)			
		Rolandic operculum (ROL)			
		Inferior frontal gyrus, opercular part (IFGoperc)			
ROI 8	6.03	Middle cingulate and paracingulate gyri (MCC)	R	−1.228	0.226
		Precuneus (PCUN)			
		Paracentral lobule (PCL)			
		Supplementary motor area (SMA)			

Regions were identified by setting the threshold to ≥5% of the maximum ROI-weight rank. Negative *t*-test values reflect significantly more DC in identified regions of interest for the MCID group. The value of *p* was based on two-sample *t*-tests. ROI, regions of interest; DC, degree centrality; L, left, i.e., contralesional hemisphere; R, right, i.e., ipsilesional hemisphere.

of stroke (Fan et al., 2015; Tang et al., 2016; Hensel et al., 2022) and concluded that hemispheric interactions in stroke patients were frequently characterized by abnormalities, in terms of balance and competition (Casula et al., 2021). Our results demonstrated that the abnormal form of FC exhibited was not entirely consistent between the different groups. In the MCID group, there were decreased FCs between the ipsilesional ROIs and the bilateral cerebral hemispheres, whereas the contralesional ROIs mainly presented decreased FCs between the ipsilesional hemisphere. In the N-MCID group, the decreased FCs were generally either between the ipsilateral or contralateral hemispheres of the ROIs. The lower FCs were found to be restored and can reach or even exceed the level of

TABLE 4 Functional connectivity (FC) of feature ROIs in the resting state and acupuncture state.

Feature ROIs	Groups	Resting state			Acupuncture state		
		Clusters	Brain regions	*F*-value (peak)	Clusters	Brain regions	*F*-value (peak)
ROI 1	MCID vs. HC	1	Bilateral Cerebellum	−2.576	1	Left Middle occipital gyrus Middle temporal gyrus Angular gyrus	−2.577
		2	Bilateral Medial cingulate gyrus Paracentral lobule Right Supplementary motor area	−2.578	2	Bilateral Cerebellum	−2.579
	N-MCID vs. HC	1	Left Inferior frontal gyrus, triangular part Inferior frontal gyrus pars orbitalis	−2.586	NA		
		2	Left Cerebellum	−2.577			
	MCID vs. N-MCID	NA			1	Right Lingual gyrus Cerebellum Calcarine fissure and surrounding cortex Cuneate Inferior occipital gyrus	−2.576
ROI 2	MCID vs. HC	1	Bilateral Thalamus Caudate nucleus	−2.582	1	Right Pallidus Putamen Insula Hippocampus Amygdala Caudate nucleus	−2.576
		2	Bilateral Medial cingulate gyrus Supplementary motor area	−2.605	2	Bilateral Anterior cingulate and paracingulate gyri Medial cingulate and paracingulate gyri Right Supplementary motor area	−2.580
		3	Right Precentral gyrus Postcentral gyrus Supplementary motor area	−2.578			
		4	Right Rolandic operculum Inferior frontal gyrus, opercular part Temporal pole: Superior temporal gyrus Superior temporal gyrus	−2.589			
		5	Left Insula Rolandic operculum	−2.578			

(Continued)

TABLE 4 (Continued)

Feature ROIs	Groups	Resting state			Acupuncture state		
		Clusters	Brain regions	*F*-value (peak)	Clusters	Brain regions	*F*-value (peak)
	N-MCID vs. HC	1	Bilateral Anterior cingulate and paracingulate gyri Medial cingulate and paracingulate gyri	−2.593	NA		
		2	Left Supramarginal gyrus Middle temporal gyrus	−2.577			
	MCID vs. N-MCID	NA			NA		
ROI 3	MCID vs. HC	1	Right Superior parietal gyrus Postcentral gyrus Precuneus	−2.576	1	Right Precentral gyrus Postcentral gyrus	−2.579
		2	Right Precentral gyrus Middle frontal gyrus	−2.584	2	Left Middle occipital gyrus Inferior occipital gyrus	−2.578
		3	Right Caudate nucleus Thalamus	−2.594			
		4	Right Rolandic operculum Inferior frontal gyrus, opercular part	−2.580			
	N-MCID vs. HC	1	Right Caudate nucleus Thalamus	−2.583	NA		
	MCID vs. N-MCID	NA			NA		
ROI 4	MCID vs. HC	1	Right Middle occipital gyrus Superior occipital gyrus Precuneus	−2.578	1	Right Middle temporal gyrus Middle occipital gyrus Superior occipital gyrus	−2.578
		2	Right Precuneus Calcarine fissure and surrounding cortex Lingual gyrus	−2.586	2	Right Calcarine fissure and surrounding cortex Lingual gyrus	−2.577
		3	Right Superior parietal gyrus Precuneus posterior central gyrus	−2.580	3	Bilateral Precuneus	−2.577
	N-MCID vs. HC	1	Bilateral Posterior cingulate gyrus	4.595	NA		
	MCID vs. N-MCID	NA			1	Left Middle temporal gyrus Supramarginal gyrus Angular gyrus	−2.584

(Continued)

TABLE 4 (Continued)

Feature ROIs	Groups	Resting state			Acupuncture state		
		Clusters	Brain regions	*F*-value (peak)	Clusters	Brain regions	*F*-value (peak)
ROI 5	MCID vs. HC	NA			1	Left Inferior parietal gyrus Superior temporal gyrus Superior parietal gyrus Supramarginal gyrus Middle temporal gyrus Precuneus Posterior central gyrus	−2.577
					2	Bilateral Cingulate gyrus Left Supplementary motor area	−2.577
					3	Left Insula Inferior frontal gyrus, opercular part Precentral gyrus Rolandic operculum	−2.578
	N-MCID vs. HC	1	Right Caudate nucleus Thalamus	−2.581	1	Left Precuneus Cerebellum Posterior cingulate gyrus Bilateral Lingual gyrus	4.324
					2	Bilateral Middle frontal gyrus pars orbitalis Gyrus rectus	3.941
	MCID vs. N-MCID	1	Left Cerebellum Lingual gyrus Middle occipital gyrus Inferior occipital gyrus	−2.577	1	Bilateral Cerebellum	−2.576
					2	Left Middle temporal gyrus Inferior temporal gyrus Heschl's gyrus	−2.577
ROI 6	MCID vs. HC	NA			1	Left Inferior frontal gyrus pars orbitalis Middle frontal gyrus Insula Inferior frontal gyrus, triangular part Temporal pole: superior temporal gyrus Anterior cingulate and paracingulate gyri Inferior frontal gyrus, opercular part Superior frontal gyrus, medial Superior frontal gyrus Superior temporal gyrus	−2.577
					2	Bilateral Cerebellum	−2.578

(Continued)

TABLE 4 (Continued)

Feature ROIs	Groups	Resting state			Acupuncture state		
		Clusters	Brain regions	*F*-value (peak)	Clusters	Brain regions	*F*-value (peak)
					3	Left Inferior temporal gyrus Middle temporal gyrus	−2.579
					4	Left Inferior parietal gyrus Angular gyrus Middle temporal gyrus supramarginal gyrus	−2.577
	N-MCID vs. HC	NA			NA		
	MCID vs. N-MCID	NA			1	Bilateral Cerebellum	−2.578
					2	Left Precuneus Superior parietal gyrus Inferior parietal gyrus Posterior central gyrus	−2.577
					3	Left Inferior temporal gyrus Middle temporal gyrus Inferior occipital gyrus	−2.576
ROI 7	MCID vs. HC	1	Bilateral Posterior central gyrus Precuneus Left Precentral gyrus Right Superior parietal gyrus	−2.578	1	Left Posterior central gyrus Precentral gyrus Paracentral lobule Supramarginal gyrus Inferior parietal gyrus Right Supplementary motor area Paracentral lobule	−2.576
		2	Left Middle temporal gyrus Superior temporal gyrus Insula	−2.576	2	Right Supramarginal gyrus Inferior parietal gyrus Angular gyrus	3.771
		3	Bilateral Supplementary motor area Left Superior frontal gyrus Paracentral lobule	−2.580	3	Left Middle temporal gyrus Superior temporal gyrus Middle occipital gyrus Inferior occipital gyrus Insula	−2.577
	N-MCID vs. HC	1	Bilateral Anterior cingulate and paracingulate gyri Medial cingulate and paracingulate gyri	−2.584	NA		
		2	Right Insula Caudate nucleus Putamen	−2.578			
	MCID vs. N-MCID	NA			NA		

(Continued)

TABLE 4 (Continued)

Feature ROIs	Groups	Resting state			Acupuncture state		
		Clusters	Brain regions	*F*-value (peak)	Clusters	Brain regions	*F*-value (peak)
ROI 8	MCID vs. HC	1	Left Middle temporal gyrus Inferior temporal gyrus	4.207	1	Left Angular gyrus Middle temporal gyrus Middle occipital gyrus	4.495
		2	Right Precuneus Superior parietal gyrus Superior occipital gyrus Cuneate	−2.583	2	Right Superior temporal gyrus supramarginal gyrus Rolandic operculum Heschl's gyrus Precentral gyrus	−2.576
		3	Right Precentral gyrus Postcentral gyrus Supramarginal gyrus	−2.576	3	Left Middle occipital gyrus Middle temporal gyrus Inferior occipital gyrus Inferior temporal gyrus	−2.581
		4	Right Superior parietal gyrus Postcentral gyrus Precuneus	−2.579			
		5	Right Caudate nucleus Thalamus	−2.582			
		6	Right Middle frontal gyrus Superior frontal gyrus	−2.579			
		7	Left Superior parietal gyrus Precuneus	−2.596			
	N-MCID vs. HC	1	Bilateral Precuneus	−2.578	1	Left Angular gyrus Middle temporal gyrus Middle occipital gyrus Inferior parietal gyrus	4.066
	MCID vs. N-MCID	NA			NA		

healthy controls (Park et al., 2011; Chen et al., 2020; Hensel et al., 2022) during motor recovery. Thus, the regulation and balance of interhemispheric inhibition can enhance post-stroke motor recovery (Boddington and Reynolds, 2017).

In the acupuncture state, we found that the MCID group still exhibited decreased FCs, but the range of brain regions with lower FC was reduced compared to the resting state. These results showed that acupuncture eliminated abnormal FCs between the contralesional ROIs with the ipsilesional brain regions, and between the ipsilesional ROIs within the ipsilesional brain regions. While in the N-MCID group, acupuncture restored the decreased FCs and even increased FCs between a part of the ipsilesional ROIs (ROI 5, ROI 8) with the contralesional brain regions. It could be speculated that acupuncture may have potential and specific targets of effects between different groups. Previous research considered that acupuncture can selectively adjust brain regions thought to be involved in mediating stroke recovery *via* functional plasticity (Li et al., 2006) and modulate functional connectivity between brain regions, brain networks, and hemispheres, which may be a beneficial effect of acupuncture to promote motor recovery from stroke (Qi et al., 2014; Li et al., 2015; Han et al., 2020). These findings above have similarities to our results, we suggested that acupuncture can modulate the bilateral hemispheres through feature ROIs to restore brain functional connectivity in stroke patients toward healthy controls and has its unique pattern of effects.

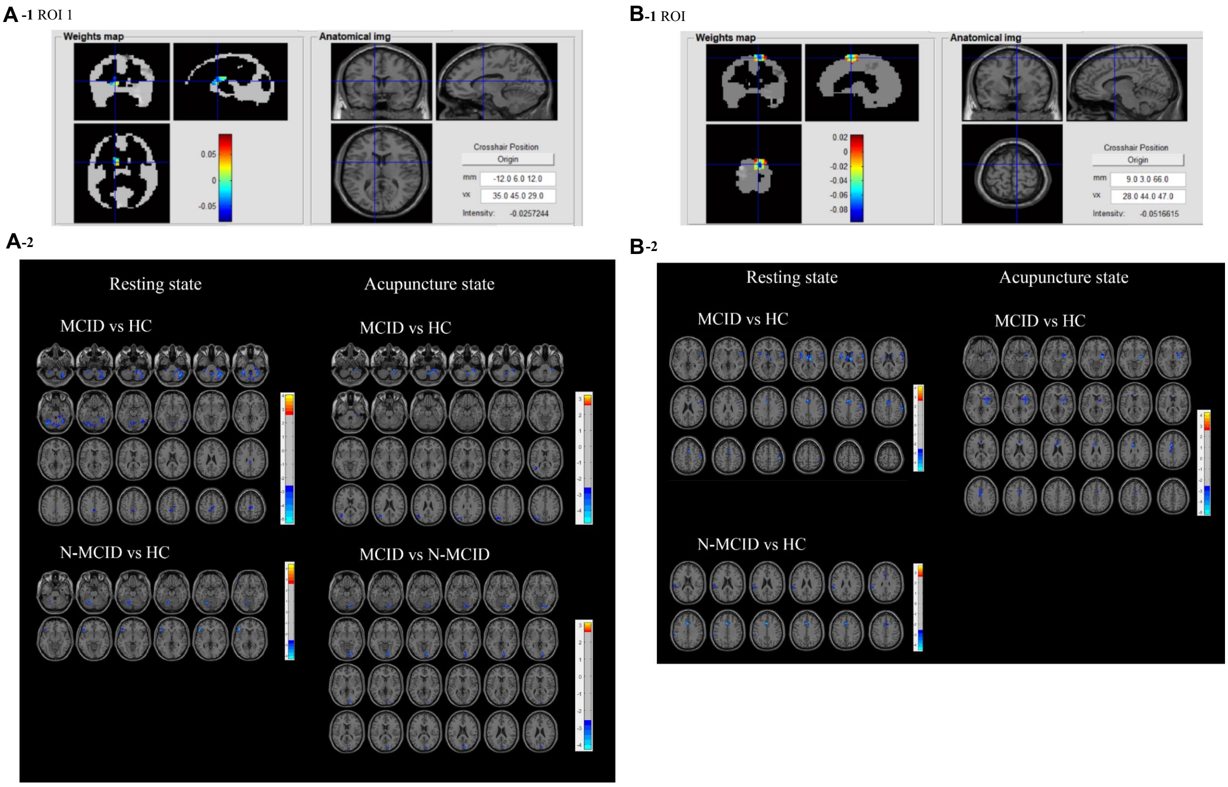

FIGURE 4
(A1,B1) Present the anatomical position and weights distribution of feature ROI 1 and ROI 2 Red indicates high weight and blue indicates low weight. **(A2,B2)** Plots of FC differences between feature ROI 1 and ROI 2 with the whole brain in the resting and acupuncture states. The blue clusters in the former group indicate lower FC between thee brain regions with the ROI, while the red clusters indicate higher FC between these brain regions with the ROI compared to the latter group. FC, functional connectivity; ROI, regions of interest; MCID, minimal clinically important difference; N-MCID, non-minimal clinically important difference. The FCs of the other ROIs are shown in Supplementary Figure S1.

Notably, we noticed that the MCID group exhibited a wider range of brain regions with abnormal FCs than the N-MCID group, both in the resting and acupuncture states, although there was no statistically significant difference between the two groups. There are two possible reasons for this result, the first is that the MCID group has a higher class accuracy (82.14%) in machine learning. Therefore, the feature ROIs we extracted may be more representative for the MCID group. The second is that the basis of the grouping caused a more discrete distribution of the degree of motor impairment among the patients in the N-MCID group, most patients with limited recovery from severe impairments or littler recovery from mild impairments. It has been suggested that the outcome of brain function remodeling is related to the degree of initial damage. Different patterns of functional and structural reorganization of brain function exist in patients with different levels of deficits, leading to different prognoses. Changes in brain functional remodeling are often associated with clinical evaluation in patients with a mild degree of impairment and good recovery. In contrast, there is no correlation between patients with poor recovery and severe impairments (Lin et al., 2019; Jimenez-Marin et al., 2022). As a result, the N-MCID group may not express more differences.

There are several limitations to this study. The first is the follow-up time. We investigated the changes in FMA within 2 weeks of recovery from motor deficits. During the follow-up, there were some subjects drop out from the study, which could influence the accuracy of research. The period of recovery is 6 months after the stroke. Large-quantity and long-term longitudinal observations may uncover functional reorganization throughout the motor recovery period. Secondly, to avoid other redundant distractions, we chose a single acupoint for this study. However, this was not consistent with real clinical therapeutic protocols. In the future, studies about multiple acupoints can be carried out to reveal the mechanisms of acupuncture. The third limitation is the subgroup analysis. The focus of this study was on motor improvement in patients, but the recovery of motor function was related to the degree of initial motor impairment. Further subgroup analysis of the degree of initial impairment could provide a deeper understanding of stroke rehabilitation.

5. Conclusion

In this study, we applied a machine learning approach to identify the feature ROIs that can predict the classification of the MCID for motor improvement after ischemic stroke, and then compared the brain functional connectivity and acupuncture effects of these brain regions. Motor impairment and recovery result from the co-regulation of the bilateral cerebral hemispheres, and different brain functional response patterns exist in patients with different motor outcomes. Acupuncture can modulate the bilateral hemispheres through feature ROIs and eliminate abnormal functional connectivity to promote motor recovery after ischemic stroke. Our study can provide potential neuroimaging features for motor recovery and mechanisms of acupuncture on functional organization after stroke, and may expand research thoughts of machine learning and fMRI in clinical applications.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine Institutional Review Boards. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ML and ZD were involved in literature search, data analyses and writing of the manuscript. JZ contributed to the experimental design. RL and LJ were involved in data analyses and reviewing the manuscript. LX and XY contributed to the clinical observations. MZ and TX contributed to the subjects' recruitment. JW and WW were involved in clinical diagnosis for stroke patients. CC contributed to FMA evaluations. JF designed the imaging approaches and consulted through the study. YZ designed the study protocol and sought funding. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1143239/full#supplementary-material

References

Ashburner, J., and Friston, K. J. (2005). Unified segmentation. *NeuroImage* 26, 839–851. doi: 10.1016/j.neuroimage.2005.02.018

Birch, S., and Robinson, N. (2022). Acupuncture as a post-stroke treatment option: a narrative review of clinical guideline recommendations. *Phytomedicine* 104:154297. doi: 10.1016/j.phymed.2022.154297

Boddington, L. J., and Reynolds, J. N. J. (2017). Targeting interhemispheric inhibition with neuromodulation to enhance stroke rehabilitation. *Brain Stimul.* 10, 214–222. doi: 10.1016/j.brs.2017.01.006

Bushnell, C., Bettger, J. P., Cockroft, K. M., Cramer, S. C., Edelen, M. O., Hanley, D., et al. (2015). Chronic stroke outcome measures for motor function intervention trials: expert panel recommendations. *Circ. Cardiovasc. Qual. Outcomes* 8, S163–S169. doi: 10.1161/CIRCOUTCOMES.115.002098

Carter, A. R., Astafiev, S. V., Lang, C. E., Connor, L. T., Rengachary, J., Strube, M. J., et al. (2010). Resting interhemispheric functional magnetic resonance imaging connectivity predicts performance after stroke. *Ann. Neurol.* 67, 365–375. doi: 10.1002/ana.21905

Casula, E. P., Pellicciari, M. C., Bonni, S., Spano, B., Ponzo, V., Salsano, I., et al. (2021). Evidence for interhemispheric imbalance in stroke patients as revealed by combining transcranial magnetic stimulation and electroencephalography. *Hum. Brain Mapp.* 42, 1343–1358. doi: 10.1002/hbm.25297

Chen, J., Sun, D., Zhang, S., Shi, Y., Qiao, F., Zhou, Y., et al. (2020). Effects of home-based telerehabilitation in patients with stroke. *Neurology* 95, e2318–e2330. doi: 10.1212/WNL.0000000000010821

Collaborators, G. B. D. Stroke (2021). Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet Neurol.* 20, 795–820. doi: 10.1016/S1474-4422(21)00252-0

Craddock, R. C., James, G. A., Holtzheimer, P. E., Hu, X. P., and Mayberg, H. S. (2012). A whole brain fMRI atlas generated via spatially constrained spectral clustering. *Hum. Brain Mapp.* 33, 1914–1928. doi: 10.1002/hbm.21333

Cramer, S. C., Sur, M., Dobkin, B. H., O'Brien, C., Sanger, T. D., Trojanowski, J. Q., et al. (2011). Harnessing neuroplasticity for clinical applications. *Brain* 134, 1591–1609. doi: 10.1093/brain/awr039

Deo, R. C. (2015). Machine learning in medicine. *Circulation* 132, 1920–1930. doi: 10.1161/CIRCULATIONAHA.115.001593

Dimyan, M. A., and Cohen, L. G. (2011). Neuroplasticity in the context of motor rehabilitation after stroke. *Nat. Rev. Neurol.* 7, 76–85. doi: 10.1038/nrneurol.2010.200

Embry, T. W., and Piccirillo, J. F. (2020). Minimal clinically important difference reporting in randomized clinical trials. *JAMA Otolaryngol. Head Neck Surg.* 146, 862–863. doi: 10.1001/jamaoto.2020.1586

Fan, Y. T., Wu, C. Y., Liu, H. L., Lin, K. C., Wai, Y. Y., and Chen, Y. L. (2015). Neuroplastic changes in resting-state functional connectivity after stroke rehabilitation. *Front. Hum. Neurosci.* 9:546. doi: 10.3389/fnhum.2015.00546

Friston, K. J., Williams, S., Howard, R., Frackowiak, R. S., and Turner, R. (1996). Movement-related effects in fMRI time-series. *Magn. Reson. Med.* 35, 346–355. doi: 10.1002/mrm.1910350312

Gladstone, D. J., Danells, C. J., and Black, S. E. (2002). The Fugl-Meyer assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabil. Neural Repair* 16, 232–240. doi: 10.1177/154596802401105171

Graff-Radford, J., Williams, L., Jones, D. T., and Benarroch, E. E. (2017). Caudate nucleus as a component of networks controlling behavior. *Neurology* 89, 2192–2197. doi: 10.1212/WNL.0000000000004680

Han, X., Jin, H., Li, K., Ning, Y., Jiang, L., Chen, P., et al. (2020). Acupuncture modulates disrupted whole-brain network after ischemic stroke: evidence based on graph theory analysis. *Neural Plast.* 2020:8838498. doi: 10.1155/2020/8838498

Handelman, G. S., Kok, H. K., Chandra, R. V., Razavi, A. H., Lee, M. J., and Asadi, H. (2018). eDoctor: machine learning and the future of medicine. *J. Intern. Med.* 284, 603–619. doi: 10.1111/joim.12822

Hensel, L., Lange, F., Tscherpel, C., Viswanathan, S., Freytag, J., Volz, L. J., et al. (2022). Recovered grasping performance after stroke depends on interhemispheric frontoparietal connectivity. *Brain* 146, 1006–1020. doi: 10.1093/brain/awac157

Jimenez-Marin, A., De Bruyn, N., Gooijers, J., Llera, A., Meyer, S., Alaerts, K., et al. (2022). Multimodal and multidomain lesion network mapping enhances prediction of sensorimotor behavior in stroke patients. *Sci. Rep.* 12:22400. doi: 10.1038/s41598-022-26945-x

Kang, S. Y., and Kim, J. S. (2008). Anterior cerebral artery infarction: stroke mechanism and clinical-imaging study in 100 patients. *Neurology* 70, 2386–2393. doi: 10.1212/01.wnl.0000314686.94007.d0

Klingbeil, J., Brandt, M. L., Wawrzyniak, M., Stockert, A., Schneider, H. R., Baum, P., et al. (2022). Association of lesion location and depressive symptoms poststroke. *Stroke* 53, e467–e471. doi: 10.1161/STROKEAHA.122.039068

Lanckriet, G. R., De Bie, T., Cristianini, N., Jordan, M. I., and Noble, W. S. (2004). A statistical framework for genomic data fusion. *Bioinformatics* 20, 2626–2635. doi: 10.1093/bioinformatics/bth294

Li, G., Jack, C. R. Jr., and Yang, E. S. (2006). An fMRI study of somatosensory-implicated acupuncture points in stable somatosensory stroke patients. *J. Magn. Reson. Imaging* 24, 1018–1024. doi: 10.1002/jmri.20702

Li, M. K., Li, Y. J., Zhang, G. F., Chen, J. Q., Zhang, J. P., Qi, J., et al. (2015). Acupuncture for ischemic stroke: cerebellar activation may be a central mechanism following Deqi. *Neural Regen. Res.* 10, 1997–2003. doi: 10.4103/1673-5374.172318

Lian, C., Liu, M., Wang, L., and Shen, D. (2022). Multi-task weakly-supervised attention network for dementia status estimation with structural MRI. *IEEE Trans Neural Netw Learn Syst* 33, 4056–4068. doi: 10.1109/TNNLS.2021.3055772

Lin, D. J., Cloutier, A. M., Erler, K. S., Cassidy, J. M., Snider, S. B., Ranford, J., et al. (2019). Corticospinal tract injury estimated from acute stroke imaging predicts upper extremity motor recovery after stroke. *Stroke* 50, 3569–3577. doi: 10.1161/STROKEAHA.119.025898

Liu, F., Chen, C., Hong, W., Bai, Z., Wang, S., Lu, H., et al. (2022). Selectively disrupted sensorimotor circuits in chronic stroke with hand dysfunction. *CNS Neurosci. Ther.* 28, 677–689. doi: 10.1111/cns.13799

Liu, G., Tan, S., Peng, K., Dang, C., Xing, S., Xie, C., et al. (2019). Network change in the ipsilesional cerebellum is correlated with motor recovery following unilateral pontine infarction. *Eur. J. Neurol.* 26, 1266–1273. doi: 10.1111/ene.13974

Malec, J. F., and Ketchum, J. M. (2020). A standard method for determining the minimal clinically important difference for rehabilitation measures. *Arch. Phys. Med. Rehabil.* 101, 1090–1094. doi: 10.1016/j.apmr.2019.12.008

Murphy, T. H., and Corbett, D. (2009). Plasticity during stroke recovery: from synapse to behaviour. *Nat. Rev. Neurosci.* 10, 861–872. doi: 10.1038/nrn2735

Page, S. J., Fulk, G. D., and Boyne, P. (2012). Clinically important differences for the upper-extremity Fugl-Meyer scale in people with minimal to moderate impairment due to chronic stroke. *Phys. Ther.* 92, 791–798. doi: 10.2522/ptj.20110009

Pan, C., Li, G., Jing, P., Chen, G., Sun, W., Miao, J., et al. (2022). Structural disconnection-based prediction of poststroke depression. *Transl. Psychiatry* 12:461. doi: 10.1038/s41398-022-02223-2

Pandian, S., Arya, K. N., and Kumar, D. (2016). Minimal clinically important difference of the lower-extremity Fugl-Meyer assessment in chronic-stroke. *Top. Stroke Rehabil.* 23, 233–239. doi: 10.1179/1945511915Y.0000000003

Park, C. H., Chang, W. H., Ohn, S. H., Kim, S. T., Bang, O. Y., Pascual-Leone, A., et al. (2011). Longitudinal changes of resting-state functional connectivity during motor recovery after stroke. *Stroke* 42, 1357–1362. doi: 10.1161/STROKEAHA.110.596155

Qi, J., Chen, J., Huang, Y., Lai, X., Tang, C., Yang, J., et al. (2014). Acupuncture at Waiguan (SJ5) and sham points influences activation of functional brain areas of ischemic stroke patients: a functional magnetic resonance imaging study. *Neural Regen. Res.* 9, 293–300. doi: 10.4103/1673-5374.128227

Qian, Z., Lv, D., Lv, Y., and Bi, Z. (2019). Modeling and quantification of impact of psychological factors on rehabilitation of stroke patients. *IEEE J. Biomed. Health Inform.* 23, 683–692. doi: 10.1109/JBHI.2018.2827100

Schrouff, J., Rosa, M. J., Rondina, J. M., Marquand, A. F., Chu, C., Ashburner, J., et al. (2013). PRoNTo: pattern recognition for neuroimaging toolbox. *Neuroinformatics* 11, 319–337. doi: 10.1007/s12021-013-9178-1

Stangeland, H., Orgeta, V., and Bell, V. (2018). Poststroke psychosis: a systematic review. *J. Neurol. Neurosurg. Psychiatry* 89, 879–885. doi: 10.1136/jnnp-2017-317327

Stinear, C. M. (2017). Prediction of motor recovery after stroke: advances in biomarkers. *The Lancet Neurology* 16, 826–836. doi: 10.1016/S1474-4422(17)30283-1

Stinear, C. M., Lang, C. E., Zeiler, S., and Byblow, W. D. (2020). Advances and challenges in stroke rehabilitation. *Lancet Neurol* 19, 348–360. doi: 10.1016/S1474-4422(19)30415-6

Sullivan, K. J., Tilson, J. K., Cen, S. Y., Rose, D. K., Hershberg, J., Correa, A., et al. (2011). Fugl-Meyer assessment of sensorimotor function after stroke. *Stroke* 42, 427–432. doi: 10.1161/STROKEAHA.110.592766

Tang, C., Zhao, Z., Chen, C., Zheng, X., Sun, F., Zhang, X., et al. (2016). Decreased functional connectivity of homotopic brain regions in chronic stroke patients: a resting state fMRI study. *PLoS One* 11:e0152875. doi: 10.1371/journal.pone.0167851

Tazoe, T., and Perez, M. A. (2014). Selective activation of ipsilateral motor pathways in intact humans. *J. Neurosci.* 34, 13924–13934. doi: 10.1523/JNEUROSCI.1648-14.2014

Tu, Y., Cao, J., Bi, Y., and Hu, L. (2021). Magnetic resonance imaging for chronic pain: diagnosis, manipulation, and biomarkers. *Sci. China Life Sci.* 64, 879–896. doi: 10.1007/s11427-020-1822-4

Wang, Y., Wang, L., Wang, Y., Lu, M., Xu, L., Liu, R., et al. (2022). Sensorimotor responses in post-stroke hemiplegic patients modulated by acupuncture at Yanglingquan (GB34): a fMRI study using Intersubject functional correlation (ISFC) analysis. *Front. Neurol.* 13:900520. doi: 10.3389/fneur.2022.1065942

Wang, L., Yu, C., Chen, H., Qin, W., He, Y., Fan, F., et al. (2010). Dynamic functional reorganization of the motor execution network after stroke. *Brain* 133, 1224–1238. doi: 10.1093/brain/awq043

Ward, N. S., Brown, M. M., Thompson, A. J., and Frackowiak, R. S. (2003). Neural correlates of motor recovery after stroke: a longitudinal fMRI study. *Brain* 126, 2476–2496. doi: 10.1093/brain/awg245

Wen, H., Liu, Y., Rekik, I., Wang, S., Chen, Z., Zhang, J., et al. (2017). Multi-modal multiple kernel learning for accurate identification of Tourette syndrome children. *Pattern Recogn.* 63, 601–611. doi: 10.1016/j.patcog.2016.09.039

Wu, P., Mills, E., Moher, D., and Seely, D. (2010). Acupuncture in poststroke rehabilitation: a systematic review and meta-analysis of randomized trials. *Stroke* 41, e171–e179. doi: 10.1161/STROKEAHA.109.573576

Wu, P., Zhou, Y. M., Zeng, F., Li, Z. J., Luo, L., Li, Y. X., et al. (2016). Regional brain structural abnormality in ischemic stroke patients: a voxel-based morphometry study. *Neural Regen. Res.* 11, 1424–1430. doi: 10.4103/1673-5374.191215

Yan, C. G., Wang, X. D., Zuo, X. N., and Zang, Y. F. (2016). DPABI: Data Processing & Analysis for (resting-state) brain imaging. *Neuroinformatics* 14, 339–351. doi: 10.1007/s12021-016-9299-4

You, B., Wen, H., and Jackson, T. (2021). Identifying resting state differences salient for resilience to chronic pain based on machine learning multivariate pattern analysis. *Psychophysiology* 58:e13921. doi: 10.1111/psyp.13921

Zeng, L. L., Wang, H., Hu, P., Yang, B., Pu, W., Shen, H., et al. (2018). Multi-site diagnostic classification of schizophrenia using discriminant deep learning with functional connectivity MRI. *EBioMedicine* 30, 74–85. doi: 10.1016/j.ebiom.2018.03.017

Zhang, S. H., Liu, M., Asplund, K., and Li, L. (2005). Acupuncture for acute stroke. *The Cochrane database of systematic reviews* 2:CD003317. doi: 10.1002/14651858.CD003317.pub2

Zhang, J., Su, J., Wang, M., Zhao, Y., Zhang, Q. T., Yao, Q., et al. (2017). The sensorimotor network dysfunction in migraineurs without aura: a resting-state fMRI study. *J. Neurol.* 264, 654–663. doi: 10.1007/s00415-017-8404-4

Zuo, X. N., Ehmke, R., Mennes, M., Imperati, D., Castellanos, F. X., Sporns, O., et al. (2012). Network centrality in the human functional connectome. *Cereb. Cortex* 22, 1862–1875. doi: 10.1093/cercor/bhr269

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Magnetic resonance imaging of white matter in Alzheimer's disease: a global bibliometric analysis from 1990 to 2022

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Background: Alzheimer's disease (AD) is a common, progressive, irreversible, and fatal neurodegenerative disorder with rapidly increasing worldwide incidence. Although much research on magnetic resonance imaging (MRI) of the white matter (WM) in AD has been published, no bibliometric analysis study has investigated this issue. Thus, this study aimed to provide an overview of the current status, hotspots, and trends in MRI of WM in AD.

Methods: We searched for records related to MRI studies of WM in AD from 1990 to 2022 in the Web of Science Core Collection (WOSCC) database. CiteSpace (version 5.1.R8) and VOSviewer (version 1.6.19) software were used for bibliometric analyses.

Results: A total of 2,199 articles were obtained from this study. From 1990 to 2022, the number of published articles showed exponential growth of $y = 4.1374e^{0.1294x}$, with an average of 17.9 articles per year. The top country and institutions were the United States and the University of California Davis, accounting for 44.52 and 5.32% of the total studies, respectively. The most productive journal was Neurology, and the most co-cited journal was Lancet Neurology. Decarli C was the most productive author. The current research frontier trend focuses on the association between small vessel disease and AD, the clinical application and exploration of diffusion MRI, and related markers.

Conclusion: This study provides an in-depth overview of publications on MRI of WM in AD, identifying the current research status, hotspots, and frontier trends in the field.

KEYWORDS

Alzheimer's disease, white matter, magnetic resonance imaging, CiteSpace, VOSviewer, bibliometric analysis

1. Introduction

Alzheimer's disease (AD) is a neurological degenerative disease with an insidious and progressive onset (Ben Miled et al., 2020) and is considered a serious problem for both individual health and government healthcare systems worldwide (Nabizadeh et al., 2022). White matter (WM) lesions are a common finding in AD and may contribute to dementia severity. Although previous studies have explored the neural mechanisms underlying AD, these mechanisms are not well understood.

In recent years, with the development of neuroimaging technologies, magnetic resonance imaging (MRI) has provided a new perspective to enhance our understanding of AD mechanisms. It provides valuable insights into the structure and function of neural networks (Chard et al., 2021). Many studies have used MRI to investigate WM changes and pathological features of AD, including WM lesions (Vermeer et al., 2003), mild cognitive impairment (Annweiler et al., 2013), Pittsburgh compound B (PiB)-induced WM pathology (Glodzik et al., 2015), diffuse MRI (Tseng et al., 2022), small vessel disease (Acharya et al., 2019), and risk factors (Artero et al., 2004). Although these studies have enhanced our understanding of the imaging mechanism of AD using MRI, little attention has been paid to the current research status, hotspots, and frontier trends in this field.

In recent years, bibliometric analysis has been widely used to explore academic publications (Kim and Park, 2021). It applies mathematical and statistical methods, bibliometrics, and data-mining algorithms to visualize the co-citation network of scientific research and identify trends and structures within a knowledge domain (Kim and Park, 2021). By interpreting the information in the network map, researchers can quickly and accurately understand the research status, hotspots, and trends of this topic in the field (Chen, 2017). In this study, we conducted a bibliometric analysis of publications related to MRI of WM in patients with AD in the Web of Science Core Collection (WOSCC) between 1990 and 2022. This study provides insights and perspectives into the literature on the MRI of WM in AD to better understand the current research status, hotspots, and frontier trends.

2. Methods

2.1. Study design

This study was retrospectively reviewed using bibliometric analysis. We performed a literature search using the WOSCC database. Bibliometric analysis was performed using VOSviewer and CiteSpace software. The number of publications, countries, institutions, authors, keywords, and references, as well as their associations, were analyzed.

2.2. Data acquisition

The search strategy was as follows: TS = ("Alzheimer's* disease" AND "white matter" AND "MRI" AND "brain"). The time span was between 1990 and 2022. Only the original articles and reviews published in English were included. The full records and all references are explained in a plain text format. Consequently, 2199 articles were obtained.

2.3. Statistical analysis

All available data were collected in WOSCC and imported to Microsoft Excel 2022, CiteSpace (version 5.1.R8) (Chen, 2017), and VOSviewer (version 1.6.19) (Van Eck and Waltman, 2010) for performing bibliometric analysis.

Microsoft Excel 2022 was used to draw a trend chart of the annual output of the 2199 records. We used VOSviewer to construct a network of countries, institutions, authors, co-cited journals, and keyword co-ocurrence. We applied CiteSpace to perform keyword clustering and keyword citation burst. The node in each map indicates a country, institution, author, co-cited journal, or a keyword. The size of the node (country, institution, or author) was proportional to the number of publications. The larger the node, the greater is the number of publications. The links between the nodes indicate their collaboration. For co-cited journals, the size of the node is proportional to the number of total citations; the larger the node, the greater is the number of total citations. The line between them refers to the co-citation of the journals. The size of the keywords is proportional to their frequency, with a larger node signifying a higher keyword frequency. The connection between them indicates keyword co-occurrence.

We set the parameters of CiteSpace as follows: time span (1990–2022), time slice (3 years), node type (country, institution, author, keyword, cited reference, or cited journal), node threshold (topn = 50), and pruning (pathfinder or MST algorithm). Detailed information is available at http://cluster.cis.drexel.edu/~cchen/citespace/ and https://www.vosviewer.com/.

3. Results

3.1. The trends of publication outputs

The annual number of publications is shown in Figure 1. As can be seen from the figure, the number of publications in the field of MRI studies on WM in AD has shown an increasing trend, and the fitted curve index was $y = 4.1374e^{0.1294x}$. The first such study was published in 1990. The overall number of publications fluctuates from 1990 to 2018, peaking in 2018. In particular, the number of publications fluctuated slowly and steadily from 2018 to 2022. However, all of these publications were over 145 annually, except for 2022, because the search date was 24 October 2022, and the annual publications in 2022 were incomplete.

3.2. Distribution and collaboration of countries

The data showed that 26 countries contributed to research on MRI studies of WM in AD. Figure 2A shows a world map of productive countries. The top 10 most productive countries are listed in Table 1. The United States was the most productive

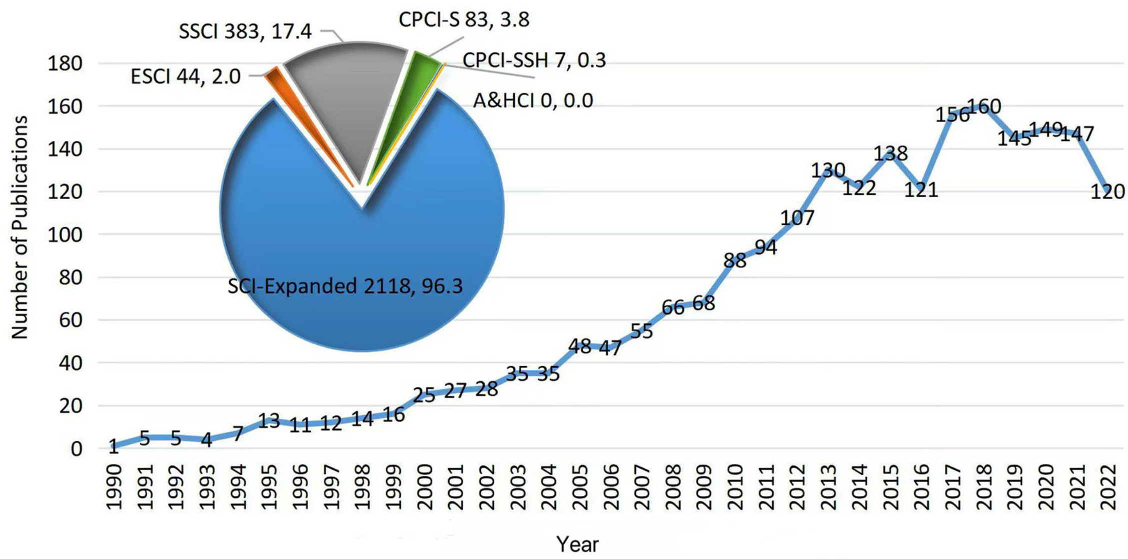

FIGURE 1
The number and trend of annual publication.

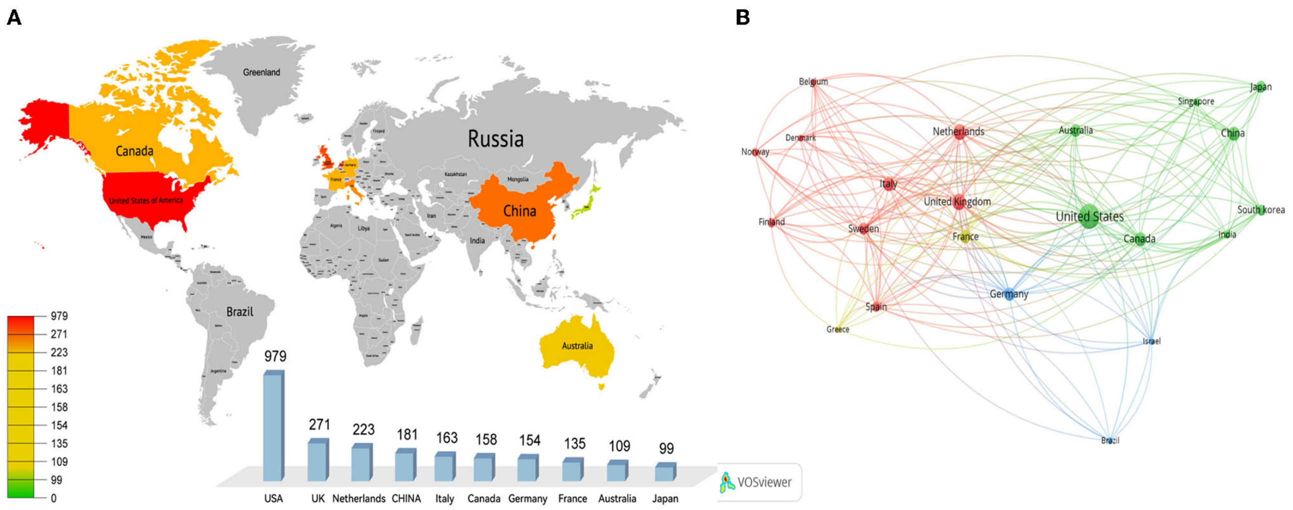

FIGURE 2
Cooperative network of country distribution **(A)** and collaboration **(B)**.

country ($n = 979$ articles), accounting for approximately 44.52% of the total literature, followed by the United Kingdom (271 articles), Netherlands (223 articles), China (181 articles), Italy (163 articles), Canada (158 articles), Germany (154 articles), France (135 articles), Australia (109 articles), and Japan (99 articles). The country with the highest centrality was the United States (0.25), followed by the United Kingdom (0.19), Canada (0.18), and France (0.10). This shows that the four countries in the field of international recognition research results are higher, and their impact is greater.

A cooperative network for collaborations by VOSviewer is shown in Figure 2B. A total of 263 collaborations were identified among these 26 countries. The nodes of the United States, the United Kingdom, Netherlands, and China are larger, which means that they had more collaborations than other countries. This shows that many countries have focused on MRI studies of WM in AD and have formed close international collaborative networks.

3.3. Collaboration of institutions

The cooperative network of institutions and their collaborations by VOSviewer are shown in Figure 3. There were 75 institutions with 725 collaborations. The top 10 countries with the highest outputs are listed in Table 1. The most productive institution was the University of California

TABLE 1 Top 10 productive countries and institutions.

Ranking	Country	Frequency (articles)	Centrality	Ranking	Institution	Frequency (articles)	Centrality
1	United States	979	0.25	1	Univ Calif Davis	117	0.11
2	United Kingdom	271	0.19	2	Univ Calif San Francisco	91	0.09
3	Netherlands	223	0.06	3	Univ Calif Los Angeles	77	0.11
4	China	181	0.03	4	Boston Univ	66	0.05
5	Italy	163	0.09	5	Univ College London	65	0.09
6	Canada	158	0.18	6	National Institute Accountants	57	0.02
7	Germany	154	0.05	7	Mayo Clin	56	0.09
8	France	135	0.10	8	Johns Hopkins Univ	54	0.06
9	Australia	109	0.04	9	Harvard Med Sch	52	0.01
10	Japan	99	0.01	10	Univ Toronto	51	0.05

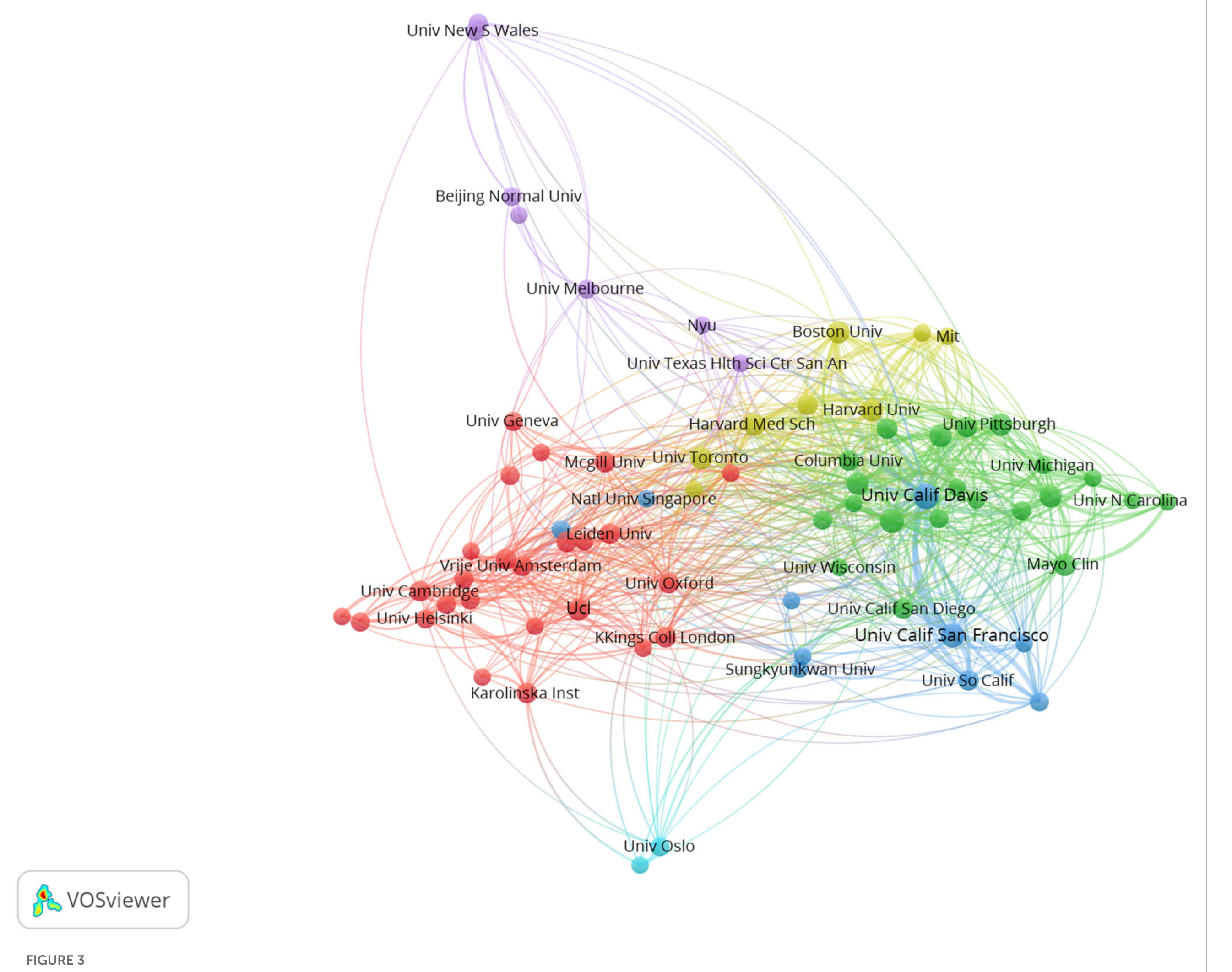

FIGURE 3
Cooperative network of institutions.

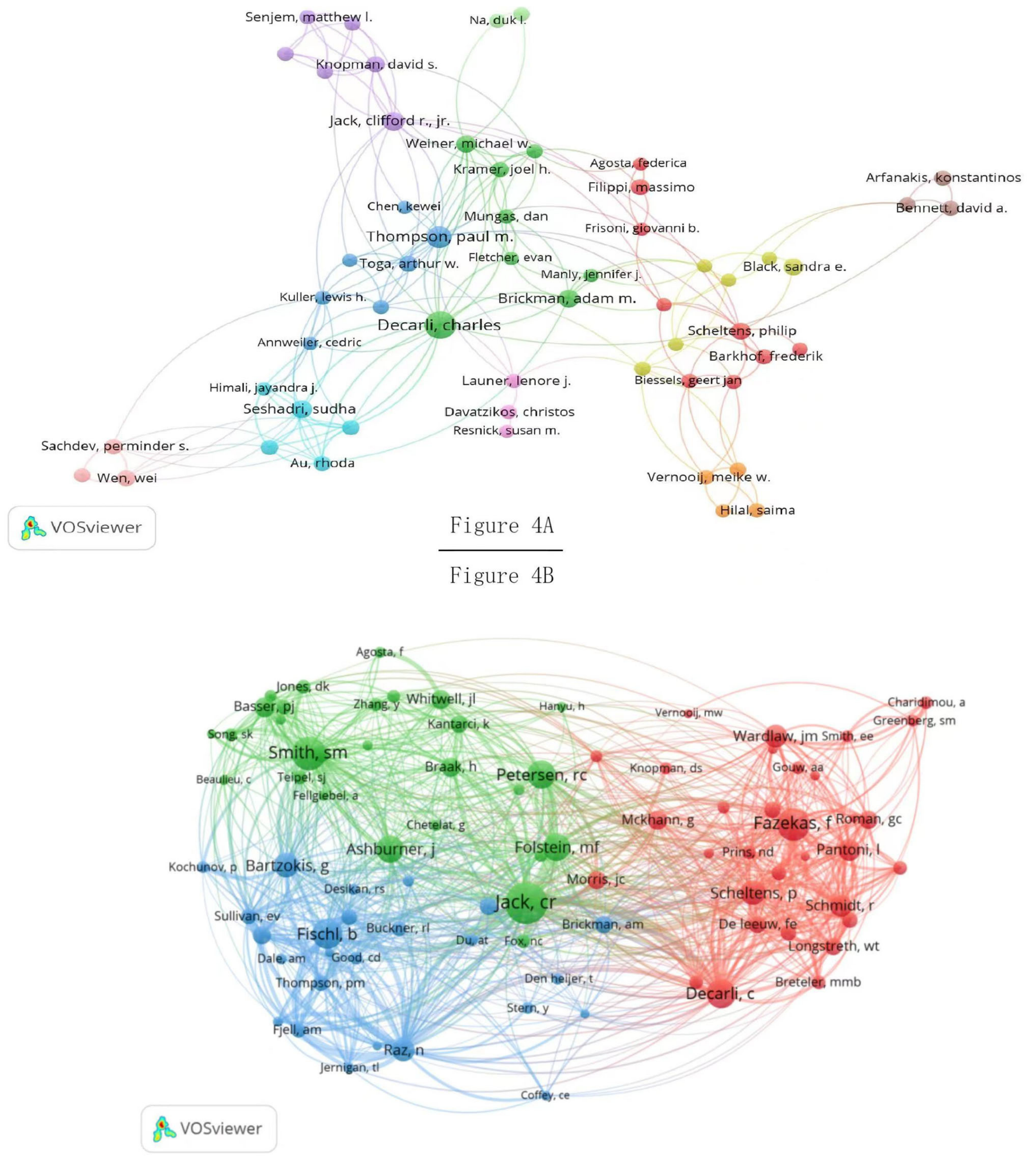

FIGURE 4
Cooperative network of productive **(A)** and co-cited **(B)** authors.

Davis (117 articles), followed by the University of California San Francisco (91 articles), and the University of California Los Angeles (77 articles). The University of California Davis and University of California Los Angeles had the highest centrality of 0.11, followed by the Mayo Clinic, University of California San Francisco, and University College London at 0.09.

3.4. Authors and co-cited authors

The authors' cooperative network using VOSviewer is shown in Figure 4A. A total of 61 authors and 201 collaborations between them contributed to this study. The top 10 productive authors are listed in Table 2. Decarli C had the highest output in the field with 86 publications. This was followed by Thompson PM (46 articles),

TABLE 2 Top ten productive and co-cited authors.

Productive author ranking	Author	Frequency (articles)	Centrality	Co-cited author ranking	Author	Frequency (citations)
1	DeCarli C	86	0.09	1	Jack CR	786
2	Thompson PM	46	0.22	2	Smith SM	593
3	Jack CR	45	0.14	3	Fazekas F	557
4	Scheltens P	44	0.34	4	Fischl B	506
5	Weiner MW	36	0.10	5	Decarli C	483
6	Barkhof F	31	0.04	6	Petersen RC	467
7	Toga AW	30	0.09	7	Folstein MF	443
8	Brickman AM	26	0.08	8	Ashburner J	429
9	Knopman DS	25	0.01	9	Bartzokis G	388
10	Seshadri S	25	0.02	10	Scheltens P	378

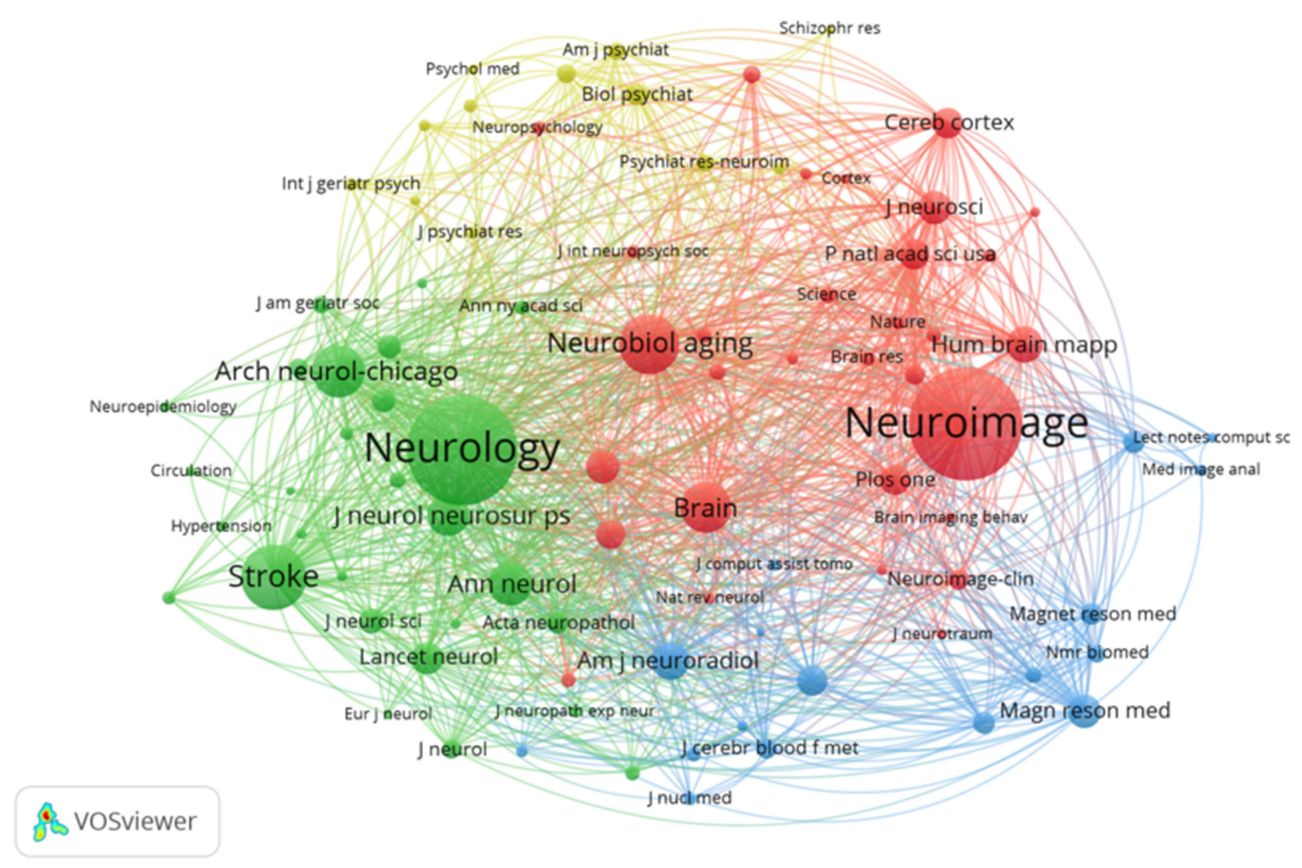

FIGURE 5
Cooperative network of co-cited journals.

Jack CR (45 articles), Scheltens P (44 articles), Weiner MW (36 articles), Barkhof F (31 articles), Toga AW (30 articles), Brickman AM (26 articles), Knopman DS (25 articles), and Seshadri S (25 articles). Scheltens P (0.34) had the highest centrality, followed by Thompson PM (0.22), Jack CR (0.14), and Weiner MW (0.10). Cooperation mainly revolves around high-yield authors and has formed good collaborative association.

The results of the authors' co-citation analysis network by using VOSviewer are shown in Figure 4B. Among the 34,280 authors, 86 authors (0.25%) were cited more than 100 times, 31 contributors (0.09%) were cited at least 300 times, and 13 authors (0.04%) were cited more than 500 times. The top ten co-cited authors were cited more than 350 times (Table 2), among which Jack CR was the most cited author (786 citations), followed by Smith SM (593 citations), Fazekas F (557 citations), Fischl B (506 citations), Decarli C (483 citations), Petersen RC (467 citations), Folstein MF (443 citations), Ashburner J (429 citations), Bartzokis G (388 citations), and Scheltens P (378 citations).

3.5. Journals and co-cited journals

The cooperative network of co-cited journals by VOSviewer is shown in Figure 5. The top 10 journals with the highest outputs are listed in Table 3. Neurology (162 articles) was the most productive journal, followed by Neuroimage (129 articles), Neurobiology of Aging (110 articles), and Journal of Alzheimer's Disease (89 articles). Neurology (impact factor (IF), 11.800) and stroke (IF, 10.170) were the most influential journals.

The top 10 journals with the most frequent co-citations are listed in Table 3. The most frequently co-cited journal was Neurology (1,079 citations, IF, 11.800), followed by Neuroimage (863 citations, IF, 7.400), Neurobiology of Aging (753 citations, IF, 5.133), and Brain (740 citations, IF, 15.255). The journals with high centrality were Neurology (0.12) and the Neurobiology of Aging (0.11). In addition, Lancet Neurology had the highest impact (IF, 59.935). Journals with high outputs and frequent citations, especially for Neurology and Neuroimage, play an important role in this field.

3.6. Research hotspots

Keywords are the high induction and central ideas of an article (Small, 1973; Chen, 2010). The research hotspots in MRI research in the WM of AD have mainly been investigated using the keywords co-occurrence, clustering, and co-citation.

3.6.1. Keyword co-occurrence and clustering

A keyword co-occurrence network with 89 keywords and 3,403 keyword co-occurrences was built using VOSviewer (Figure 6). The top 10 keywords for frequency and centrality are listed in Table 4. AD had the highest frequency (2,031 times) with the largest corresponding node, followed by MRI (958 times), dementia (626 times), brain (582 times), WM (444 times), mild cognitive impairment (375 times), WM hyperintensity (293 times), atrophy (240 times), WM lesions (221 times), and risk factors (206 times). In terms of centrality, age (0.48) was the highest at 0.48, followed by MRI (0.44), lesions (0.35), healthy elderly subjects (0.34), and risk factors (0.31), all of which were over 0.30.

CiteSpace software was used to build keyword clustering with Q = 0.7582 and S = 0.8097, indicating that the clustering result was scientific, reasonable, and significant. There are nine clusters of keywords as follows: #0 aging, #1 senile dementia, #2 magnetic resonance imaging, #3 mild cognitive impairment, #4 voxel-based morphometry, #5 leukoencephalopathy, #6 progression, #7 registration, and #8 cortex. Cluster numbering and font size are mainly based on the cluster size; that is, the smaller the label, the larger the cluster font, indicating that the cluster contains more keywords.

Keyword clusters are presented in Table 5. The results show that each cluster index is within a reasonable range, indicating that a single cluster is better and that the module is more homogenous. Cluster #0 contained 17 keywords with the first five feature words of aging, visual retention test, sex difference, longitudinal CT, and temporal lobe. Cluster #1 included 17 keywords with the first

TABLE 3 Top ten productive and co-cited journals.

Ranking	Journal	Frequency (articles)	IF(2021)	Q(2021)	Ranking	Co-cited journal	Frequency (citations)	Centrality	IF(2021)	Q(2021)
1	Neurology	162	11.800	Q1	1	Neurology	1079	0.12	11.800	Q1
2	NeuroImage	129	7.400	Q1	2	NeuroImage	863	0.09	7.400	Q1
3	Neurobiology of Aging	110	5.133	Q2	3	Neurobiology of Aging	753	0.11	5.133	Q2
4	Journal of Alzheimer's Disease	89	4.160	Q2	4	Brain	740	0.06	15.255	Q1
5	Frontiers in Aging Neuroscience	65	5.702	Q1	5	Ann Neurol	671	0.07	17.274	Q1
6	PloS One	55	3.752	Q2	6	Archives of neurology	628	0.05	0.000	N/A
7	NeuroImage-Clinical	53	4.891	Q2	7	Stroke	628	0.03	10.170	Q1
8	Stroke	53	10.170	Q1	8	Journal of Neurology Neurosurgery and Psychiatry	597	0.03	13.651	Q1
9	Human Brain Mapping	48	5.399	Q1	9	PloS One	578	0.02	3.752	Q2
10	Journal of the Neurological Sciences	38	4.553	Q2	10	Journal of Alzheimer's Disease	564	0.01	4.160	Q2

IF, impact factor; Q, quartile; Q1, first quartile; Q2, second quartile.

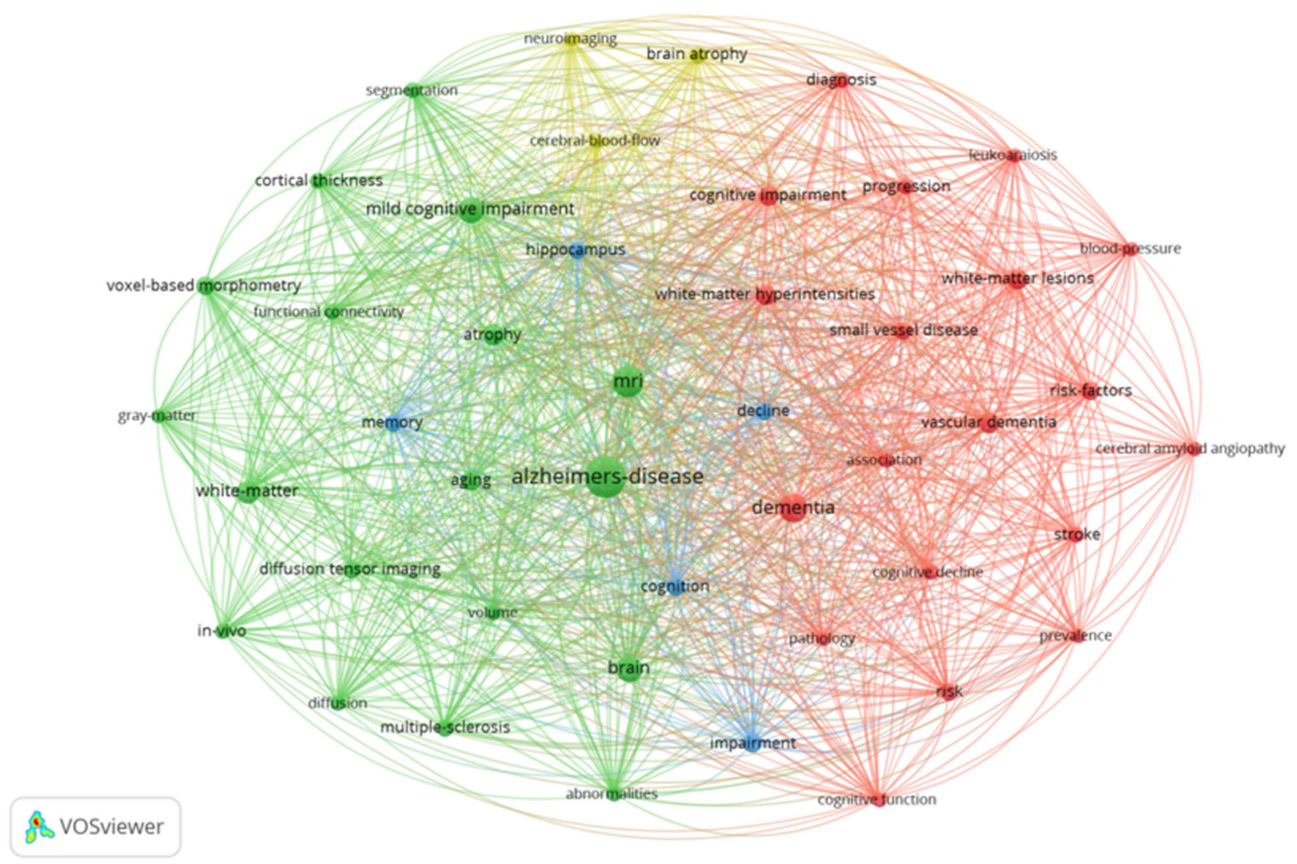

FIGURE 6
Keyword co-occurrence network.

TABLE 4 Top 10 keywords with centrality and frequency.

Ranking	Frequency	Keyword	Centrality	Ranking	Centrality	Keyword	Frequency
1	2031	Alzheimer's disease	0.30	1	0.48	Age	129
2	958	MRI	0.44	2	0.44	Mri	958
3	626	Dementia	0.22	3	0.35	Lesion	176
4	448	Brain	0.18	4	0.34	Healthy elderly subject	4
5	444	White matter	0.21	5	0.31	Risk factor	206
6	375	Mild cognitive impairment	0.01	6	0.30	Alzheimer's disease	2031
7	293	White matter hyperintensity	0.06	7	0.27	Memory	133
8	240	Atrophy	0.24	8	0.25	Hyperintensity	60
9	221	White matter lesion	0.02	9	0.25	Leukoencephalopathy	9
10	206	Risk factor	0.31	10	0.24	Atrophy	240

five feature words of senile dementia, risk, depression, atrophy, and Lewy body. Cluster #2 included 15 keywords and the first five features were MRI, leukoaraiosis, high signal lesions, DTI, and cerebral atrophy. Cluster #3 consisted of 15 keywords, and the first five ones were mild cognitive impairment, white matter hyperintensity, gait velocity, medial temporal atrophy, and older adults. Cluster #4 had 15 keywords, and the first five words were voxel-based morphometry, PET, connectivity, diffusion tensor imaging, and val(158)met genotype. Cluster #5 had 14 keywords, and the first five words were leukoencephalopathy, diffusion tensor imaging, leukoencephalopathies, epsilon 4 allele, and subcortical lesion. Cluster #6 involved 13 keywords, with the first five feature words being progression, risk factor, blood pressure, small vessel disease, and insulin resistance. Cluster #7 had 11 keywords, and the first five feature words extracted were registration, onset, ferritin, substantia nigra, and signal hyperintensity. Cluster #8

TABLE 5 Keywords cluster.

ID Ranking (#)	Size	Silhouette	Top-term (LLR)
0	17	0.708	aging (14.34, 0.001); visual retention test (11.29, 0.001); sex difference (11.29, 0.001); longitudinal ct (11.29, 0.001); temporal lobe (10.46, 0.005)
1	17	0.821	senile dementia (9.31, 0.005); risk (8.77, 0.005); depression (7.64, 0.01); atrophy (7.36, 0.01); lewy body (6.93, 0.01)
2	15	0.717	magnetic resonance imaging (16.41, 1.0E-4); leukoaraiosis (10.89, 0.001); high signal lesions (10.44, 0.005); dti (8.07, 0.005); cerebral atrophy (7.81, 0.01)
3	15	0.95	mild cognitive impairment (15.84, 1.0E-4); white matter hyperintensity (11.53, 0.001); gait velocity (10.02, 0.005); medial temporal atrophy (10.02, 0.005); older adults (10.02, 0.005)
4	15	0.859	voxel based morphometry (17.49, 1.0E-4); pet (12.67, 0.001); connectivity (9.83, 0.005); diffusion tensor imaging (8.86, 0.005); val(158)met genotype (6.59, 0.05)
5	14	0.858	leukoencephalopathy (28.97, 1.0E-4); diffusion tensor imaging (18.02, 1.0E-4); leukoencephalopathies (17.47, 1.0E-4); epsilon 4 allele (17.47, 1.0E-4); subcortical lesion (17.47, 1.0E-4)
6	13	0.954	progression (17.96, 1.0E-4); risk factor (15.99, 1.0E-4); blood pressure (15.02, 0.001); small vessel disease (13.79, 0.001); insulin resistance (9.27, 0.005)
7	11	0.859	registration (20.31, 1.0E-4); onset (13.53, 0.001); ferritin (13.53, 0.001); substantia nigra (13.53, 0.001); signal hyperintensity (13.53, 0.001)
8	11	0.952	cortex (21.16, 1.0E-4); white matter (16.39, 1.0E-4); neuroinflammation (11.51, 0.001); williams syndrome (10.57, 0.005); fractal complexity (10.57, 0.005)

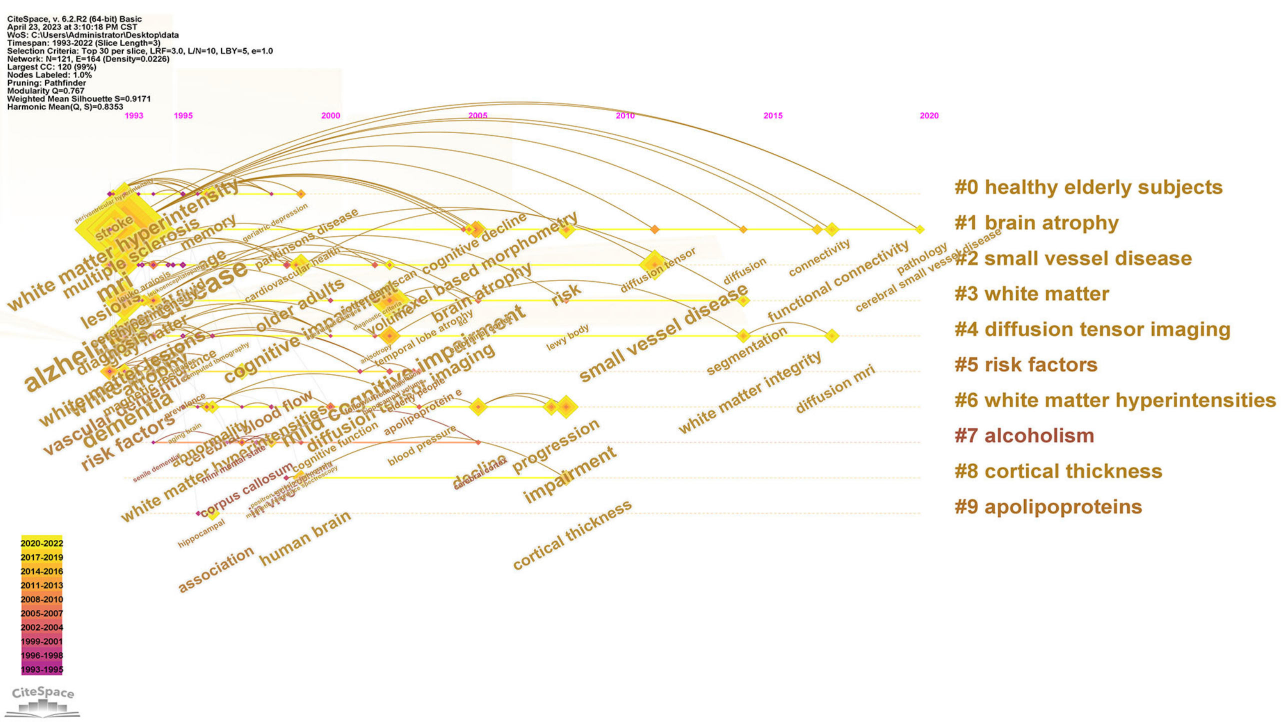

FIGURE 7
Timeline viewer of keywords cluster.

contained 11 keywords, with the first five words being the cortex, the white matter, neuroinflammation, Williams syndrome, and fractal complexity.

The timeline viewer of MRI in the WM of AD is drawn based on CiteSpace software (Figure 7), which visually presents the phased hotspots of this issue from the time dimension. From 1990 to 1999, research focused on the health of the elderly, and the main keywords were multiple sclerosis, geriatric depression, and Alzheimer's disease. From 1999 to 2009, the study focused on carrier protein and risk factors, and the main keywords were related to the brain, cognitive impairment, Alzheimer's disease, temporal lobe, and alcoholism. From 2009 to 2022, the research mainly focused on brain atrophy, diffusion tensor imaging, and the white matter, and the main keywords were associated with

TABLE 6 Top five frequently co-cited references.

Ranking	Title	Author	Journal	Frequency (citations)	Year
1	Neuroimaging standards for research into small vessel disease and its contribution to aging and neurodegeneration	Wardlaw JM	Lancet Neurology	125	2013
2	White matter hyperintensities, cognitive impairment and dementia: an update	Prins ND	Nature Reviews Neurology	64	2015
3	FSL	Jenkinson M	NeuroImage	46	2012
4	What are White Matter Hyperintensities Made of? Relevance to Vascular Cognitive Impairment	Wardlaw JM	Journal of the American Heart Association	44	2015
5	White matter hyperintensities are a core feature of Alzheimer's disease: Evidence from the dominantly inherited Alzheimer network	Lee S	Annals of Neurology	41	2016

TABLE 7 Top five co-cited references of high centrality.

Ranking	Title	Author	Journal	Frequency	Year
1	Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study	De Leeuw FE	Journal of Neurology Neurosurgery and Psychiatry	0.84	2001
2	Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people - The cardiovascular health study	Longstreth WT	Stroke	0.80	1996
3	About "axial" and "radial" diffusivities	Wheeler-Kingshott CAM	Magnetic Resonance in Medicine	0.74	2009
4	White matter changes in mild cognitive impairment and AD: A diffusion tensor imaging study	Medina D	Neurobiology of Aging	0.61	2006
5	Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study	Garde E	Lancet	0.35	2000

small vessel disease, functional connectivity, white matter integrity, and pathology.

3.6.2. Co-cited reference

The five most frequently co-cited references are listed in Table 6, and the top five centrality references are listed in Table 7. "Neuroimaging standards for research into small vessel disease and its contribution to aging and neurodegeneration" was the most co-cited article on WM of AD by MRI with 125 citations (Wardlaw et al., 2013). It was published by Wardlaw JM in Lancet Neurology in 2013 (Wardlaw et al., 2013). This study reported neuroimaging criteria for the study of small vessel diseases and their effects on aging and neurodegeneration (Wardlaw et al., 2013), and the results have important implications for the standardization of image interpretation of common neurodegenerative pathophysiology and clinical features (Wardlaw et al., 2013). "Prevalence of cerebral white matter lesions in elderly people: A population-based magnetic resonance imaging study The Rotterdam Scan Study" was the article with the highest centrality of 0.84 (Leeuw et al., 2001). It was published by De Leeuw Fe in the Journal of Neurology Neurosurgery and Psychiatry in 2001 (Leeuw et al., 2001). The prevalence and degree of cerebral WM lesions increase with age, and women tend to have a higher degree of WM lesions than men (Leeuw et al., 2001).

3.7. Research trend

The results of the research trend were carried out through keyword citation bursts using the CiteSpace software, as shown in Figure 8. The main set in the process was the Minimum Duration (1 year) and γ (3.33). In the graph, Begin and End indicate the start and end times of the burst, respectively, and Red indicates the time span of the burst.

As can be seen from the diagram, "magnetic resonance" began in 1991 and lasted until 2010 with a burst intensity (12.6637), which was the longest burst duration, indicating the importance of MRI for research in this field. The highest burst intensity word was "vascular dementia" (24.1938) and then followed by "WM lesion" (16.2700). The burst time period of "small vessel disease," "diffusion MRI," and "biomarker" were 2016–2022, 2017–2022, and 2018–2022, respectively, and they all lasted to the present. This indicates that these are current and future research hotspots and trends.

4. Discussion

Data were obtained from MRI studies of WM in AD from 1990 to 2022 in the WOSCC database using the CiteSpace software. This study investigated the countries, institutions, authors, journals, keywords, and reference co-citations in this field

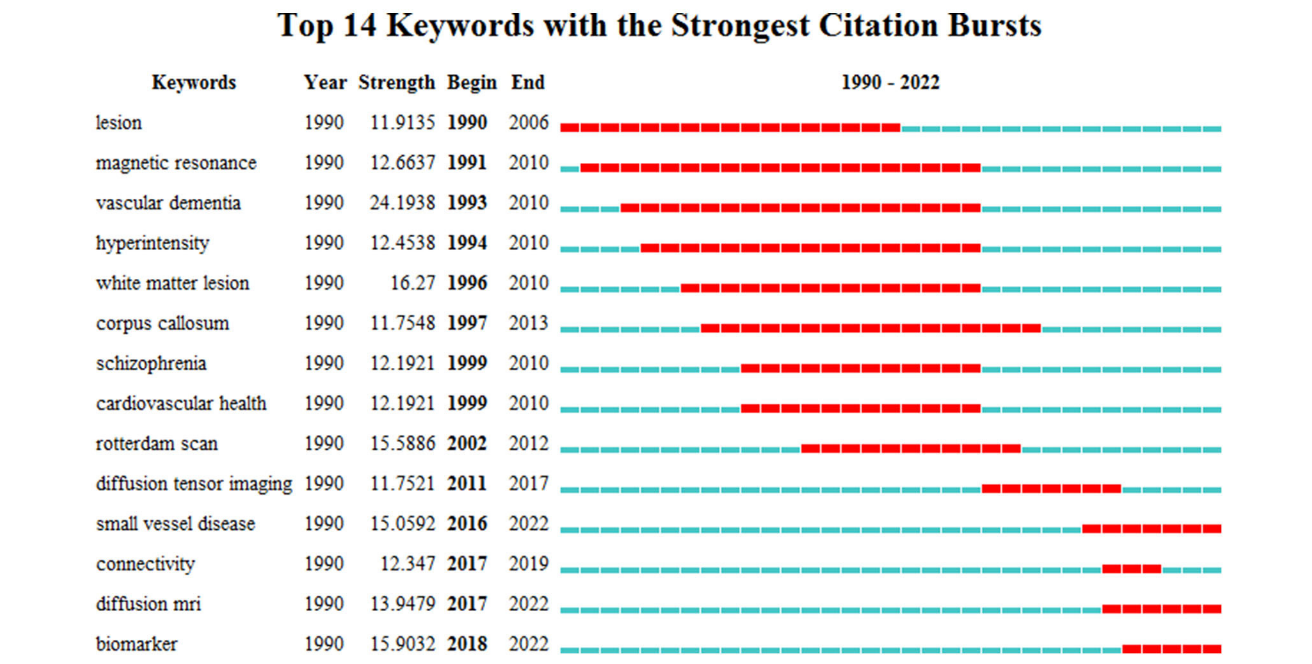

FIGURE 8
Top 14 keywords with the strongest citation bursts.

and comprehensively explored the research status, hotspots, and trends in the present domain.

4.1. Research status of MRI studies of WM in AD

An increasing trend was observed in the number of publications in this field, with a fitted curve index of $y = 4.1374e^{0.1294x}$. The average number of publications was 17.9. The United States was the most productive country, accounting for 44.52% of the total literature, followed by England, the Netherlands, China, and Italy, with relatively high-yielding outputs. There is a close cooperation network between countries. England, Canada, and France were the most influential countries.

Among the institutions, the University of California Davis was the most productive, accounting for 5.32% of the total number of studies. An institutional network is closely connected, forming a strong cooperative relationship. The University of California Davis, University of California Los Angeles, and Mayo Clinic had higher centrality, indicating a larger impact in this field. Research results from these institutions are of great significance for the development of this field.

From the authors' perspective, a good cooperative relationship has been established between authors in this field, and cooperation mainly revolves around high-yield authors. Among them, DeCarli et al. (2004) was the most productive author, accounting for approximately 3.91% of the total literature and is mainly involved in the development of mild cognitive impairment to dementia (DeCarli et al., 2004); the relationship between high-intensity WM volume and stroke, mild cognitive impairment, dementia, and mortality (Debette et al., 2010); the relationship between high-intensity WM around the ventricle, high-intensity WM in the deep and total WM load (DeCarli et al., 2005); and the diagnosis of AD using MRI (Cuenco et al., 2008).

Scheltens P was the author with the highest centrality, mainly involving the clinical application of structural MRI in AD (Frisoni et al., 2010); the relationship between high WM intensity in the elderly, cerebral amyloid angiopathy, and dementia (Tanskanen et al., 2013); the quantitative method of high WM intensity on MRI (Gao et al., 2011); and the WM change scale (Wahlund et al., 2001).

In terms of journals, Neurology accounted for 7.37% with an IF of 11.800 (2021), and it was the basis for the first 10 journals with the most influential factors. The journal with the highest number of citations was Neurology and the journal with the highest IF was Lancet Neurology (59.935). The journals with the highest centrality were Neurology (0.12) and Neurobiology of Aging (0.11). The study found that Neurology and Neuroimage were the top two journals in terms of the number of publications and total citations between 1990 and 2022, contributing to the development of the field.

4.2. Hotspots and research status on MRI of WM in AD

According to the keyword burst, the research fields of MRI on WM in AD were AD, MRI, dementia, brain, WM, mild cognitive impairment, WM hyperintensity, atrophy, WM lesions, and risk factors. In terms of centrality, age, MRI, lesion, healthy elderly subjects, and risk factors were all greater than 0.30, ranking among the top five most influential factors. The age of onset is a key factor in determining the obvious characteristics of patients with cognitive impairment, such as pathological burden and structural changes (Jang et al., 2016).

Based on clustering, the results were scientific and reliable, and were divided into nine types: #0 aging, #1 senile dementia, #2 magnetic resonance imaging, #3 mild cognitive impairment, #4 voxel-based morphometry, #5 leukoencephalopathy, #6 progression, #7 registration, and #8 cortex. WM lesions, mild cognitive impairment, high WM intensity, MRI findings, and risk factors were the main research focuses. These findings suggest that leukoencephalopathy is a potential risk factor for memory and cognitive impairment (No et al., 2019), whereas the synergistic effect between high WM intensity and hippocampal atrophy and the interaction between vascular and degenerative processes may be an important determinant of dementia (Wu et al., 2002), increased risk of neurological abnormalities associated with parietal region lacunae (parietal lobe space), and high-intensity WM (Camarda et al., 2015).

MRI is an important tool and method in the study of recognition, and it is of great significance in the diagnosis of AD with the aid of MRI. Structural MRI can explore the underlying pathophysiology through histopathology (Reijmer and Van Veluw, 2016), and functional magnetic resonance imaging (fMRI) can be used to detect brain function in patients (Yang et al., 2015), multimodal MRI is widely used to detect vascular cognitive impairment (Xia et al., 2022). Rs-fMRI can detect leukoaraiosis and a wide range of brain dysfunction (Cheng et al., 2017). In addition, other MRI techniques such as small vessel disease-related MRI, neuromelanin-sensitive MRI, diffusion-weighted imaging, cerebrovascular abnormality-related MRI, resting fMRI, and proton magnetic resonance spectroscopy can provide imaging features that can predict the degree of cognitive impairment in AD (Hou and Shang, 2022). Early studies have shown that mild cognitive impairment is a precursor to early clinical signs of AD, and changes in WM volume during this period may be of great value in clinical practice (Pergher et al., 2020). It has become the focus of epidemiology, neuroimaging, biomarkers, neuropathology, disease mechanisms, and clinical trials (Petersen et al., 2009).

From the perspective of reference co-citation, literature with high co-citation and centrality has been published in relatively high-influence international publications with high academic reference values. Among them, the research of highly co-cited literature mainly involves neuroimaging criteria of small vessel disease and its effect on aging and neurodegeneration (Wardlaw et al., 2013), the latest development of high-intensity WM, cognitive impairment, and dementia (Prins and Scheltens, 2015), the FSL (FMRIB software library) MRI brain imaging data comprehensive analysis tool library (Jenkinson et al., 2012), the composition of high-intensity WM (Wardlaw et al., 2015), and high-intensity WM as a core characteristic of MRI evidence of AD (Lee et al., 2016).

The high centrality studies were mainly related to the prevalence of WM lesions in the elderly (Leeuw et al., 2001), the potential risk factors and clinical manifestations of WM manifestations on brain MRI (Longstreth et al., 1996), the "Axial" and "Radial" diffusion rates of WM pathological regions (Wheeler-Kingshott and Cercignani, 2009), the changes of WM in mild cognitive impairment and AD (Medina et al., 2006), the age-related mental decline and high intensity of WM in healthy octogenarians (Garde et al., 2000).

In terms of keyword burst, the "magnetic resonance" burst lasted the longest, and the "vascular dementia" burst intensity was the largest at 24.1938. The burst time of "small vessel disease," "diffusion MRI," and "biomarker" were 2016–2022, 2017–2022, and 2018–2022, respectively, and they all lasted to the present. Best et al. identified increased perivascular space in the deep WM of the brain as a risk factor for intracranial hemorrhage in patients taking oral anticoagulants in a prospective initial cohort study (Best et al., 2020). Camarda's study assessed WM hyperintensities using the APOE genotype and normal cognition, mild cognitive impairment and AD brain imaging studies, and the visual scoring scale. The APOE ε3 allele may be associated with cerebrovascular diseases, especially in the frontal and parietal-occipital lobes (Camarda et al., 2022). In terms of diffusion MRI, Andersen's study showed that demyelination and axonal degeneration reduced fractional-order anisotropy in normal WM, which can be routinely imaged using diffusion tensor imaging (Andersen et al., 2020). In addition, researchers such as David S (David et al., 2022) and Chang YL (Chang et al., 2021) have used diffusion MRI. Neuroimaging biomarkers have been used in the clinical diagnosis, differential diagnosis, treatment, and prognosis of Parkinson's disease and AD (Li et al., 2022).

Gomar et al. (2011) reported that biomarkers are becoming increasingly important for understanding the neurodegeneration associated with AD (Gomar et al., 2011). Beaudin et al. (2022) suggested that small vessel disease and other vascular factors increase the risk of AD, and decreased cerebrovascular reactivity is a central feature of cerebral amyloid angiopathy, which can serve as another biomarker for disease severity and cognitive impairment (Beaudin et al., 2022). A prospective study of high-intensity MRI in WM and biomarkers such as neurofilament chain and glial fibrillary acidic protein revealed that serum GFAP is a promising fluid biomarker because it is associated not only with clinical severity but also with cognitive function (Huss et al., 2022). Ford et al. (2022) found that blood–brain barrier dysfunction is a hallmark of aging and aging-related diseases, including small vessel disease of the brain and AD. A novel biomarker of blood–brain barrier dysfunction is the blood–brain barrier water exchange rate [k(W)], as measured by diffusion-weighted arterial spin labeling (DW-ASL) MRI (Ford et al., 2022). In summary, the research field of MRI in WM of AD will focus on the association between small vessel disease and AD, and the clinical application and exploration of diffusion MRI. In particular, image marker research has a larger scope for further development.

4.3. Limitations

This study has several limitations. First, this study only obtained literature from the WOSCC database, and articles not covered by the WOSCC were not included. Second, all studies were published in English, and records in other languages were not included. Third, some original studies were manually reported; thus, some potential studies may have been lost. Fourth, the classification of clusters may be insufficiently precise because of the software.

5. Conclusion

This study investigated the current research status, hotspots, and frontier trends in MRI of WM in patients with AD from 1990 to 2022. The present research situation indicates that the research field of MRI in the WM of AD has great potential for development. Research hotspots include WM disorders, mild cognitive impairment, high-intensity WM, MRI, and risk factors mainly involved in neuroimaging, neuropathology, vascular pathology, and epidemiology. Research trends include the relationship between small vessel disease and AD, clinical applications and exploration of diffusion MRI, and research on related imaging markers.

Author contributions

J-hY, Q-hZ, XY, and X-LL: concept and design. XY, PW, X-CS, and AL: data curation. XY and X-CS: formal analysis. X-LL, D-NC, L-WZ, and Q-hZ: funding acquisition. X-LL, L-WZ, and GY: investigation and project administration. XY, X-CS, and AL: methodology. J-hY, Q-hZ, XY, X-CS, Z-YW, and X-LL: resources. XY: software. Q-hZ, X-LL, and GY: supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

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References

Acharya, A., Liang, X., Tian, W., Jiang, C., and Han, Y. (2019). White matter hyperintensities relate to basal ganglia functional connectivity and memory performance in aMCI and SVMCI. *Front. Neurosci.* 13, 1204. doi: 10.3389/fnins.2019.01204

Andersen, K. W., Lasič, S., Lundell, H., Nilsson, M., Topgaard, D., Sellebjerg, F., et al. (2020). Disentangling white-matter damage from physiological fibre orientation dispersion in multiple sclerosis. *Brain. Commun.* 2, fcaa077. doi: 10.1093/braincomms/fcaa077

Annweiler, C., Beauchet, O., Bartha, R., and Montero-Odasso, M. (2013). Slow gait in MCI is associated with ventricular enlargement: results from the gait and brain study. *J. Neural. Transm.* 120, 1083–1092. doi: 10.1007/s00702-012-0926-4

Artero, S., Tiemeier, H., Prins, N. D., Sabatier, R., and Breteler, M. M. B. (2004). Neuroanatomical localisation and clinical correlates of white matter lesions in the elderly. *J. Neurol. Neurosurg. Ps.* 75, 1304–1308. doi: 10.1136/jnnp.2003.023713

Beaudin, A. E., McCreary, C. R., Mazerolle, E. L., Gee, M., Sharma, B., Subotic, A., et al. (2022). Cerebrovascular reactivity across the entire brain in cerebral amyloid angiopathy. *Neurology.* 98, e1716–e1728. doi: 10.1212/WNL.0000000000200136

Ben Miled, A., Yeferny, T., and Ben Rabeh, A. (2020). MRI Images analysis method for early stage Alzheimer's disease detection. *IJCSNS.* 20, 214–220. doi: 10.22937/IJCSNS.2020.20.09.26

Best, J. G., Barbato, C., Ambler, G., Du, H., Banerjee, G., Wilson, D., et al. (2020). Association of enlarged perivascular spaces and anticoagulant-related intracranial hemorrhage. *Neurol.* 95, 2192–2199. doi: 10.1212/WNL.0000000000010788

Camarda, C., Torelli, P., Camarda, R., Battaglini, I., and Gagliardo, C. (2015). Isolated, subtle, neurological abnormalities in neurologically and cognitively healthy aging subjects. *J. Neurol.* 262, 1328–1339. doi: 10.1007/s00415-015-7716-5

Camarda, C., Torelli, P., Pipia, C., Sottile, G., and Cilluffo, G. (2022). APOE genotypes and brain imaging classes in normal cognition, mild cognitive impairment, and Alzheimer's disease: a longitudinal study. *Curr. Alzheimer. Res.* 17, 766–780. doi: 10.2174/1567205017666201109093314

Chang, Y. L., Chao, R. Y., Hsu, Y. C., and Chen, T. F. (2021). White matter network disruption and cognitive correlates underlying impaired memory awareness in mild cognitive impairment. *Neuroimage. Clin.* 30, 102626. doi: 10.1016/j.nicl.2021.102626

Chard, D. T., Alahmadi, A. A., Audoin, B., Charalambous, T., Enzinger, C., Hulst, H. E., et al. (2021). Mind the gap: from neurons to networks to outcomes in multiple sclerosis. *Nat. Rev. Neurol.* 17, 173–184. doi: 10.1038/s41582-020-00439-8

Chen, C. (2010). The structure and dynamics of co-citation clusters: a multiple-perspective co-citation analysis. *JASIST.* 61, 1386–1409. doi: 10.1002/asi.21309

Chen, C. (2017). Science mapping: a systematic review of the literature. *JDIS.* 2, 1–40. doi: 10.1515/jdis-2017-0006

Cheng, R., Qi, H., Liu, Y., Zhao, S., Li, C., Liu, C., et al. (2017). Abnormal amplitude of low-frequency fluctuations and functional connectivity of resting-state functional magnetic resonance imaging in patients with leukoaraiosis. *Brain. Behav.* 7, e00714. doi: 10.1002/brb3.714

Cuenco, K. T., Green, R. C., Zhang, J., Lunetta, K., Erlich, P. M., Cupples, L. A., et al. (2008). Magnetic resonance imaging traits in siblings discordant for Alzheimer disease. J. *Neuroimaging*. 18, 268–275. doi: 10.1111/j.1552-6569.2007.00191.x

David, S., Brown, L. L., Heemskerk, A. M., Aron, E., and Leemans, A. (2022). Sensory processing sensitivity and axonal microarchitecture: identifying brain structural characteristics for behavior. *Brain. Struct. Funct*. 227, 2769–2785. doi: 10.1007/s00429-022-02571-1

Debette, S., Beiser, A., DeCarli, C., Au, R., Himali, J. J., Kelly-Hayes, M., et al. (2010). Association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality the framingham offspring study. *Stroke*. 41, 600–606. doi: 10.1161/STROKEAHA.109.570044

DeCarli, C., Fletcher, E., Ramey, V., and Harvey, D. (2005). Anatomical mapping of white matter hyperintensities (WMH)-Exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke*. 36, 50–55. doi: 10.1161/01.STR.0000150668.58689.f2

DeCarli, C., Mungas, D., Harvey, D., Reed, B., Weiner, M., Chui, H., et al. (2004). Memory impairment, but not cerebrovascular disease, predicts progression of MCI to dementia. *Neurol*. 63, 220–227. doi: 10.1212/01.WNL.0000130531.90205.EF

Ford, J. N., Zhang, Q., Sweeney, E. M., Merkler, A. E., Leon, d. e., Gupta, M. J., et al. (2022). Quantitative water permeability mapping of blood-brain-barrier dysfunction in aging. *Front. Aging. Neurosci*. 14, 867452. doi: 10.3389/fnagi.2022.867452

Frisoni, G. B., Fox, N. C., Jack, C. R., and Scheltens, P. (2010). The clinical use of structural MRI in Alzheimer disease. *Nat. Rev. Neurol*. 6, 67–77. doi: 10.1038/nrneurol.2009.215

Gao, F. Q., Swartz, R. H., Scheltens, P., Leibovitch, F. S., Kiss, A., Honjo, K., et al. (2011). Complexity of MRI white matter hyperintensity assessments in relation to cognition in aging and dementia from the sunnybrook dementia study. *J. Alzheimers. Dis*. 26, 379–388. doi: 10.3233/JAD-2011-0058

Garde, E., Mortensen, E. L., Krabbe, K., and Rostrup, E. (2000). Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study. *Lancet*. 356, 628–634. doi: 10.1016/S0140-6736(00)02604-0

Glodzik, L., Rusinek, H., Li, J., Zhou, C., Tsui, W., Mosconi, L., et al. (2015). Reduced retention of Pittsburgh compound B in white matter lesions. *Eur. J. Nucl. Med. Mol. Imaging*. 42, 97–102. doi: 10.1007/s00259-014-2897-1

Gomar, J. J., Bobes-Bascaran, M. T., Conejero-Goldberg, C., Davies, P., and Goldberg, T. E. (2011). Utility of combinations of biomarkers, cognitive markers, and risk factors to predict conversion from mild cognitive impairment to alzheimer disease in patients in the alzheimer's disease neuroimaging initiative. *Arch. Gen. Psychiatry*. 68, 961–969. doi: 10.1001/archgenpsychiatry.2011.96

Hou, Y. B., and Shang, H. F. (2022). Magnetic resonance imaging markers for cognitive impairment in Parkinson's disease: current view. *Front. Aging. Neurosci*. 14, 788846. doi: 10.3389/fnagi.2022.788846

Huss, A., Abdelhak, A., Mayer, B., Tumani, H., Müller, H. P., Althaus, K., et al. (2022). Association of serum GFAP with functional and neurocognitive outcome in sporadic small vessel disease. *Biomedicines*. 10, 1869. doi: 10.3390/biomedicines10081869

Jang, Y. K., Kwon, H., Kim, Y. J., Jung, N. Y., San Lee, J., Lee, J., et al. (2016). Early-vs late-onset subcortical vascular cognitive impairment. *Neurol*. 86, 527–534. doi: 10.1212/WNL.0000000000002357

Jenkinson, M., Beckmann, C. F., and Behrens, T. E. (2012). FSL. *Neuroimage*. 62, 782–790. doi: 10.1016/j.neuroimage.2011.09.015

Kim, A. R., and Park, H. Y. (2021). Theme trends and knowledge-relationship in lifestyle research: a bibliometric analysis. *Int. J. Environ. Res. Public. Health*. 18, 7503. doi: 10.3390/ijerph18147503

Lee, S., Viqar, F., Zimmerman, M. E., Narkhede, A., Tosto, G., Benzinger, T. L., et al. (2016). White matter hyperintensities are a core feature of Alzheimer's disease: evidence from the dominantly inherited Alzheimer network. *Ann. Neurol*. 79, 929–939. doi: 10.1002/ana.24647

Leeuw, D., de Groot, F. E., Achten, J. C., Oudkerk, E., Ramos, M., Heijboer, L. M. P., et al. R. (2001). Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J. Neurol. Neurosurg. Psychiatry*. 70, 9–14. doi: 10.1136/jnnp.70.1.9

Li, X. L., Gao, R. X., Zhang, Q., Li, A., Cai, L. N., Zhao, W. W., et al. (2022). A bibliometric analysis of neuroimaging biomarkers in Parkinson disease based on Web of Science. *Medicine*. 101, e30079. doi: 10.1097/MD.0000000000030079

Longstreth, W. T., Manolio, T. A., Arnold, A., Burke, G. L., Bryan, N., Jungreis, C. A., et al. (1996). Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people - The cardiovascular health study. *Stroke*. 27, 1274–1282. doi: 10.1161/01.STR.27.8.1274

Medina, D., deToledo-Morrell, L., Urresta, F., Gabrieli, J. D., Moseley, M., Fleischman, D., et al. (2006). White matter changes in mild cognitive impairment and AD: a diffusion tensor imaging study. *Neurobiol. Aging*. 27, 663–672. doi: 10.1016/j.neurobiolaging.2005.03.026

Nabizadeh, F., Balabandian, M., Rostami, M. R., Ward, R. T., Ahmadi, N., Pourhamzeh, M., et al. (2022). Plasma p-tau181 associated with structural changes in mild cognitive impairment. *Aging. Clin. Exp. Res*. 34, 2139–2147. doi: 10.1007/s40520-022-02148-2

No, H. J., Yi, H. A., Won, K. S., and Chang, H. W. (2019). Association between white matter lesions and the cerebral glucose metabolism in patients with cognitive impairment. *Rev. Esp. Med. Nucl. Ima*. 38, 160–166. doi: 10.1016/j.remnie.2019.01.005

Pergher, V., Schoenmakers, B., Demaerel, P., and Tournoy, J. (2020). Differential impact of cognitive impairment in mci patients: a case-based. Case. *Rep. Neurol*. 12, 222–231. doi: 10.1159/000507977

Petersen, R. C., Roberts, R. O., Knopman, D. S., Boeve, B. F., Geda, Y. E., Ivnik, R. J., et al. (2009). Mild Cognitive impairment ten years later. *Arch. Neurol*. 66, 1447–1455. doi: 10.1001/archneurol.2009.266

Prins, N. D., and Scheltens, P. (2015). White matter hyperintensities, cognitive impairment and dementia: an update. *Nat. Rev. Neurol*. 11, 157–165. doi: 10.1038/nrneurol.2015.10

Reijmer, Y. D., and Van Veluw, S. J. (2016). Ischemic brain injury in cerebral amyloid angiopathy. *J. Cerebr. Blood. F. Met*. 36, 40–54. doi: 10.1038/jcbfm.2015.88

Small, H. (1973). Co-citation in scientific literature: a new measure of the relationship between publications. *JASIS*. 24, 265–269. doi: 10.1002/asi.4630240406

Tanskanen, M., Kalaria, R. N., Notkola, I. L., Mäkelä, M., Polvikoski, T., Myllykangas, L., et al. (2013). Relationships between white matter hyperintensities, cerebral amyloid angiopathy and dementia in a population-based sample of the oldest old. *Curr. Alzheimer. Res*. 10, 1090–1097. doi: 10.2174/15672050113106660177

Tseng, W. Y. I., Hsu, Y. C., and Kao, T. W. (2022). Brain age difference at baseline predicts clinical dementia rating change in approximately two years. *J. Alzheimers. Dis*. 86, 613–627. doi: 10.3233/JAD-215380

Van Eck, N., and Waltman, L. (2010). Software survey: vosviewer, a computer program for bibliometric mapping. *Scientometrics*. 84, 523–538. doi: 10.1007/s11192-009-0146-3

Vermeer, S. E., Prins, N. D., Heijer, D., Hofman, T., and Koudstaal, A. P. J. (2003). Silent brain infarcts and the risk of dementia and cognitive decline. *NEJM*. 348, 1215–1222. doi: 10.1056/NEJMoa022066

Wahlund, L. O., Barkhof, F., Fazekas, F., Bronge, L., Augustin, M., Sjogren, M., et al. (2001). A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke*. 32, 1318–1322. doi: 10.1161/01.STR.32.6.1318

Wardlaw, J. M., Hernandez, M. C. V., and Munoz-Maniega, S. (2015). What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. *J. Am. Heart. Assoc*. 4, 001140. doi: 10.1161/JAHA.114.001140

Wardlaw, J. M., Smith, E. E., Biessels, G. J., Cordonnier, C., Fazekas, F., Frayne, R., et al. (2013). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet. Neurol*. 12, 822–838. doi: 10.1016/S1474-4422(13)70124-8

Wheeler-Kingshott, C. A. M., and Cercignani, M. (2009). About "axial" and "radial" diffusivities. *Magn. Reson. Med*. 61, 1255–1260. doi: 10.1002/mrm.21965

Wu, C. C., Mungas, D., Petkov, C. I., Eberling, J. L., Zrelak, P. A., Buonocore, M. H., et al. (2002). Brain structure and cognition in a community sample of elderly Latinos. *Neurol*. 59, 383–391. doi: 10.1212/WNL.59.3.383

Xia, M. H., Li, A., Gao, R. X., Li, X. L., Zhang, Q., Tong, X., et al. (2022). Research hotspots and trends of multimodality MRI on vascular cognitive impairment in recent 12 years: a bibliometric analysis. *Medicine*. 101, e30172. doi: 10.1097/MD.0000000000030172

Yang, R. L., Li, X. L., and Wang, F. (2015). Progress of acupuncture therapy for functional brain imaging under different functional status. *J. Inform. Tradit. Chin. Med*. 1, 122–125.

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Acupuncture with twirling reinforcing and reducing manipulation shows a control of hypertension and regulation of blood pressure-related target brain regions in spontaneously hypertensive rat: a preliminary resting-state functional MRI study

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Aim: To observe the effects of acupuncture manipulations on blood pressure and brain function in spontaneously hypertensive rats and elucidate the anti-hypertensive effect of the manipulations' central mechanism.

Methods: This study used acupuncture twirling reinforcing, acupuncture twirling reducing, and acupuncture twirling uniform reinforcing-reducing manipulations to act on the bilateral TaiChong point of rats. The depth of acupuncture was 1.5–2mm, and twisting was performed at a frequency of 60 times/min within ±360° for 3min, followed by the needle being retained for 17min. Functional magnetic resonance imaging was performed at the end of the intervention. Regional homogeneity and amplitude of low-frequency fluctuations were used to assess the differences in brain regions in each group of rats, and the core brain region (left hypothalamus) among the differential brain regions was selected as the seed for functional connectivity analysis.

Results: (1) The anti-hypertensive effect was achieved by acupuncture manipulations, and the anti-hypertensive effect of twirling reducing manipulation on spontaneously hypertensive rats was better than that of twirling uniform reinforcing-reducing and twirling reinforcing manipulations. (2) After regional homogeneity and amplitude of low-frequency fluctuations analyses, the hypothalamus, the brain region related to blood pressure, was activated in the twirling uniform reinforcing-reducing manipulation group; the corpus callosum and cerebellum were activated in the twirling reinforcing manipulation group; and the hypothalamus, olfactory bulb, corpus callosum, brainstem, globus pallidum, and striatum were activated in the twirling reducing manipulation group. (3) According to the functional connectivity analysis, different acupuncture manipulations increased the functional connections between seed points and the brainstem, olfactory bulb, and cerebellum, etc.

Conclusion: These results suggest that acupuncture manipulations achieved the hypotensive effect and the twirling reducing manipulation had a better hypotensive effect on spontaneously hypertensive rats than twirling uniform reinforcing-reducing and twirling reinforcing manipulations; the central mechanism of the anti-hypertensive effect of twirling reinforcing and reducing manipulation may be related to the activation of brain regions associated with blood pressure regulation and the functional connections between them. Furthermore, brain regions involved in motor control, cognition, and hearing were also activated. We hypothesize that activation of these brain regions may help prevent or mitigate the onset and progression of hypertensive brain damage.

KEYWORDS

acupuncture, twirling reinforcing and reducing manipulation, spontaneously hypertensive rats, resting-state functional MRI, central mechanism

1. Introduction

Essential hypertension (EH) is a chronic, clinically frequent, and common disease affecting approximately 116 million adults in the United States and over 1 billion global adults. Although it is one of the leading causes of morbidity and mortality in the cardiovascular system, there is no definitive cure for this disease (Malpas, 2010; Gu et al., 2015; Nishijima et al., 2015; Yang et al., 2017; Carey et al., 2022). The number of adults with high blood pressure is estimated to reach 1.5 billion worldwide by 2025; this situation poses an increasingly serious challenge to global public health, thus treatment of hypertension has become a key issue that needs to be addressed (Kearney et al., 2005). Studies have found that a 10 mmHg reduction in systolic blood pressure can decrease the risk of cardiovascular disease by about 20%–30% (Malpas, 2010). Clinically, hypertension is mainly treated with drugs, including thiazides and angiotensin-converting enzyme inhibitors (Carey et al., 2022). These drugs have certain limitations, such as dependence, higher costs, and adverse effects (Calhoun et al., 2014; Oparil and Schmieder, 2015; Goshgarian and Gorelick, 2019; Flack and Adekola, 2020; Reeve et al., 2020). Numerous clinical and experimental studies have proven that acupuncture is an efficient complementary alternative therapy for reducing high blood pressure (Wang et al., 2018; Yang et al., 2022; Zhang et al., 2022). Compared with drug therapy, acupuncture to lower blood pressure does not cause adverse reactions associated with drug therapy, such as side effects of drugs, meanwhile reducing dependence on antihypertensive drugs and reducing the cost of medication, making it an effective option for controlling hypertension and its complications. In traditional Chinese medicine, the Taichong point (LR 3) is considered an infusion point and the original point of the Foot Turk's Yin Liver meridian, which regulates the meridians, pacifies the liver, submerges the yang, and treats liver yang hyperactivity. Modern studies clearly established the unique advantages and efficacy of acupuncture at the "Taichong" point in decreasing blood pressure (Lu et al., 2015; Luo et al., 2019; Zhang et al., 2021a). One study found that acupuncture at LR 3 led to a rapid onset of action in treating hypertension by reducing systolic and diastolic blood pressure within 20 min (Han and Liang, 2021). Acupuncture twirling reinforcing and reducing manipulation (TRRM) is a key factor in the efficacy of acupuncture for decreasing blood pressure. The twirling uniform reinforcing-reducing (TUR) manipulation, twirling reinforcing (TRF) manipulation and twirling reducing (TRD) manipulation are the basic manipulations of TRRM. TUR is the twisting of the needle forward and backward with the same force after the needle is pierced into the skin at a certain depth; TRF means that after piercing the needle into the skin to a certain depth, the thumb applies a forward or backward twisting force to the needle and is heavier in the forward direction and lighter in the backward direction; TRD is a lighter force with the thumb forward and a heavier force with the thumb backward. TRRM is a treatment method based on the theory of the Nei Jing: treating excess with expelling, and treating deficiency with reinforcement, which means that if the evil Qi is strong and the positive Qi is not yet weakened, acupuncture can be used to treat by reducing, and if the positive Qi is weak and the body is poor, acupuncture can be used to treat by reinforcement. TRRM can achieve the multitarget regulation of blood pressure via the neuroendocrine system, cytokines, signal transduction pathways, and other pathways (Lu et al., 2017; Liang et al., 2021; Wu et al., 2021). However, the mechanisms underlying its anti-hypertensive effects remain unclear.

Functional magnetic resonance imaging (fMRI) is a common visualization technique used to reveal local brain functions (Zeng et al., 2012; Liu et al., 2014; Ko et al., 2016; Zhang et al., 2021b). Owing to its advantages of high temporal and spatial resolution, lack of radiation, fast imaging speed, and noninvasive nature, fMRI has become the most generally used neuroimaging technique in the study of the central mechanisms of acupuncture and the brain's response to acupuncture stimulation (Kim et al., 2020; Huang et al., 2022). Resting-state fMRI (rs-fMRI), based on a blood oxygenation level-dependent (BOLD) technique, provides a wide variety of methods and tools for studying functional brain imaging metrics. Regional homogeneity (ReHo) is an imaging metric that describes the local functional activity of the brain and is used to evaluate the temporal synchronization and co-ordination of neuronal activity in the local brain areas (Zhang et al., 2019; Shan et al., 2020). Increased ReHo indicates the convergence of neuronal synchronization in local brain areas, whereas increased ReHo indicates dyssynchronization of nerve element activity. The amplitude of low-frequency fluctuation (ALFF) is an amplitude analysis of the low-frequency band of the BOLD signal, which is a reflection of the level of spontaneous activity of each voxel at rest from an energy point of view (Liang et al., 2014; Wang et al., 2016). An increase in ALFF value indicates increased excitability in that brain region, whereas a decrease in ALFF value suggests that decreased neurons' excitability in that brain region (Yang et al., 2018). ALFF and ReHo deliver different information about different types of neuronal activity and are complementary methods ways to study changes in the whole brain. The combination of ALFF and ReHo may be a more comprehensive assessment of the pathophysiology of brain dysfunction than any of the approaches on their own (Li et al., 2018). Seed-point-based functional connectivity (FC) involves the analysis of the overall functional activity of the brain to determine whether FC exists between brain regions in a time series. The above three analytical methods are widely used in acupuncture to study the central mechanisms (Fang et al., 2015; Zhang et al., 2018, 2021a,b; Sun et al., 2021).

Using positron emission computed tomography, we found that the hypotensive effect of acupuncture with TRRM could be exerted by increasing glucose metabolism in different target brain regions (Guo et al., 2018). However, the central regulation of blood pressure is a complex process involving several brain regions (Chen et al., 2013), and the central mechanism of TRRM in decreasing blood pressure remains unclear. Different brain imaging techniques use different principles and can reflect neurophysiological and pathological mechanisms from different perspectives. Therefore, the present study used rs-fMRI to assess the local brain function indices ReHo, ALFF, and FC in spontaneously hypertensive rats (SHRs) to elucidate other potential central mechanisms of blood pressure regulation by TRRM. This provides some objective scientific basis for the clinical application of the TRRM. Figure 1 shows a flowchart.

2. Materials and methods

2.1. Animal preparation

Thirty-two male SHRs at 12 weeks old and eight Wistar–Kyoto (WKY) rats at 12 weeks old were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. in China (License No.SCXK (Jing)20210006). The experimental animals were housed according to specific standards for pathogen-free and supplied food and water *ad libitum* for 1 week. The rearing temperature was controlled throughout the study at 20 ± 1°C, and alternating light and dark lighting (12 h, 12 h) was used. All laboratory animal experiments were conducted in strict compliance with the World Health Organization International Guidelines for Biomedical Research Involving Animals and were approved by the Animal Care and Use Committee of Beijing University of Traditional Chinese Medicine (BUCM-4-2021040804-2140).

Thirty-two SHRs at 12 weeks old were randomly divided into four groups ($n = 8$/group): model group, twirling uniform reinforcing-reducing (TUR)manipulation group, twirling reinforcing (TRF) manipulation group, and twirling reducing (TRD) manipulation group. Eight WKY rats at 12 weeks old were used as control group.

2.2. Intervention methods

The LR 3 is located in the dorsal recess of the first and second metatarsal bones on the dorsal surface of the hind limbs. Before the operation, the rat was completely drilled into a comfortable soft mouse sleeve with its head fixed and both hind limbs exposed.

Needling intervention with the right hand as the stabbing hand was performed daily at 14:00 p.m. by a professional technician with the help of a metronome pronunciation frequency of 3 min at 60 times/min to facilitate the control of the twisting frequency and time. The duration of the intervention was 28 days (intermittently, 1 day per week, once per day).

In the TUR group, needles (0.18 mm × 13 mm, Beijing Zhongyan Taihe Medical Instrument Co., Ltd., Beijing, China) were inserted directly into LR 3 bilaterally for 1.5–2 mm. Twisting was then performed at a frequency of 60 times per minute within ±360° for 3 min, during which the thumb was twisted forward and backward with the same force. The needle retention time was 17 min.

In the TRF group, needles were inserted directly into LR 3 bilaterally for 1.5–2 mm. Twisting was then performed at a frequency of 60 times per minute within ±360° for 3 min, during which the thumb force forward was heavier and the thumb force backward was lighter. The needle retention time was 17 min.

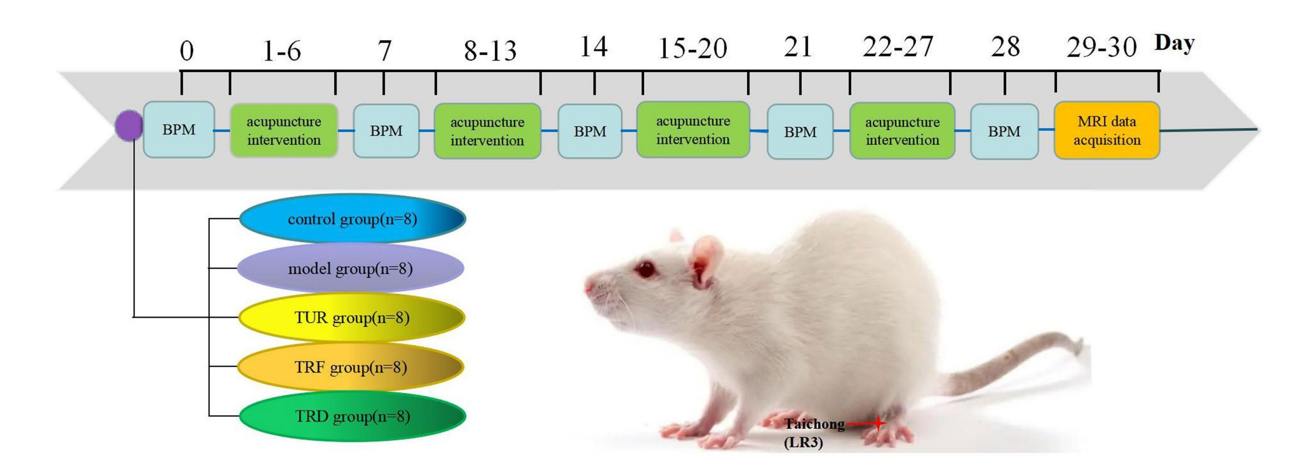

FIGURE 1
Experimental flowchart. TUR, twirling uniform reinforcing-reducing; TRF, twirling reinforcing; TRD, twirling reducing; BPM, blood pressure measurement.

In the TRD group, needles were inserted directly into LR 3 bilaterally for 1.5–2 mm. Twisting was performed at a frequency of 60 times per minute within ±360° for 3 min, during which the thumb force forward was lighter and the thumb force backward was heavier. The needle retention time was 17 min.

In the model and control groups, the rats were operated under the same constraints as the first three groups for 20 min of fixation without acupuncture treatment.

2.3. Blood pressure measurement

The rats were preheated at 37°C (constant temperature adjustment with thermostat) for approximately 2 min at room temperature of 22 ± 2°C, and the systolic blood pressure in the quiet and awake state was measured using a noninvasive blood pressure monitor (BP-2010E, Softron Biotechnology, Beijing, China). The blood pressure of each rat was measured three times, and the mean recorded blood pressure was used as the baseline value. Blood pressure was measured in all rats on day 0 (1 d before acupuncture) and on days 7, 14, 21, and 28. Blood pressure values were measured between 8:00 a.m. and 11:00 a.m. each day to keep errors to a minimum. The mesh and tail sleeve sizes should be appropriate during blood pressure measurements, and movements should be gentle to avoid fluctuations.

2.4. MRI data acquisition

A 7.0 T small animal live MRI scanner (PharmaScan 70/16 US, Bruker, Germany) with a dedicated small-animal cranial surface coil was used for scanning. Before scanning, each rat was injected intramuscularly with a dexmedetomidine hydrochloride injection (100 ug/mL) in the posterior lateral thigh at a dose of 0.02 mL per 100 g of rat body weight, and anesthesia was induced using a mixture of 5% isoflurane/95% oxygen. During the scan, the rat was placed in a prone position on a rat specific scanning platform, the rat's head was held in place with dental hooks and ear sticks, and a 2% isoflurane/98% oxygen mixture was administered using a mask. First, T2-weighted sequences were scanned, followed by BOLD sequences. During the scanning process, a physiological detector monitored body temperature, respiration rate, and heart rate in real time (Model 1025, Small Animal Instruments Inc., Stony Brook, NY, United States). T2-weighted imaging was performed using T2-turboRARE sequence with the following parameters: repetition time (TR) = 5,500 ms; echo time (TE) = 33 ms; the number of averages = 2, slice thickness = 0.5 mm, percent phase field view = 100, acquisition matrix = 256 × 256; bandwidth = 152.587; and flip angle = 90°. BOLD-based rs-fMRI was also conducted using the T2Star_FID_EPI sequence with the following parameters: TR = 2,000 ms, TE = 11 ms, number of averages = 1, slice thickness = 0.5 mm, percent phase field view = 80, bandwidth = 2,500, acquisition matrix = 80 × 48, and flip angle = 90°.

2.5. Magnetic resonance data preprocessing and index calculation

Data preprocessing and metrics calculation were based on the Statistical Parametric Mapping (SPM) 12 package, REST package, FC toolkit, and the Data Processing & Analysis of Brain Imaging (DPABI) package of the MATLAB platform. The following procedures were used: (1) Format Conversion: Raw functional and T2 images were transformed from medical digital imaging and communication formats to the neuroimaging informatics initiative format. (2) Starting time point removal: When the functional image is acquired initially, the BOLD signal is often unstable; therefore, the first 10 time points must be removed. (3) Voxel augmentation: The SPM package is designed based on the actual size of the human brain, and the rat brain is much smaller than the human brain; therefore, the acquired rat MRI images need to be magnified 10 times to fit the package operation. (4) Slice timing: The data of all voxels were adjusted as if they were scanned simultaneously, thus making the acquisition time of all voxel BOLD signals theoretically consistent within a time point. (5) Realignment: A small amount of movement of each rat's head was corrected between different time points in the scan. The exclusion criterion was a movement of more than one voxel size (magnified voxel size of 3 × 3 × 3 mm). (6) Reorientation: The average functional phase is homeopathically corrected, and the homeopathic correction matrix of the average functional phase is applied to the functional phase file; the T2 phase also needs to be corrected. (7) Normalization: The brains of all subjects were aligned to a uniform standard space to resolve the differences in brain shape between rats and the inconsistent spatial position of the head during scanning and to facilitate subsequent statistics. (8) Smooth: High-frequency noise is reduced from image deformation during spatial normalization and improves statistical validity; the smooth kernel size is two to three times the magnified voxel size. (9) Calculation of indicator: The REST and SPM12 toolkits must be loaded to calculate ALFF and ReHo. The calculation of ALFF was performed after the spatial smoothing, and ALFF was the selected range of 0.01–0.08 Hz. The calculation of ReHo was performed after the spatial normalization, and the noise in the frequency band below 0.01 Hz (low frequency) and above 0.08 Hz (high frequency) needed to be removed before ReHo calculation. The FC, SPM12, and DPABI toolkits must be loaded for the FC calculations. The calculation of FC was performed after the spatial smoothing.

2.6. Statistical analysis

2.6.1. Systolic pressure data analysis

SPSS 20.0 statistical analysis software was used to process the blood pressure measurements. The data are presented as mean ± standard deviation, and the results were analyzed by two-way analysis of variance (ANOVA) for repeated measurements. Statistical significance was set at $p < 0.05$.

2.6.2. fMRI data analysis

The magnetic resonance data were modeled using a general linear model. Data were analyzed using one-way ANOVA with a post-hoc two-sample *t*-test. Areas with significant ALFF and ReHo changes between the two groups were determined based on $p < 0.005$ (uncorrected) and clusters >5 voxels Areas with significant FC changes between the two groups were determined based on $p < 0.005$ (uncorrected) and clusters >2 voxels.

2.6.3. Correlation analysis of systolic blood pressure and ReHo/ALFF

We performed a Pearson correlation analysis between systolic blood pressure and ReHo/ALFF values after TRRM. $p < 0.05$ was considered significant difference.

3. Results

3.1. Effects of TRRM on systolic blood pressure in SHRs

The blood pressure of rats was significantly higher in model group on days 0, 7, 14, 21, and 28 of the experiment ($p < 0.01$) than in the control group, indicating that the hypertension data of the SHR model remained stable. Before the experiment, on day 0 of acupuncture, there was not statistically meaningful difference among the model, TUR, TRF, and TRD groups, suggesting that each group's baseline blood pressure levels were consistent and comparable. On days 14th, 21st, and 28th days of acupuncture, the blood pressure of the SHRs in each acupuncture group was markedly below than that of the model group ($p < 0.01$). On the 21st and 28th days of acupuncture, compared with the TRF, the blood pressure of rats in the TUR group was no statistically meaningful between groups ($p > 0.05$), whereas the blood pressure of rats in the TRD group was markedly lower ($p < 0.01$). On the 28th day of acupuncture, the blood pressure of rats in the TRD group was significantly lower than that of rats in the TUR group ($p < 0.05$). This indicates that TRRM has a certain effect on decreasing blood pressure, and TRD has the most significant effect on decreasing blood pressure (Figure 2).

3.2. ReHo analysis

The brain regions with decreased ReHo in the model group compared to that in the control group were the entorhinal cortex (left and right), olfactory bulb (left and right), hippocampal dentate gyrus (left), and brainstem (left and right), etc. (Figure 3A). The brain regions with increased ReHo in the TRF group compared to that in the model group were the brainstem (right), striatum (left), basal forebrain region (left), etc. (Figure 3B). The brain regions with increased ReHo in the TUR group compared to that in the model group were the hypothalamus (right), brainstem (right), primary visual cortex (right), etc. (Figure 3C). The brain regions with increased ReHo in the TRD group compared to that in the model group were the entorhinal cortex (left), primary motor cortex (left), etc. (Figure 3D). The locations of brain regions with significant differences are presented in Supplementary Table S1.

In this study, we focused on brain regions with a callback effect. We intersected brain regions with decreased ReHo values in the model group compared to that in the control group and brain regions with increased ReHo values in the treatment group compared to that in the model group to obtain the callback brain regions. These callback brain regions are critical brain regions for acupuncture intervention. Callback brain regions in the TUR group included the entorhinal cortex (left), hippocampal dentate gyrus (left and right), hypothalamus (left), etc. Callback brain regions in the TRF group included the brainstem (right), corpus callosum (right), cerebellum (right), basal forebrain region (left), etc. Callback brain regions in the TRD group included the entorhinal cortex (left), basal forebrain region (left), brainstem (left and right), descending corticofugal pathways and globus pallidum (left), hypothalamus (left), etc.

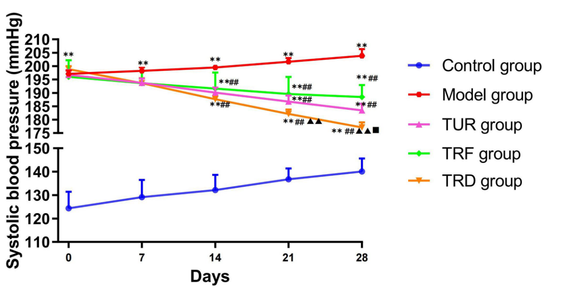

FIGURE 2
Evolution of systolic blood pressure measured in each group. All data are expressed as the mean±SD (n=8/group). **p<0.01 vs. control group; ##p<0.01 vs. model group; ▲▲p<0.01 vs. TRF group; ■p<0.05 vs. TUR group. TUR, twirling uniform reinforcing-reducing manipulation; TRF, twirling reinforcing manipulation; TRD, twirling reducing manipulation.

3.3. ALFF analysis

The brain regions with decreased ALFF in the model group compared to that in the control group were the hypothalamus (left), entorhinal cortex (left and right), hippocampal dentate gyrus (left and right), etc. (Figure 4A). The brain regions with increased ALFF in the TRF group compared to that in the model group were the cerebellum (left and right), striatum (right), primary motor cortex (right), etc. (Figure 4B). The brain regions with increased ALFF in the TUR group compared to that in the model group were the hypothalamus (left), basal forebrain region (right), primary visual cortex (left), etc. (Figure 4C). The brain regions with increased ALFF in the TRD group compared to that in the model group were the thalamus (left and right), olfactory bulb (left and right), brainstem (left and right), etc. (Figure 4D). The locations of brain regions with significant differences are presented in Supplementary Table S2.

Similar to the ReHo analysis above, we focused on callback brain regions. Callback brain regions in the TUR group included the entorhinal cortex (right), corpus callosum (right), olfactory bulb (left), hypothalamus (left), etc. Callback brain regions in the TRF group included the entorhinal cortex (left), hippocampus (left), cerebellum (left and right), etc. Callback brain regions in the TRD group included the entorhinal cortex (left and right), hypothalamus (left), striatum (left and right), hippocampal dentate gyrus (left), etc.

3.4. Correlation analysis of systolic blood pressure and ReHo/ALFF

We intersected the callback brain regions based on ReHo analysis and the callback brain regions based on ALFF analysis to obtain common callback brain regions. These brain regions are usually the core brain regions for acupuncture intervention. The common callback brain regions in the TUR group included the hypothalamus

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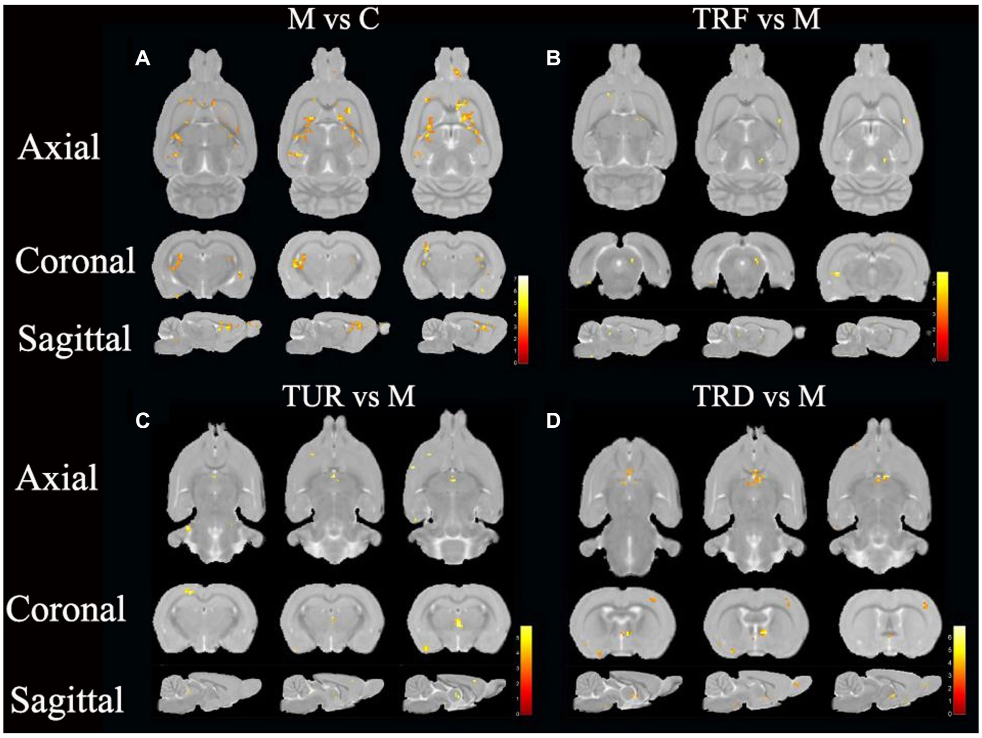

FIGURE 3
ReHo difference brain regions map. **(A)** Brain regions with decreased ReHo in the model group versus the control group (M vs. C); **(B)** Brain regions with increased ReHo in the TRF group versus the model group (TRF vs. M); **(C)** Brain regions with increased ReHo in the TUR group versus the model group (TUR vs. M); **(D)** Brain regions with increased ReHo in the TRD group versus the model group (TRD vs. M). The color bars were used to signify the *t*-value of the group analysis (the color is brighter; the *t*-value is higher).

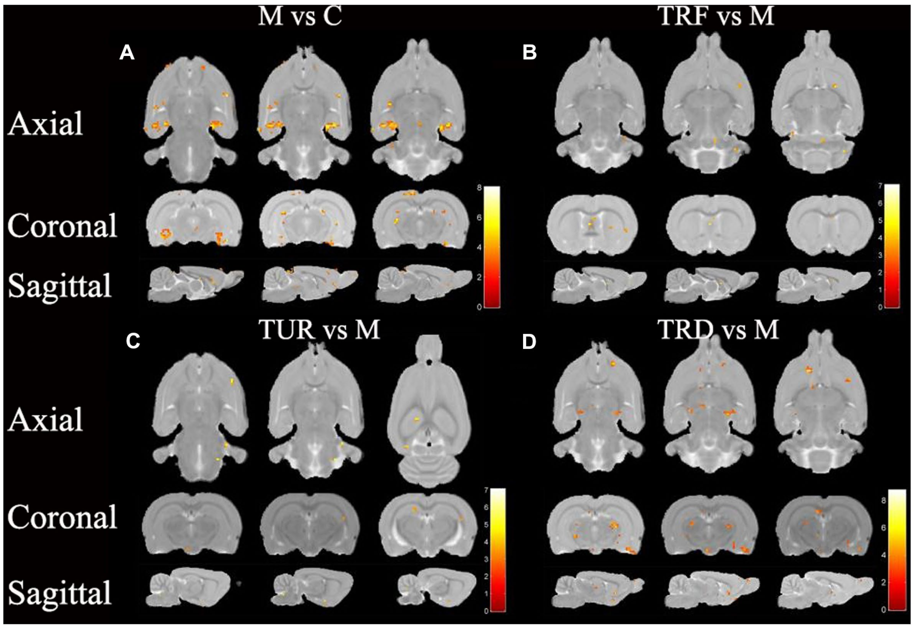

FIGURE 4
ALFF difference brain regions map. **(A)** Brain regions with decreased ALFF in the model group versus the control group (M vs. C); **(B)** Brain regions with increased ALFF in the TRF group versus the model group (TRF vs. M); **(C)** Brain regions with increased ALFF in the TUR group versus the model group (TUR vs. M); **(D)** Brain regions with increased ALFF in the TRD group versus the model group (TRD vs. M). The color bars were used to signify the *t*-value of the group analysis (the color is brighter; the *t*-value is higher).

(left) and hippocampal dentate gyrus (left). The common callback brain regions in the TRF group included the corpus callosum (right) and cerebellum (left and right). The common callback brain regions of the TRD group included the basal forebrain region (left), brainstem (right), descending corticofugal pathways and globus pallidum (left), striatum (right), hypothalamus (left), thalamus (left and right), preLimbic system (left), olfactory bulb (left), corpus callosum (left and right), secondary auditory cortex (left), primary somatosensory cortex forelimb (left and right), primary motor cortex (left), and entorhinal cortex (left).

The hypothalamus is closely related to blood pressure regulation. Based on the above ReHo/ALFF results, we found that the brain areas activated by TRRM included the left hypothalamus, we performed a Pearson correlation analysis between systolic blood pressure and ReHo/ALFF values of the left hypothalamus. The results revealed a negative correlation between systolic blood pressure and ALFF values in TUR group ($p=0.022$, $r=-0.781$; Figure 5A), as well as a negative correlation between systolic blood pressure and ReHo values in TRD group ($p=0.013$, $r=-0.818$; Figure 5B).

3.5. FC analysis

Based on the results obtained by ReHo and ALFF, the left hypothalamus, a brain region closely related to blood pressure, was selected as the seed point and analyzed for FC in the whole brain to obtain differential brain regions. The brain regions with decreased FC in the model group compared to that in the control group were the brainstem (left and right), basal forebrain region (left and right), olfactory bulb (right), primary motor cortex (left and right), etc. (Figure 6A). Brain regions with increased FC in the TRF group compared to that in the model group were the thalamus (left), hippocampal dentate gyrus (left), etc. (Figure 6B). The brain regions with increased FC in the TUR group compared to that in the model group were the cerebellum (right), the primary somatosensory cortex (left and right), etc. (Figure 6C). The brain regions with increased FC in the TRD group compared to that in the model group were the cerebellum (left and right), thalamus (left and right), olfactory bulb (left and right), etc. (Figure 6D). The locations of brain regions with significant differences are presented in Supplementary Table S3.

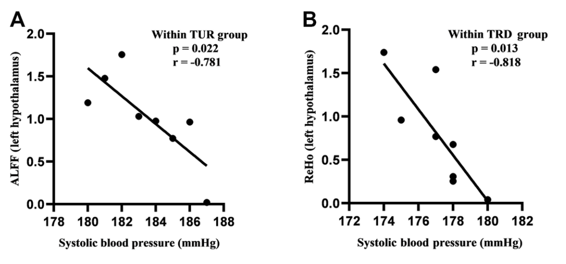

FIGURE 5
Correlation analysis of systolic blood pressure and ReHo/ALFF values. **(A)** Correlation analysis of systolic blood pressure and ALFF values in the TUR group. **(B)** Correlation analysis of systolic blood pressure and ReHo values in the TRD group. Pearson's correlation was used with $p<0.05$ for statistical significance and r for correlation.

Similar to the ReHo and ALFF analysis, we focused on the callback brain regions. Callback brain regions in the TUR group included the basal forebrain region (right), periaqueductal Gray (left), striatum (left), etc. Callback brain regions in the TRF group included the brainstem (left and right), Primary cingular cortex (left and right), olfactory bulb (left), etc. Callback brain regions in the TRD group included the primary somatosensory cortex (left and right), secondary motor cortex (left and right), entorhinal cortex (left), dentate gyrus (left and right), etc.

4. Discussion

Compared with the model groups, each treatment group showed a gradual anti-hypertensive effect from day 14, and the extent of the anti-hypertensive effect varied among the treatment groups. On days 14–28 of the acupuncture intervention, the difference between the anti-hypertensive effect of each treatment and the model group was stable. The TRD group had a significantly better anti-hypertensive effect than the TRF and TUR groups. There was no statistically significant difference between the TRF and TUR groups; however, the trend of systolic blood pressure decrease in the TUR group was more evident than in the former. Acupuncture manipulations decreased the blood pressure of the SHRs, and the hypotensive effect of TRD on the SHRs was better than that of TUR or TRF. This result is consistent with a previous study (Wu et al., 2021), in which acupuncture manipulations had different hypotensive effects.

In the rs-fMRI study, we combined ALFF and ReHo analysis to identify changes in the brain regions of SHRs, which do not depend on the effects of model-generated errors and can directly suggest the presence of spontaneous neuronal activity, which is reliable, practical, and sensitive for monitoring local brain function (Li et al., 2018). After ReHo and ALFF analysis, the callback brain regions associated with blood pressure regulation that were activated in the TUR group was the hypothalamus; the TRF group included the corpus callosum and cerebellum; the TRD group included the hypothalamus, olfactory bulb, corpus callosum, brainstem, globus pallidum, and striatum. The results showed extensive regional alterations in the brain regions of SHRs. However, each acupuncture intervention group reversed some of the alterations and activated different target brain regions related to blood pressure regulation, which may explain the different anti-hypertensive effects of TRRM. The central regulatory regions of the cardiovascular system are widely distributed and include the brainstem, medulla oblongata, periaqueductal gray matter of the midbrain, hypothalamus, and amygdala (Chen et al., 2013). Some of these brain regions are the key target areas involved in central blood pressure regulation, forming a complete network. Using positron emission tomography for brain imaging, a previous study found that acupuncture with TRRM significantly reduced blood pressure in SHRs with clear central effects and achieved central regulation of blood pressure by increasing glucose metabolism levels in several target brain regions, including the hypothalamus, medulla oblongata, hippocampus, and cerebellum, similar to the activated brain regions in our findings (Guo et al., 2018).

The hypothalamus was activated in both the TUR and TRD groups. A previous study showed that the hypothalamus is the neuroendocrine center, and endocrine disorders lead to elevated cortisol levels, which is significantly associated with increased blood

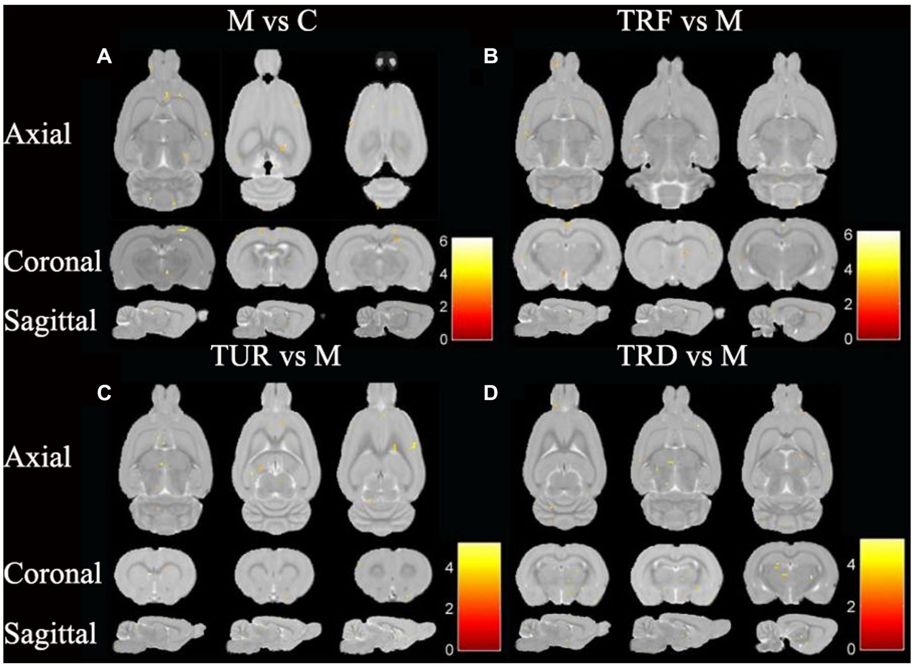

FIGURE 6
FC difference brain regions map. **(A)** Brain regions with decreased FC in the model group versus the control group (M vs. C); **(B)** Brain regions with increased FC in the TRF group versus the model group (TRF vs. M); **(C)** Brain regions with increased FC in the TUR group versus the model group (TUR vs. M); **(D)** Brain regions with increased FC in the TRD group versus the model group (TRD vs. M). The color bars were used to signify the *t*-value of the group analysis (the color is brighter; the *t*-value is higher).

pressure (Gold et al., 2005). The hypothalamic supraoptic nucleus and paraventricular nucleus secrete pressor hormone and oxytocin, which act on the pituitary system and interact with neurons of cardiovascular activity (Qin et al., 2018). It has been found that acupuncture with the supraoptic and paraventricular nuclei as seed points strengthens the functional connections between the hypothalamus and the frontal lobes, cerebellum, and insula, suggesting that these brain regions constitute a neural network structure with specific functions and elaborating the mechanism of its anti-hypertensive effects (Zheng et al., 2016). Acupuncture for EH in the hypothalamic-associated resting brain network has revealed that acupuncture can regulate the cardiovascular system via the intricate brain networks of the cortex, hypothalamus, and brainstem. After performing FC analysis using the left hypothalamus as the seed point, our results showed that different acupuncture manipulations enhanced the FC of the left hypothalamus with the brainstem, olfactory bulb, and cerebellum and that these brain regions overlapped with the blood pressure–callback brain regions activated by ReHo and ALFF analysis, which may further indicate that these brain regions play a key role in blood pressure regulation.

The brainstem is the brain region activated by TRD, and FC analysis has revealed enhanced FC between the brainstem and hypothalamus. It is located at the core of the central nervous system and has a crucial function in regulating elevated EH (Cheng et al., 2020). The current study demonstrated that the nucleus tractus solitarius and reactive oxygen species are essential factors in the neural mechanisms of hypertension by increasing central sympathetic outflow and inhibiting the pressure reflex regulation of blood pressure via their effects on the lateral ventral and nucleus tractus solitarius of the medulla oblongata (Chan and Chan, 2014). And Cheng found abnormal insulin signaling pathways in the brainstem of SHRs during hypertension, which is a potentially important indicator of a lack of brainstem metabolic disorders (Cheng et al., 2020). The pathology of hypertension involves immune, metabolic, and neuroendocrine processes and provides new insights into the dysregulation of the central nervous system associated with metabolite levels (Van Arendonk et al., 2023). The cerebellum is a specific brain region that TRD activates to maintain balance and coordinate random movements; however, its synergistic relationship with other brain regions is not fully understood. The cerebellar adrenomedullin (AM) is involved in regulating blood pressure. Dysregulation of cerebellar earthworm AM, its receptor components, and AM signaling pathway occurs during hypertension (Figueira and Israel, 2017). Connections between the cerebellum and the seed points may reveal the cerebellum's role in blood pressure regulation (Zheng et al., 2016).

Both the olfactory bulb and striatum, which are extensions of the telencephalon, were activated in the TRD group. It projects many nerve fibers to the hypothalamus. It is closely associated with the amygdala, pyriform nucleus, and hypothalamus's ventral medial and posterior nuclei, which are involved in the regulation of cardiovascular function (Edwards et al., 1993). Significantly enhanced norepinephrine transmission in the asymmetric olfactory bulb of hypertensive rats may contribute to hypertension development, maintenance, and progression (Cassinotti et al., 2018). Guil supported the correlation between olfactory bulb regulation in chronically elevated blood pressure and the powerful effects of endothelin (Guil et al., 2019). Abnormalities in the brain's dopamine system contribute to the progression of hypertension (Watanabe et al., 1997). Sawamura found high levels of dopamine in the striatum and that extracellular dopamine levels in the striatum correlated with variations in blood pressure, suggesting that dopamine in the striatum is engaged in the progression of 2 K-1C hypertension and that the striatum may be the region engaged in the development of hypertension (Sawamura and Nakada, 1996). We found that the TRD group co-activated the corpus callosum with the TUR group. In the traditional pathological sense, memory impairment and epilepsy often occur with corpus callosum lesions, and psychiatric abnormalities and limb dysfunction can occur. The corpus callosum has complex connections with the insula and limbic and paralimbic regions of the cerebral hemispheres (Shah et al., 2021). The microstructural integrity of the corpus callosum is associated with overall cognition, and appropriate blood pressure treatment may delay these changes and cause concomitant cognitive dysfunction (Gons et al., 2012). We found that the corpus callosum was activated, which may be an effect of the acupuncture technique to prevent further development of hypertension and slow the development of cerebral small vessel disease.

In the correlation analysis, we found a high negative correlation between blood pressure and brain function after acupuncture intervention, indicating that TRRM can activate the functions of brain regions related to blood pressure regulation and play an important role in lowering blood pressure.

This study has some limitations. First, we explored the central mechanism of TRRM in decreasing blood pressure from the perspective of rs-fMRI; however, we did not conduct deeper molecular mechanism research. In the future, we will investigate the relevant mechanisms from a multi-omics perspective. Second, we used only one index, systolic blood pressure, to assess changes in blood pressure in rats. Multiple indices should be used in future studies to obtain a more objective evaluation. Third, under the premise of meeting the experimental requirements (FJalal et al., 2015; Chen et al., 2017, 2021; Li et al., 2018; Jiang et al., 2022), we referred to the relevant literature and only performed the fMRI scans of each group of rats after the experimental acupuncture intervention, and did not perform the fMRI scans of each group of rats before the acupuncture intervention, which might make the experimental data incomplete. In future experiments, we will perform brain scans of each group of rats at baseline to further improve the rigor of the experimental design.

5. Conclusion

Our results showed that TRD, TRF, and TUR decreased blood pressure, with TRD having a greater effect than TRF or TUR. The three acupuncture treatments activated different target brain regions related to blood pressure regulation and enhanced the functional connection between the hypothalamus and brain regions relevant to blood pressure. This finding clarifies how the three acupuncture manipulations produce different anti-hypertensive effects. In addition, brain regions involved in vision, motor control, cognition, and hearing were activated. Activation of these regions may help prevent or reduce the development and progression of hypertensive brain damage and its complications.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of the Beijing University of Chinese Medicine (BUCM-4-2021040804-2140).

Author contributions

Y-YL participated in the experimental procedures and wrote, and revised the manuscript. J-PL participated in blood pressure measurements and graphing of the article. S-FS and K-ZY translated the manuscript. YG, JS, QX, and X-LW analyzed the data. Q-GL and MX designed and directed the experiment participated in reviewing and revising the article and provided funding. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1161578/full#supplementary-material

References

Calhoun, D. A., Booth, J. N., Oparil, S., Irvin, M. R., Shimbo, D., Lackland, D. T., et al. (2014). Refractory hypertension: determination of prevalence, risk factors, and comorbidities in a large, population-based cohort. *Hypertension* 63, 451–458. doi: 10.1161/HYPERTENSIONAHA.113.02026

Carey, R. M., Moran, A. E., and Whelton, P. K. (2022). Treatment of hypertension: a review. *JAMA* 328, 1849–1861. doi: 10.1001/jama.2022.19590

Cassinotti, L. R., Guil, M. J., Schöller, M. I., Navarro, M. P., Bianciotti, L. G., and Vatta, M. S. (2018). Chronic blockade of brain endothelin receptor type-a (ETA) reduces blood pressure and prevents catecholaminergic overactivity in the right olfactory bulb of DOCA-salt hypertensive rats. *Int. J. Mol. Sci.* 19:660. doi: 10.3390/ijms19030660

Chan, S. H., and Chan, J. Y. (2014). Brain stem NOS and ROS in neural mechanisms of hypertension. *Antioxid. Redox Signal.* 20, 146–163. doi: 10.1089/ars.2013.5230

Chen, H., Dai, J., Zhang, X., Wang, K., Huang, S., Cao, Q., et al. (2013). Hypothalamus-related resting brain network underlying short-term acupuncture treatment in primary hypertension. *Evid. Based Complement. Alternat. Med.* 2013:808971. doi: 10.1155/2013/808971

Chen, V. C., Hsu, T. C., Chen, L. J., Chou, H. C., Weng, J. C., and Tzang, B. S. (2017). Effects of taurine on resting-state fMRI activity in spontaneously hypertensive rats. *PLoS One* 12:e0181122. doi: 10.1371/journal.pone.0181122

Chen, Y., Zhao, Y., Tan, R. Y., Zhang, P. Y., Long, T., Shi, Y., et al. (2021). The influence of stomach Back-Shu and front-mu points on insular functional connectivity in functional dyspepsia rat models. *Evid. Based Complement. Alternat. Med.* 2021:2771094. doi: 10.1155/2021/2771094, 14

Cheng, Y., Sun, D., Zhu, B., Zhou, W., Lv, C., Kou, F., et al. (2020). Integrative metabolic and proteomic profiling of the brainstem in spontaneously hypertensive rats. *J. Proteome Res.* 19, 4114–4124. doi: 10.1021/acs.jproteome.0c00585

Edwards, D. A., Nahai, F. R., and Wright, P. (1993). Pathways linking the olfactory bulbs with the medial preoptic anterior hypothalamus are important for intermale aggression in mice. *Physiol. Behav.* 53, 611–615. doi: 10.1016/0031-9384(93)90162-9

Fang, J., Wang, D., Zhao, Q., Hong, Y., Jin, Y., Liu, Z., et al. (2015). Brain-gut Axis modulation of acupuncture in functional dyspepsia: a preliminary resting-state fcMRI study. *Evid. Based Complement. Alternat. Med.* 1:11. doi: 10.1155/2015/860463

Figueira, L., and Israel, A. (2017). Cerebellar adrenomedullinergic system. Role in cardiovascular regulation. *Adv. Exp. Med. Biol.* 956, 541–560. doi: 10.1007/5584_2016_48

FJalal, F. Y., Yang, Y., Thompson, J. F., Roitbak, T., and Rosenberg, G. A. (2015). Hypoxia-induced neuroinflammatory white-matter injury reduced by minocycline in SHR/SP. *J. Cereb. Blood Flow Metab.* 35, 1145–1153. doi: 10.1038/jcbfm.2015.21

Flack, J. M., and Adekola, B. (2020). Blood pressure and the new ACC/AHA hypertension guidelines. *Trends Cardiovasc. Med.* 30, 160–164. doi: 10.1016/j.tcm.2019.05.003

Gold, S. M., Dziobek, I., Rogers, K., Bayoumy, A., McHugh, P. F., and Convit, A. (2005). Hypertension and hypothalamo-pituitary-adrenal axis hyperactivity affect frontal lobe integrity. *J. Clin. Endocrinol. Metab.* 90, 3262–3267. doi: 10.1210/jc.2004-2181

Gons, R. A., Van Oudheusden, L. J., De Laat, K. F., Van Norden, A. G., Van Uden, I. W., Norris, D. G., et al. (2012). Hypertension is related to the microstructure of the corpus callosum: the RUN DMC study. *J. Alzheimers Dis.* 32, 623–631. doi: 10.3233/JAD-2012-121006

Goshgarian, C., and Gorelick, P. B. (2019). Perspectives on the relation of blood pressure and cognition in the elderly. *Trends Cardiovasc. Med.* 29, 12–18. doi: 10.1016/j.tcm.2018.05.011

Gu, D., He, J., Coxson, P. G., Rasmussen, P. W., Huang, C., Thanataveerat, A., et al. (2015). The cost-effectiveness of low-cost essential antihypertensive medicines for hypertension control in China: a modelling study. *PLoS Med.* 12:e1001860. doi: 10.1371/journal.pmed.1001860

Guil, M. J., Schöller, M. I., Cassinotti, L. R., Biancardi, V. C., Pitra, S., Bianciotti, L. G., et al. (2019). Role of endothelin receptor type a on catecholamine regulation in the olfactory bulb of DOCA-salt hypertensive rats: hemodynamic implications. *Biochim. Biophys. Acta Mol. basis Dis.* 1865:165527. doi: 10.1016/j.bbadis.2019.08.003

Guo, Q., Liu, Q., Sun, D., and Nie, B. (2018). Twirling reinforcing-reducing manipulation-central mechanism underlying anti-hypertensive effect on spontaneous hypertension in rats. *J. Tradit. Chin. Med.* 38, 391–398. doi: 10.1016/S0254-6272(18)30629-0

Han, Q. Y., and Liang, H. S. (2021). Progress of researches on mechanisms of acupuncture therapy for lowering blood pressure. *Zhen Ci Yan Jiu* 46, 700–706. doi: 10.13702/j.1000-0607.20210369

Huang, H., Yue, X., Huang, X., Long, W., Kang, S., Rao, Y., et al. (2022). Brain activities responding to acupuncture at ST36 (zusanli) in healthy subjects: a systematic review and meta-analysis of task-based fMRI studies. *Front. Neurol.* 13:930753. doi: 10.3389/fneur.2022.930753

Jiang, Y., Liu, D. F., Zhang, X., Liu, H. G., Zhang, C., and Zhang, J. G. (2022). Modulation of the rat hippocampal-cortex network and episodic-like memory performance following entorhinal cortex stimulation. *CNS Neurosci. Ther.* 28, 448–457. doi: 10.1111/cns.13795

Kearney, P. M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P. K., and He, J. (2005). Global burden of hypertension: analysis of worldwide data. *Lancet* 365, 217–223. doi: 10.1016/S0140-6736(05)17741-1

Kim, H., Mawla, I., Lee, J., Gerber, J., Walker, K., Kim, J., et al. (2020). Reduced tactile acuity in chronic low back pain is linked with structural neuroplasticity in primary somatosensory cortex and is modulated by acupuncture therapy. *NeuroImage* 217:116899. doi: 10.1016/j.neuroimage.2020.116899

Ko, S. J., Park, K., Kim, J., Kim, M., Kim, J. H., Lee, J., et al. (2016). Effect of acupuncture and its influence on cerebral activity in functional dyspepsia patients: study protocol for a randomized controlled trial. *Trials* 17:183. doi: 10.1186/s13063-016-1296-2

Li, J., Yang, R., Xia, K., Wang, T., Nie, B., Gao, K., et al. (2018). Effects of stress on behavior and resting-state fMRI in rats and evaluation of Telmisartan therapy in a stress-induced depression model. *BMC Psychiatry* 18:337. doi: 10.1186/s12888-018-1880-y

Liang, J., Wu, J., Zhang, X., Hao, X., Zeng, T., Sun, J., et al. (2021). Proteomics analysis of the hypothalamus in spontaneously hypertensive rats treated with twirling reinforcing manipulation, twirling reducing manipulation or electroacupuncture. *Exp. Ther. Med.* 21:381. doi: 10.3892/etm.2021.9812

Liang, P., Xiang, J., Liang, H., Qi, Z., and Li, K. (2014). Altered amplitude of low-frequency fluctuations in early and late mild cognitive impairment and Alzheimer's disease. *Curr. Alzheimer Res.* 11, 389–398. doi: 10.2174/1567205011666140331225335

Liu, X., Wang, S., Zhang, X., Wang, Z., Tian, X., and He, Y. (2014). Abnormal amplitude of low-frequency fluctuations of intrinsic brain activity in Alzheimer's disease. *J. Alzheimers Dis.* 40, 387–397. doi: 10.3233/JAD-131322

Lu, S., Cao, X., Ohara, H., Nakamura, Y., Izumi-Nakaseko, H., Ando, K., et al. (2015). Common parameters of acupuncture for the treatment of hypertension used in animal models. *J. Tradit. Chin. Med.* 35, 343–348. doi: 10.1016/s0254-6272(15)30108-4

Lu, J., Guo, Y., Guo, C. Q., Shi, X. M., Du, N. Y., Zhao, R. L., et al. (2017). Acupuncture with reinforcing and reducing twirling manipulation inhibits hippocampal neuronal apoptosis in spontaneously hypertensive rats. *Neural Regen. Res.* 12, 770–778. doi: 10.4103/1673-5374.206648

Luo, X., Huang, J., Yu, J., and Tang, C. (2019). Effect of Taichong (LR 3) acupuncture in spontaneously hypertensive rats. *J. Tradit. Chin. Med.* 39, 74–80. doi: 10.19852/j.cnki.jtcm.2019.01.009

Malpas, S. C. (2010). Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol. Rev.* 90, 513–557. doi: 10.1152/physrev.00007.2009

Nishijima, H., Haga, R., Suzuki, C., and Tomiyama, M. (2015). Asymmetric posterior reversible encephalopathy syndrome due to hypertensive encephalopathy. *Intern. Med.* 54, 993–994. doi: 10.2169/internalmedicine.54.3762

Oparil, S., and Schmieder, R. E. (2015). New approaches in the treatment of hypertension. *Circ. Res.* 116, 1074–1095. doi: 10.1161/CIRCRESAHA.116.303603

Qin, C., Li, J., and Tang, K. (2018). The Paraventricular nucleus of the hypothalamus: development, function, and human diseases. *Endocrinology* 159, 3458–3472. doi: 10.1210/en.2018-00453

Reeve, E., Jordan, V., Thompson, W., Sawan, M., Todd, A., Gammie, T. M., et al. (2020). Withdrawal of antihypertensive drugs in older people. *Cochrane Database Syst. Rev.* 6:CD012572. doi: 10.1002/14651858

Sawamura, T., and Nakada, T. (1996). Role of dopamine in the striatum, renin-angiotensin system and renal sympathetic nerve on the development of two-kidney, one-clip Goldblatt hypertension. *J. Urol.* 155, 1108–1111. doi: 10.1016/S0022-5347(01)66401-2

Shah, A., Jhawar, S., Goel, A., and Goel, A. (2021). Corpus callosum and its connections: a fiber dissection study. *World Neurosurg.* 151, e1024–e1035. doi: 10.1016/j.wneu.2021.05.047

Shan, X., Qiu, Y., Pan, P., Teng, Z., Li, S., Tang, H., et al. (2020). Disrupted regional homogeneity in drug-naive patients with bipolar disorder. *Front. Psych.* 11:825. doi: 10.3389/fpsyt.2020.00825

Sun, R., He, Z., Ma, P., Yin, S., Yin, T., Liu, X., et al. (2021). The participation of basolateral amygdala in the efficacy of acupuncture with deqi treating for functional dyspepsia. *Brain Imaging Behav.* 15, 216–230. doi: 10.1007/s11682-019-00249-7

Van Arendonk, J., Neitzel, J., Steketee, R. M. E., Van Assema, D. M. E., Vrooman, H. A., Segbers, M., et al. (2023). Diabetes and hypertension are related to amyloid-beta burden in the population-based Rotterdam study. *Brain* 146, 337–348. doi: 10.1093/brain/awac354

Wang, J. J., Chen, X., Sah, S. K., Zeng, C., Li, Y. M., Li, N., et al. (2016). Amplitude of low-frequency fluctuation (ALFF) and fractional ALFF in migraine patients: a resting-state functional MRI study. *Clin. Radiol.* 71, 558–564. doi: 10.1016/j.crad.2016.03.004

Wang, X. R., Yang, J. W., Ji, C. S., Zeng, X. H., Shi, G. X., Fisher, M., et al. (2018). Inhibition of NADPH oxidase-dependent oxidative stress in the rostral Ventrolateral medulla mediates the antihypertensive effects of acupuncture in spontaneously hypertensive rats. *Hypertension* 71, 356–365. doi: 10.1161/HYPERTENSIONAHA.117.09759

Watanabe, Y., Fujita, M., Ito, Y., Okada, T., Kusuoka, H., and Nishimura, T. (1997). Brain dopamine transporter in spontaneously hypertensive rats. *J. Nucl. Med.* 38, 470–474.

Wu, J., Zeng, T., Liang, J., Zhang, X., Xie, Q., Lv, T., et al. (2021). Effects of different acupuncture manipulations on protein expression in the parietal cortex of spontaneously hypertensive rats. *J. Tradit. Chin. Med. Sci.* 8, 257–264. doi: 10.1016/j.jtcms.2021.07.009

Yang, L., Yan, Y., Wang, Y., Hu, X., Lu, J., Chan, P., et al. (2018). Gradual disturbances of the amplitude of low-frequency fluctuations (ALFF) and fractional ALFF in Alzheimer Spectrum. *Front. Neurosci.* 12:975. doi: 10.3389/fnins.2018.00975

Yang, J. W., Ye, Y., Wang, X. R., Li, F., Xiao, L. Y., Shi, G. X., et al. (2017). Acupuncture attenuates renal sympathetic activity and blood pressure via beta-adrenergic receptors in spontaneously hypertensive rats. *Neural. Plast.* 2017:8696402. doi: 10.1155/2017/8696402, 9

Yang, K., Zhang, P., Lv, T., Wu, J., and Liu, Q. (2022). Acupuncture at Taichong and Zusanli points exerts hypotensive effect in spontaneously hypertensive rats by metabolomic analysis. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 1207:123352. doi: 10.1016/j.jchromb.2022.123352

Zeng, F., Qin, W., Ma, T., Sun, J., Tang, Y., Yuan, K., et al. (2012). Influence of acupuncture treatment on cerebral activity in functional dyspepsia patients and its relationship with efficacy. *Am. J. Gastroenterol.* 107, 1236–1247. doi: 10.1038/ajg.2012.53

Zhang, J., Cai, X., Wang, Y., Zheng, Y., Qu, S., Zhang, Z., et al. (2019). Different brain activation after acupuncture at combined acupoints and single Acupoint in hypertension patients: an Rs-fMRI study based on ReHo analysis. *Evid. Based Complement. Alternat. Med.* 2019:5262896. doi: 10.1155/2019/5262896

Zhang, J., Lyu, T., Yang, Y., Wang, Y., Zheng, Y., Qu, S., et al. (2021a). Acupuncture at LR3 and KI3 shows a control effect on essential hypertension and targeted action on cerebral regions related to blood pressure regulation: a resting state functional magnetic resonance imaging study. *Acupunct. Med.* 39, 53–63. doi: 10.1177/0964528420920282

Zhang, S., Wang, X., Yan, C. Q., Hu, S. Q., Huo, J. W., Wang, Z. Y., et al. (2018). Different mechanisms of contralateral- or ipsilateral-acupuncture to modulate the brain activity in patients with unilateral chronic shoulder pain: a pilot fMRI study. *J. Pain Res.* 1, 505–514. doi: 10.2147/JPR.S152550

Zhang, J., Zhang, Y., Hu, L., Huang, X., Liu, Y., Li, J., et al. (2021b). Global trends and performances of magnetic resonance imaging studies on acupuncture: a Bibliometric analysis. *Front. Neurosci.* 14:620555. doi: 10.3389/fnins.2020.620555

Zhang, M., Zhu, Y., Wang, J., Li, Y., and Hua, Z. (2022). Association between acupuncture and grade 1 hypertension: a systematic review and meta-analysis. *Complement. Ther. Clin. Pract.* 49:101649. doi: 10.1016/j.ctcp.2022.101649

Zheng, Y., Zhang, J., Wang, Y., Wang, Y., Lan, Y., Qu, S., et al. (2016). Acupuncture decreases blood pressure related to hypothalamus functional connectivity with frontal lobe, cerebellum, and insula: a study of instantaneous and short-term acupuncture treatment in essential hypertension. *Evid. Based Complement. Alternat. Med.* 2016:6908710. doi: 10.1155/2016/6908710

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Bibliometric analysis of acupuncture and moxibustion treatment for mild cognitive impairment

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Objective: This study aims to analyze the current research status of acupuncture in the treatment of mild cognitive impairment (MCI) using bibliometric methods, explore current research hotspots, and predict future research trends.

Methods: Literature on acupuncture for MCI in China National Knowledge Infrastructure (CNKI) and Web of Science (WOS) databases were searched from their inception to December 31, 2022. Articles were then filtered using inclusion and exclusion criteria and imported into VOSviewer 1.6.11 and CiteSpace 6.1.6msi software for descriptive analysis of publication numbers, network analysis of author/institution collaborations, and cluster analysis of keywords, as well as analysis of keyword emergence and linear relationships with time.

Results: The Chinese and English databases included 243 and 565 relevant articles, respectively. The overall volume of Chinese and English literature was stable, with the annual volume generally increasing. In terms of countries, institutions, and authors, China had the highest number of English-language publications; however, the number of joint publications among institutions/authors was low. Research institutions were independent and dispersed, with no collaborative teams formed around a single institution/author. The hotspots in Chinese literature were “needling, treatment, electric acupuncture, nimodipine, cognitive training” and other clinical research directions. The hotspots in English literature were "acupuncture, electro-acupuncture, Alzheimer’s disease, dementia, cognitive impairment, memory, vascular dementia, mild cognitive impairment, stroke, hippocampus, injury," and other mechanisms of action.

Conclusion: The popularity of acupuncture for MCI is increasing year by year. Acupuncture for MCI, along with cognitive training, can help improve cognitive function. "Inflammation" is the frontier of acupuncture for MCI research. In the future, strengthening effective communication and cooperation among institutions, especially international cooperation, is essential for conducting high-quality research on acupuncture for MCI. This will help obtain high-level evidence and improve the output and translation of research results.

KEYWORDS

acupuncture and moxibustion, mild cognitive impairment, CiteSpace, VOSviewer, knowledge map, visual analysis

1. Introduction

Mild cognitive impairment (MCI) is a transitional state between normal aging and dementia, characterized by cognitive decline and memory loss in some patients (Tangalos and Petersen, 2018). The prevalence of MCI in adults over 60 years of age is approximately 6.7 to 25.2% (Jongsiriyanyong and Limpawattana, 2018) and increases with age. Acupuncture stimulation of corresponding acupoints can improve blood circulation (Yuan et al., 2022) and help balance Yin and Yang while dredging meridians, thereby intervening in patients' cognitive function and promoting cognitive recovery (Kuang et al., 2021). Recently, traditional Chinese medicine (TCM) acupuncture has made significant progress in treating MCI (Liu et al., 2023). With increasing research in this field, some reviews have discussed acupuncture treatment for MCI, but no scholars have conducted a systematic analysis of the profile, hotspots, and frontier of acupuncture treatment using visual analysis.

Bibliometrics refers to the interdisciplinary science that employs quantitative mathematical and statistical methods (Chen et al., 2021). It is a comprehensive knowledge system that integrates mathematics, statistics, and philology while emphasizing quantification. In particular, the application of information visualization technology and methods can intuitively display the research development process, current research status, research hotspots, and development trends. CiteSpace and VOSviewer are the most widely used software tools for literature information visualization.

VOSviewer (van Eck and Waltman, 2010) and CiteSpace (Li et al., 2022) are visual analysis software developed by Professor Van Eck of Leiden University in the Netherlands and Professor Chen Chaomei of Drexel University in the United States, respectively. VOSviewer primarily deconstructs the relationships of elements to be analyzed by distance, while CiteSpace focuses on graphics and connections, showing the strength of the relationships among the analyzed elements. The main features of both are their rich graphical presentations and clear displays, making the results of bibliometric analyses easy to interpret.

This study, based on China National Knowledge Infrastructure (CNKI) and Web of Science (WOS) databases, applies bibliometric methods and uses CiteSpace and VOSviewer software to visually analyze literature information. This approach is more intuitive and comprehensive than traditional reviews and clinical research, as it identifies the research hotspots of acupuncture and moxibustion treatment for MCI and provides insights for future research.

2. Data and methods

2.1. Literature sources and data retrieval strategy

For Chinese literature in the CNKI database, the search formula is: SU = (''针'+'针灸'+'灸'+'电针'+'毫针'+'火针'+'腕踝针'+'眼针'+'揿针'+'蜂针'+'舌针'+'腹针'+'耳针'+'头针'+'体针'+'针法'+'艾灸') AND SU = ('轻度认知障碍'+'轻度认知损伤'+'轻度认 知损害 + 'MCI' + 'SCD'). For English literature in the WOS database, the search formula is: TS = (acupuncture OR pharmacopuncture) AND TS = (mild cognitive impairment OR Cognitive Dysfunctions* OR Cognitive Impairment* OR Cognitive Disorder* OR Mild Cognitive Impairment OR Cognitive Decline* OR Mental Deterioration*). The search period ranges from the inception of the databases to December 31, 2022.

2.2. Literature inclusion and exclusion criteria

Inclusion criteria for Chinese literature: (1) literature source: CNKI; (2) literature on acupuncture for MCI. Exclusion criteria: duplicate papers, conference papers, scientific and technological achievements, and newspaper literature.

Inclusion criteria for English literature: (1) literature source: WOS; (2) literature on acupuncture treatment for MCI. Exclusion criteria: duplicate papers, conference papers, scientific and technological achievements, and newspaper documents.

2.3. Literature screening method

Two researchers independently screened the literature by reading the titles and abstracts. They excluded literature that did not meet the criteria and performed a cross-check. For any divergent literature, the decision was made through discussion.

2.4. Visual analysis

2.4.1. Literature data extraction

The screened Chinese literature is exported from CNKI in Refworks format, named as "download##.txt" for source data processing; the filtered English literature is exported in

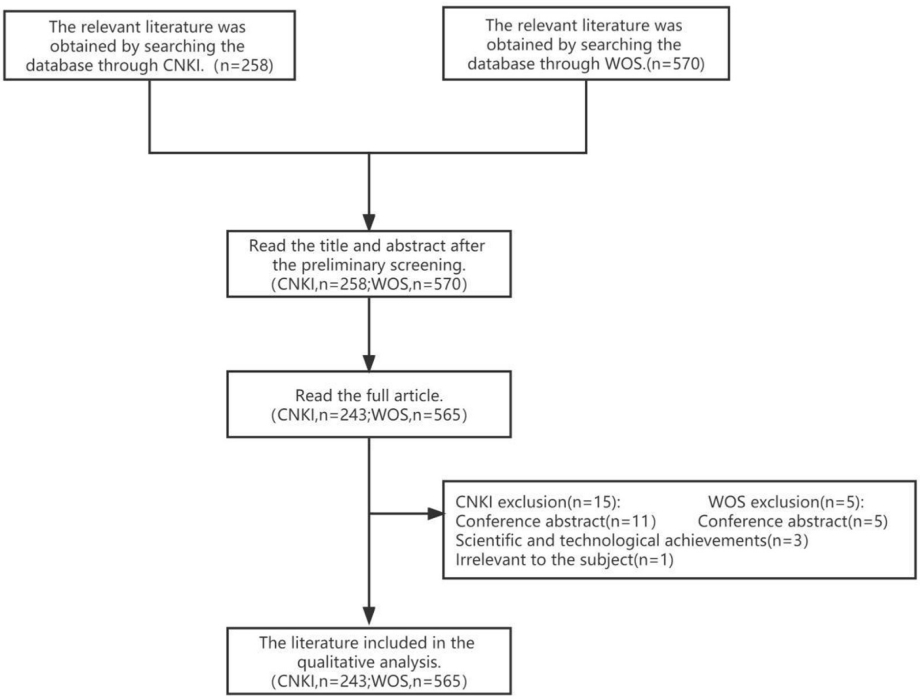

FIGURE 1
Flow chart of literature screening.

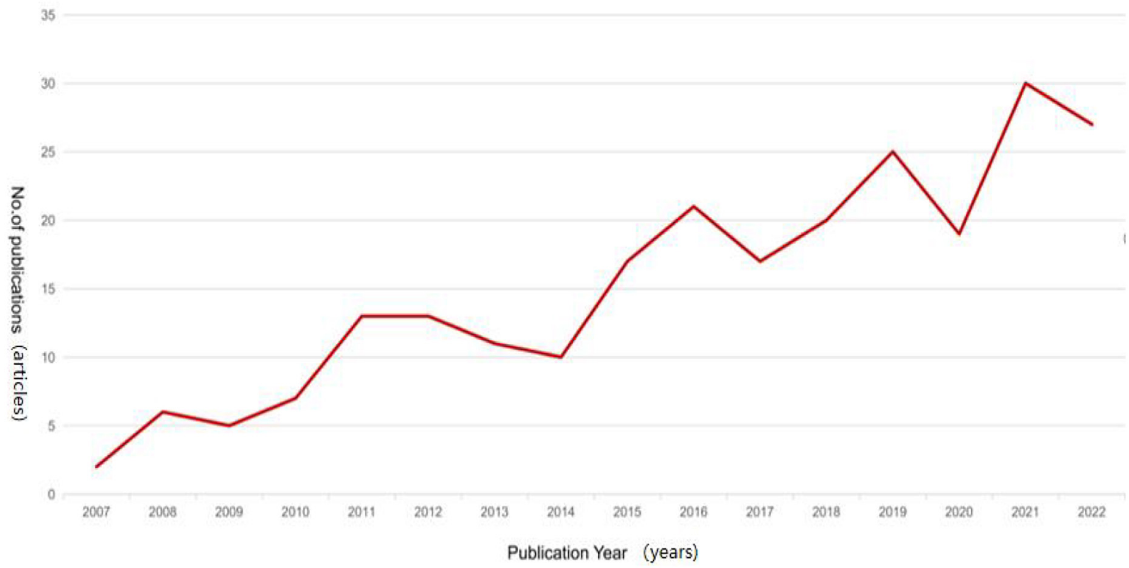

FIGURE 2
Trend chart of the annual publications of Chinese literature.

"plain text" file format and named as "download##.txt" for source data processing. The data to be analyzed include literature titles.

2.4.2. Visual software

Chinese literature is primarily analyzed using CiteSpace-6.1.6msi, while English literature employs CiteSpace-6.1.6msi and VOSviewer 1.6.11 software.

2.4.3. Literature cluster analysis

Variables such as authors, institutions, and keywords are extracted, followed by cluster analysis. The Chinese literature keyword cluster uses the LLR algorithm module in CiteSpace-6.1.6msi to draw the visual map of MCI keyword analysis for acupuncture treatment. In the keyword cluster analysis of English literature, the three visualization modules provided by VOSviewer—network visualization, overlay visualization, and density visualization—are selected for cluster analysis to generate the keyword cluster map.

2.4.3.1. Network visualization

Circles and labels of an element represent its size, which depends on the node degree, connection strength, citations, etc. The element's color represents its cluster, with different clusters in different colors. Through the view, one can examine each individual cluster, discover research hotspots through thematic co-occurrence, research communities through author collaboration, and similarities and differences between scholars on research topics through author coupling networks.

2.4.3.2. Overlay visualization

Nodes are assigned different colors based on the score or color (red, green, blue) fields in the map file. By default, the average year of the keyword is used for color mapping.

2.4.3.3. Density visualization

Each point on the map is filled with a color according to the density of the surrounding elements. Higher density areas are closer to red, while lower density areas are closer to blue. Density size depends on the number of elements in the surrounding region and the importance of these elements. Density view can be used to quickly observe important areas and the density of knowledge fields and studies.

CiteSpace provides two metrics, Modularity (Q value) and Weighted Mean Silhouette (S value), which serve as a basis for judging the mapping's effectiveness. Generally, a Q value in the [0,1] range, $Q > 0.3$, indicates that the structure of the delineated associations is significant. An S value of 0.7 indicates that the clustering is efficient and convincing. If it is above 0.5, the clustering is usually considered reasonable.

TABLE 1 Top 10 magazines in terms of publications.

Publication	Number of literature (articles)	Proportion (%)
Shanghai Acupuncture magazine	11	6.92
Chinese acupuncture	7	4.40
A Clinical Journal of Traditional Chinese Medicine	7	4.40
The Chinese Journal of Gerontology	6	3.77
Clinical Journal of Acupuncture	6	3.77
Journal of Anhui University of Traditional Chinese Medicine	5	3.15
New traditional Chinese medicine	5	3.15
The Chinese Journal of Traditional Chinese Medicine	4	2.52
Asia Pacific Traditional Medicine	4	2.52
Yunnan Journal of Traditional Chinese Medicine	4	2.52

2.4.4. Investigator cooperative network visualization analysis

In the research section, Price's Law (Wang et al., 2022) $N=0.749 \times \sqrt{max}$ is used to determine the number of core author publications.

2.4.5. Visualization of social network analysis graphs

In the visual social network analysis diagram, the size of each node represents the frequency of the analyzed variable; the edge represents the connection between variables; two variables appearing together in the same document will have an edge; the thickness of the line between nodes indicates the strength of the association; different colors indicate different clusters.

2.4.6. Keyword timeline atlas analysis

The timeline view shows the keywords according to time, indicating the hot evolution and stage characteristics of keywords in the field (Qi et al., 2020), we can analyze the frequency and growth rate of keywords and their clusters from the perspective of time and explore the hot research issues to predict the development trend (Wei et al., 2021). Keywords of the same cluster were placed on the same horizontal line, with the time corresponding to the keywords positioned at the top of the view. In the time line map, the more keywords there are, the more important the clustering field is. The size of the circle in the figure represents the key word. The larger the circle, the higher the frequency number.

2.4.7. Keyword emergence analysis

Keyword emergence is a rapid increase in the frequency of keywords within a certain period (Yan et al., 2020; Gao et al., 2021). The blue lines in the figure indicate the start and end time of the keywords, while the red lines indicate the time period from the keyword emergence to the end.Click "Burstness" in the control panel to detect emerging words, and click "Refresh" to calculate the number of upcoming words. If the number of non-emergent words or emergent words is too small, the value of Y [0,1] can be reduced until sufficient number of emergent words are obtained.

3. Results

A total of 258 Chinese documents were retrieved from CNKI, and 243 of them were included after literature screening. Additionally, 570 English documents were retrieved from WOS, and 565 of them were selected for further analysis (**Figure 1**).

3.1. Visual analysis results of the Chinese literature

3.1.1. Statistics on the number of articles published

Among the 243 selected Chinese articles, the first one was "30 cases of mild cognitive impairment treated by the combination of the Yuanluotongjing acupuncture method and oral administration of An Lishen" published in the Chinese Journal of Chinese

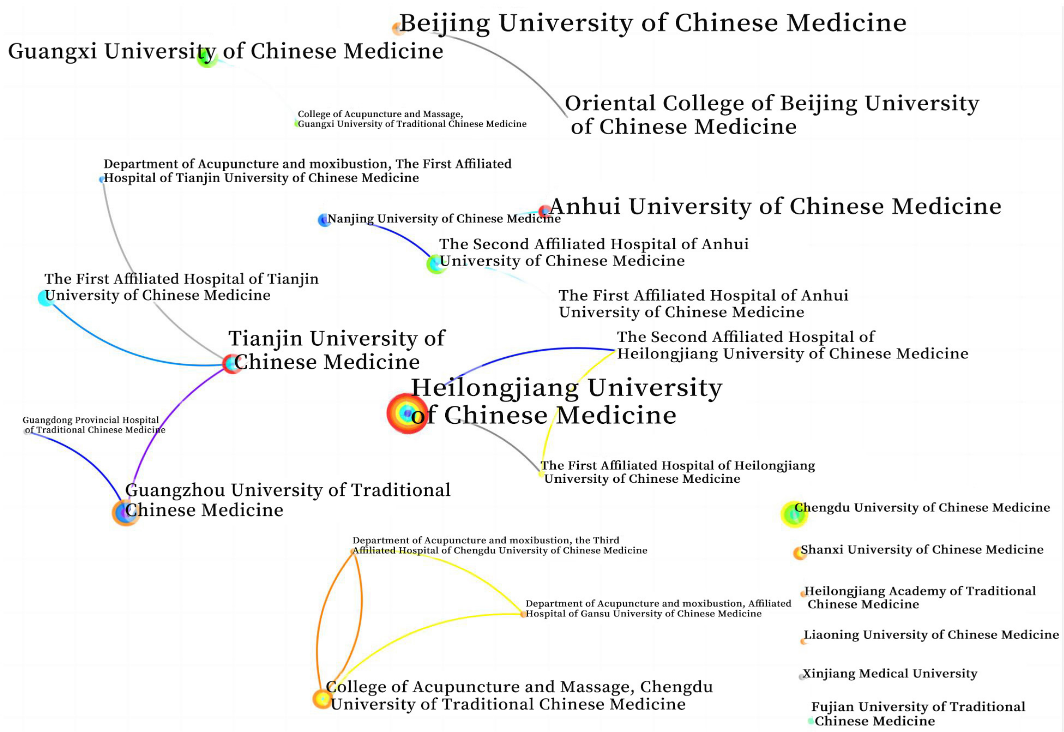

FIGURE 3
Network analysis atlas of the research institution (frequency ≥ 3 articles).

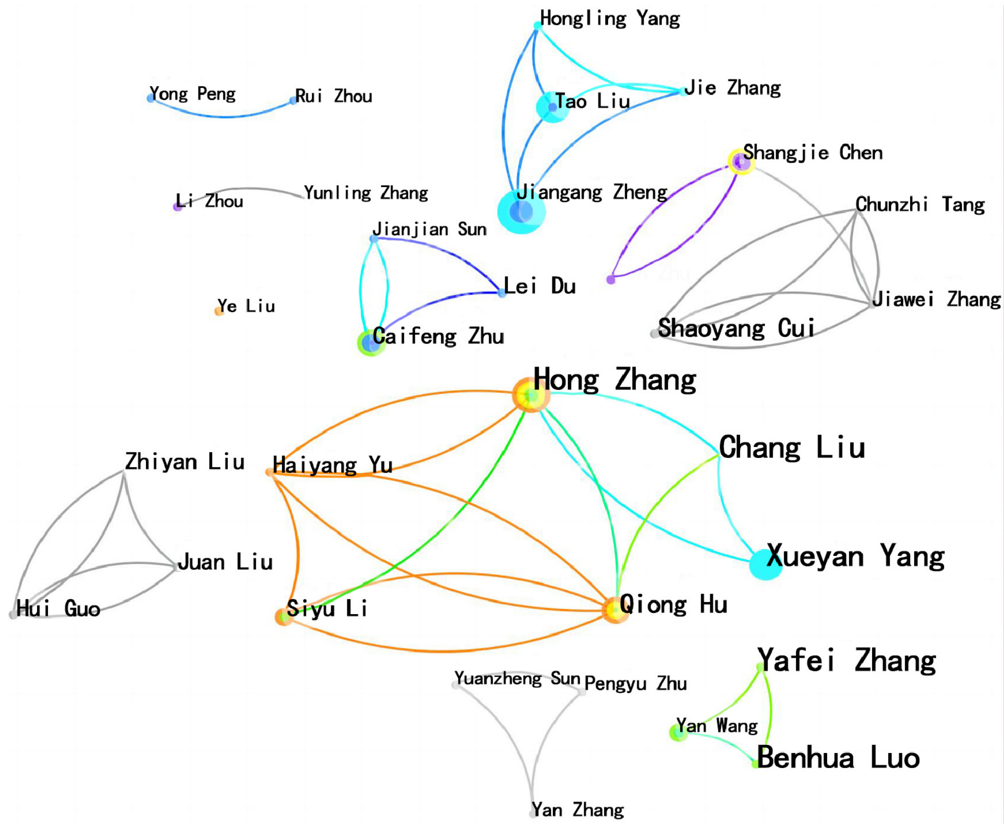

FIGURE 4
Network analysis atlas of the author (frequency ≥ 3 times).

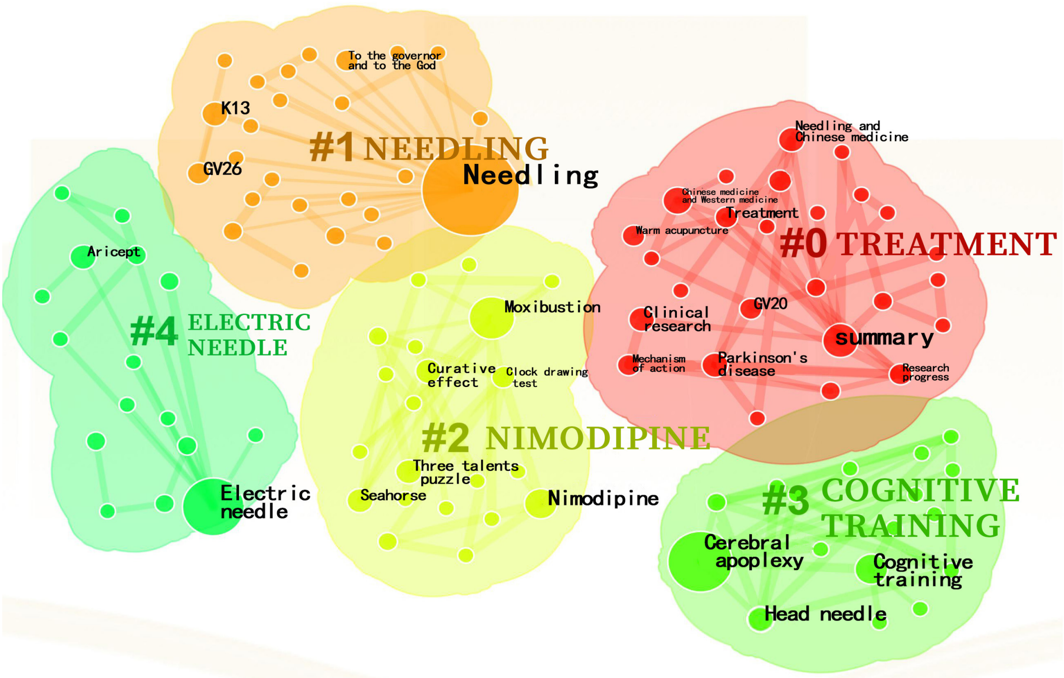

FIGURE 5
CNKI keyword cluster map (frequency ≥ 3 times).

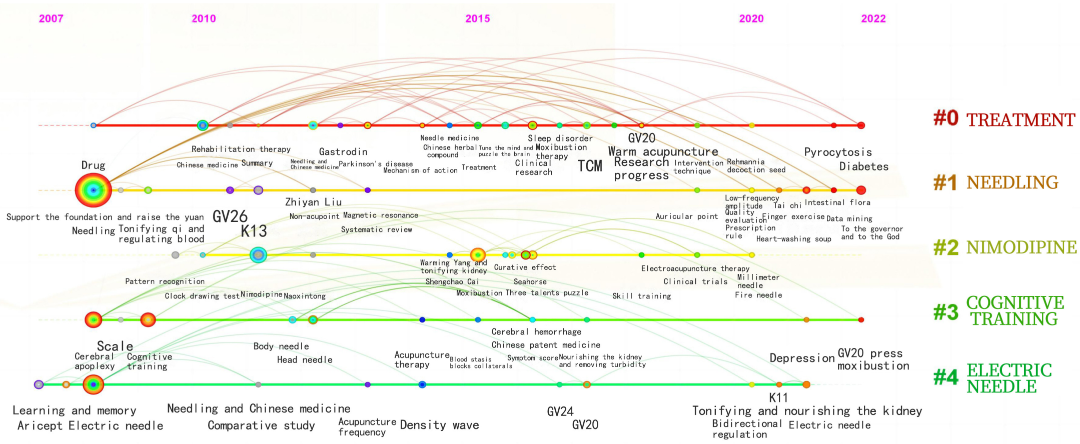

FIGURE 6
Chinese literature keyword time line chart.

Medicine Science and Technology in 2007. According to Figure 2, there was an overall upward trend in the number of annual publications from 2007 to 2022. The lowest number of publications was in 2007, with two articles, while the highest number of publications was in 2021, with 30 articles (Figure 2).

In terms of publication types, academic journals accounted for the majority of articles (159 articles), followed by doctoral and master's degree theses (84 articles). The top three journals in terms of number of publications were the Shanghai Acupuncture Journal (6.918%), Chinese Acupuncture (4.348%), and Clinical Journal of Chinese Medicine (4.348%). The top 10 journals in terms of number of publications are shown in Table 1. Approximately 24.28% of the articles were published in the above-metioned 10 journals, which mainly focused on traditional Chinese medicine, acupuncture and moxibustion, and traditional Chinese pharmacy, etc.

3.1.2. Research institution network analysis

Network analysis of the research institutions showed that there were 204 nodes, 188 edges, and a density of 0.0091. The top five institutions with the largest number of articles are: Heilongjiang University of Chinese Medicine (25 articles), Guangzhou University of Chinese Medicine (17 articles), Tianjin University of Chinese Medicine (15 articles), Chengdu University

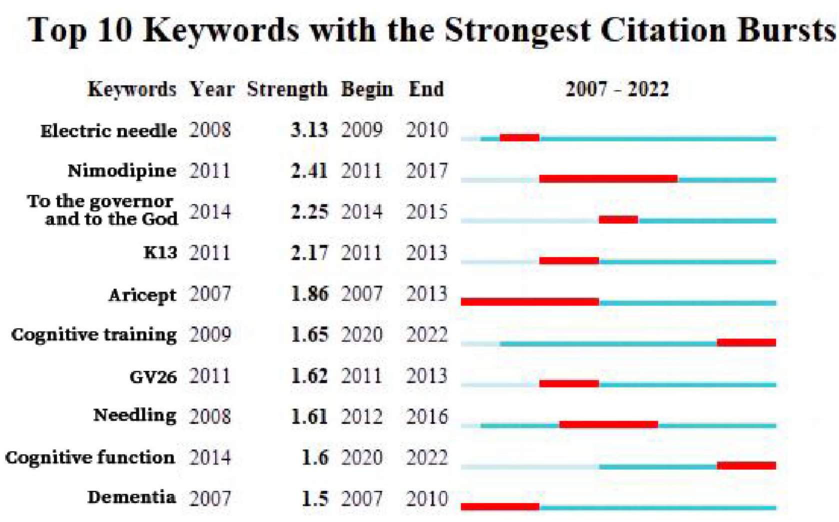

FIGURE 7
Present map of CNKI keywords.

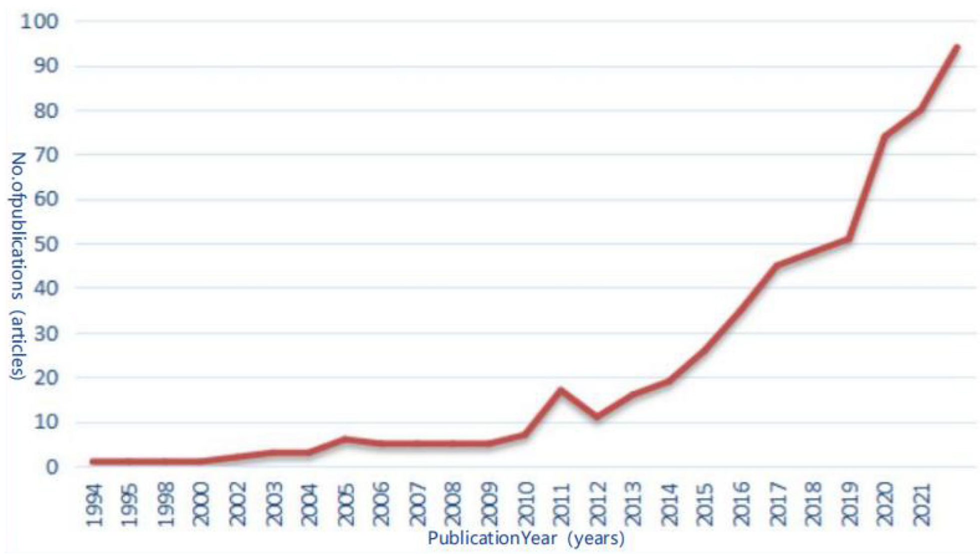

FIGURE 8
Trends in the annual number of articles published in English.

of Chinese Medicine (13 articles), and Guangxi University of Chinese Medicine (11 articles). Among them, 25 institutions had three or more publications, accounting for 12% of the total number of institutions. The relationship of the published literature network of each research team is shown in Figure 3, indicating that various institutions have little cooperation in MCI research, mainly independent research, and most of them are traditional Chinese medicine universities.

3.1.3. Author partnership network analysis

Network analysis with authors as nodes yielded 339 nodes, 604 edges and a density of 0.0106. Among the included literature, 33 authors had more than 3 publications, accounting for 10% of the total number of authors. The top 5 authors in terms of publications were Zhang Hong, Hu Qiong, Zheng Jiangang, Zhu Caifeng, and Chen Shangjie, with Zhang Hong having the highest number of publications at 13. A network visualisation of these author collaborations (Figure 4) suggests a relatively fragmented group of authors, with the larger being a core group of authors centred on Zhang Hong.

3.1.4. Keyword analysis

Through cluster analysis of Chinese literature keywords, Q value = 0.8382, S value = 0.9673, a total of 5 class keywords were identified, mainly related to needling, treatment, electric acupuncture, nimodipine, cognitive training (Figure 5). Based on the cluster map, a timeline was constructed (Figure 6). The earliest keywords was "aricept", which were studied for a long period of

TABLE 2 Top 20 journals in terms of number of articles published.

Publication	Number of literature (articles)	Proportion (%)
Medicine	38	6.73
Evidence Based Complementary and Alternative Medicine	35	6.20
Trials	21	3.72
Acupuncture in Medicine	18	3.19
Frontiers in Aging Neuroscienced	15	2.66
BMC Complementary and Alternative Medicine	12	2.12
Neural Regeneration Research	11	1.95
Cochrane Database of Systematic Reviews	10	1.77
Frontiers in Neurology	9	1.59
Journal of Alternative and Complementary Medicine	9	1.59
Neural Plasticity	8	1.42
PLOS One	8	1.42
World Journal of Acupuncture Moxibustion	8	1.42
BMJ Open	7	1.24
Journal of Acupuncture and Tuina Science	7	1.24
Explore the Journal of Science and Healing	6	1.06
Frontiers in Psychology	6	1.06
European Journal of Integrative Medicine	5	0.89
Frontiers in Neuroscience	5	0.89
Journal of Nervous and Mental	5	0.89

TABLE 3 Top 10 countries in terms of number of articles published.

Country	Number of literature (articles)
Peoples R China	328
USA	118
South Korea	51
Canada	20
Australia	16
England	16
Germany	15
Taiwan	15
Italy	8
Italy	6

time, with the research focus mainly on “treatment”, “needling”, “cognitive training” and “electric acupuncture”.

3.1.5. Keyword emergent analysis

Emergent analysis of keywords in CNKI Chinese literature generated 10 emergent words (Figure 7). Among them, the earliest emergence time was “aricept” and “dementia”, which appeared in 2007. The keywords “nimodipine” and “aricept” had a longer emergence time, while “electric needle” had the strongest intensity, with an intensity of 3.13. The keywords “Cognitive training” and “Cognitive function” are still being studied today.

3.2. Visual analysis of literature based on Web of Science

3.2.1. Statistics on the number of articles published

The first relevant paper was "TENSION-TYPE HEADACHE - PSYCHOSOMATIC CLINICAL-ASSESSMENT AND TREATMENT" published by BIONDI, M, an Italian, in the journal Psychotherapy and Psychosomatics in 1994. Over the past 30 years, the number of articles published on the topic of acupuncture and moxibustion for MCI has increased year by year. From 1994 to 2010, the number of articles on the topic of acupuncture for MCI began to increase each year, but the trend was slow. From 2012 to 2022, the number of articles increased significantly over the 10-year period, with a rapid increase in the number of articles on topics related to acupuncture for MCI starting in 2019 (Figure 8).

The type of literature was dominated by original research (66%), followed by review articles (30%). The top three journals in terms of number of publications were Medicine (38 articles, 6.73%), Evidence based complementary and alternative medicine (35 articles, 6.20%), and Trials (21 articles, 3.72%). The top 20 journals in terms of number of publications are shown in Table 2. Nearly half (43.01%) of the literature was published in these 20 journals in the areas of neuroscience, Chinese medicine, acupuncture and moxibustion, complementary alternative medicine, psychiatry, and cell biology.

3.2.2. Visual analysis of the network of issuing countries/regions and issuing institutions

The top ten countries in terms of number of publications are shown in Table 3, with China being the country with the highest number of publications. An analysis of country/region cooperation in published literature is shown in Figure 9, and there is a need to strengthen cooperation between countries. The top 12 research institutions in terms of number of publications are shown in Table 4, with 77 of these academic institutions being from China. The Capital Medical University holds the top position with 47 publications. However, there are few international collaborations on MCI research in the mapping of institutional collaboration networks, with only one collaboration between Capital Medical University and the Toyo Institute in Korea (Figure 10).

3.2.3. Researcher posting volume and network visualization analysis

A total of 2693 authors were involved in the 565 publications, with 12 authors having $\geq$ 10 publications, all from China. The authors with the highest number of publications are Liu, Cun Zhi and Chen, Lidian, with each author having published 20 articles. According to Price's law $N \approx 3$, the authors with 3 publications are the core authors in the field. The number of authors with three publications was 162. Using VOSviewer to plot the density view

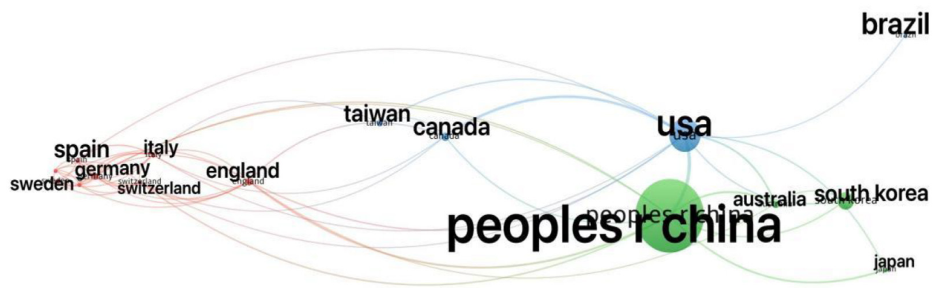

FIGURE 9
Analysis of country/regional cooperation in published literature.

of the core author collaboration network (Figure 11), six author groups were formed, most of which consisted of 5-6 authors, but the fusion component of each author group was low, suggesting that there were fewer links between the teams.

3.2.4. Visual analysis of keyword clustering

The keyword-based social network analysis is shown in Figure 12, with the main keyword clusters grouped into three broad categories:

The red area is cluster 1, with core cluster terms such as acupuncture, auricular acupuncture, cognitive behavioural therapy, randomised controlled, double-blind, efficacy, meta-analysis, anxiety, and depression, etc. The clustering themes are clinical treatments common to acupuncture for MCI, research methods, and MCI for concomitant diseases.

Cluster 2, the green area, has core terms such as electroacupuncture, cognitive impairment, memory, mechanism, stroke, vascular dementia, hippocampus, apoptosis, inflammation, oxidative stress, rat models, etc. The theme is research on mechanisms related to acupuncture for MCI in animal experiments.

TABLE 4 Academic institutions with published literature.

Name of Institution	Number of literature (articles)
Capital Medical University	47
Beijing University of Chinese Medicine	45
Guangzhou University of Chinese Medicine	35
Fujian University of Traditional Chinese Medicine	29
Tianjin University of Traditional Chinese Medicine	26
Korea Institute of Oriental Medicine KIOM	25
University of Hong Kong	21
Kyung Hee University	19
Shanghai University of Traditional Chinese Medicine	19
Harvard University	18
China Academy of Chinese Medical Sciences	16
Zhejiang Chinese Medical University	16

Cluster 3, the yellow area, has core terms such as dementia, Alzheimer's disease, mild cognitive impairment, systematic review, etc. The theme is MCI-related diseases and popular research trends.

A temporal analysis of social networks based on keywords shows that acupuncture appears earlier and more frequently than MCI, and that research on acupuncture for Alzheimer's disease or other neurological disorders predates research on acupuncture for MCI (Figure 13).

3.2.5. Analysis of keyword emergence

The keyword emergence analysis of the WOS English literature generated 11 emergent terms (Figure 14). "Cognitive therapy" was the first (1994), strongest (4.19), and longest lasting keyword (20 years) to emerge. This was followed by "controlled trials", "double-blind" "anxiety" and "rat", which emerged with a relatively long duration. "Long-term enhancement" and "dentate gyrus" began to appear in 2017 and lasted for 2 years. "Cognitive impairment" and "memory" are words that have emerged in recent years but not for long. "Inflammation" emerged in 2020 and continues today, and is considered to be at the forefront of research on acupuncture for MCI.

4. Discussion

4.1. Acupuncture for MCI research requires greater collaboration between institutions and researchers

Studies have shown that the conversion rate of MCI patients to dementia is between 8 and 15% (Jin et al., 2022). It is of great significance to prevent and treat AD during the MCI Stage. Acupuncture, as a traditional Chinese medicine treatment, has the advantages of being safe, convenient and effective, making it a potential future trend for MCI treatment.

The analysis of the number of articles published reflects the main trends in this research area (Cheng et al., 2006). The results of this study show a significant increase in Chinese literature since 2014 and a significant increase in the English literature in WOS since 2012, suggesting a gradual increase in research interest. Studies in the English literature were mainly conducted in China, emphasizing the promotion of the advantages of acupuncture for MCI worldwide.

FIGURE 10
Social network analysis graph for academic institutions with published literature.

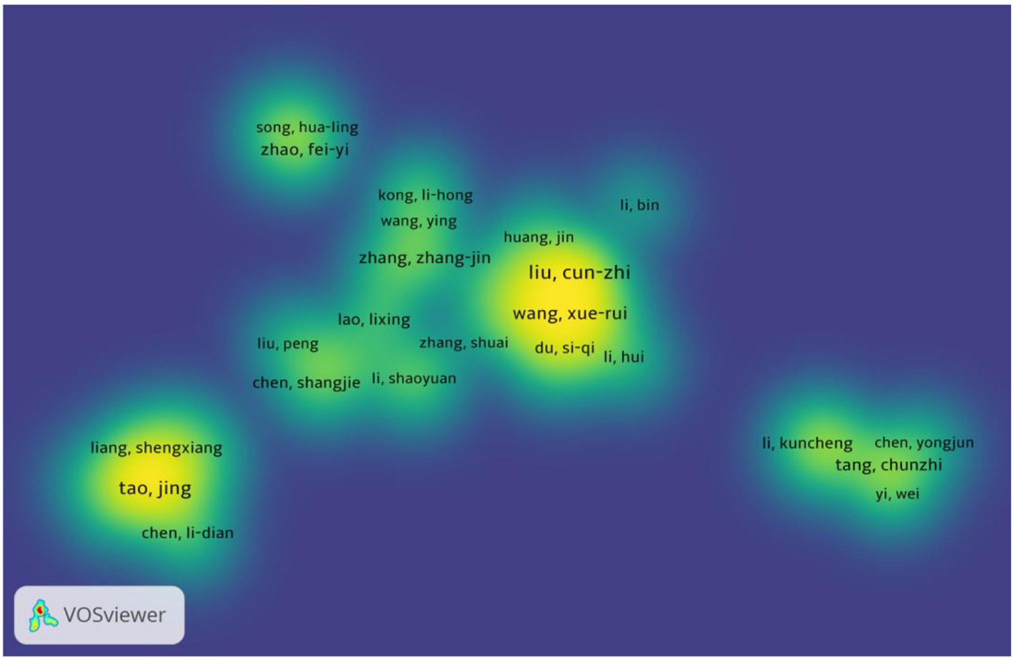

FIGURE 11
Analysis of the core author collaboration network.

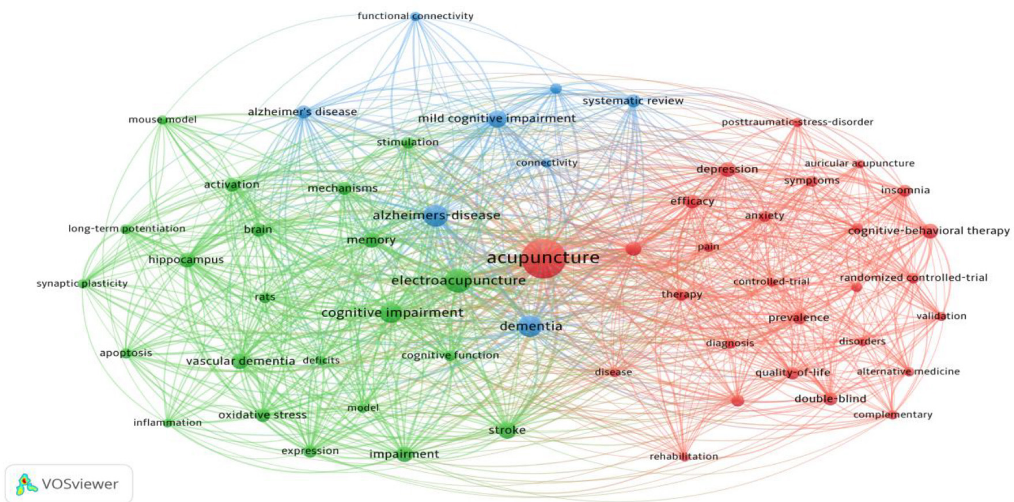

FIGURE 12
Keyword clustering map.

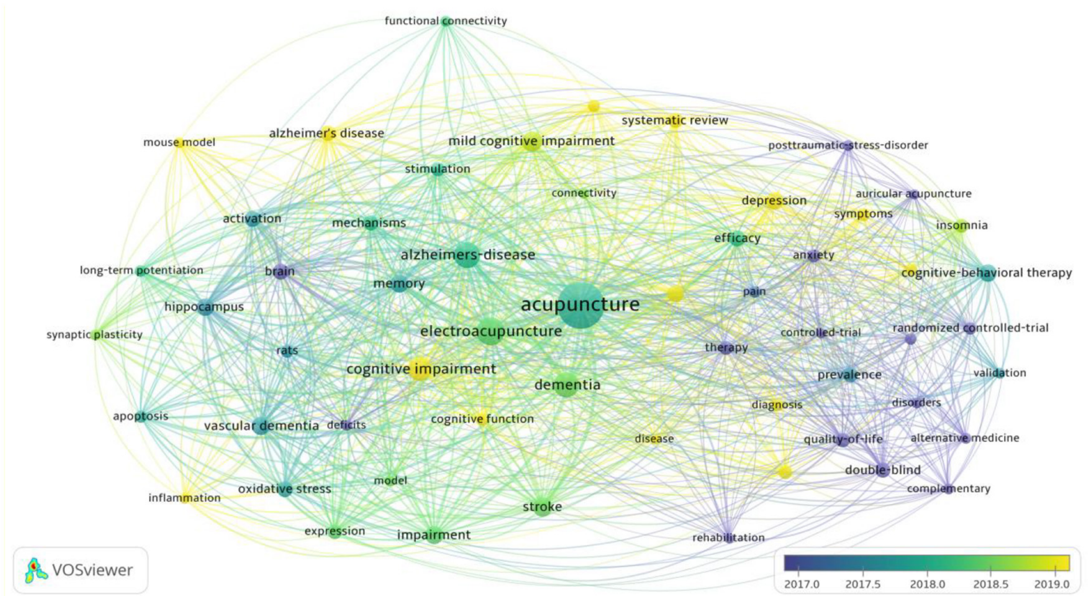

FIGURE 13
Social network timing diagram for keywords.

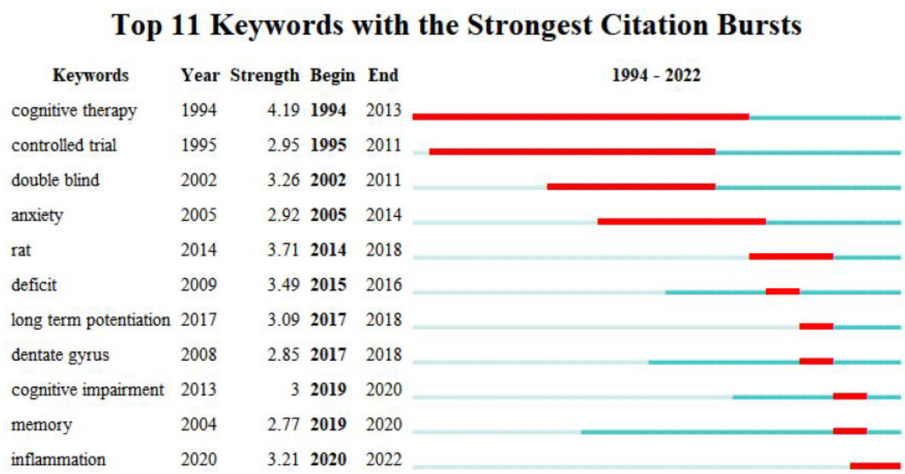

FIGURE 14
Keyword emergence map of English literature.

However, analysis of institutional/author social networks showed that there were few joint studies within China or internationally, the researcher teams were relatively independent and mainly from various TCM universities, the types of studies were mostly single-centre small-sample clinical studies, and differences in the selection of representative samples, sample sizes, and interventions all contributed to the limitations of the studies to some extent. In recent years, as the status of acupuncture and moxibustion in international medicine has increased, scholars in more and more countries have focused on the effectiveness of acupuncture and moxibustion in the treatment of diseases (Su and Wang, 2021). Future research will require greater collaboration not only between research teams within the country, but also between international institutions. To do this, efforts can be made in two ways: The first is to give full play to the guiding role of the government and various academic groups to provide policy and project support for acupuncture and moxibustion treatment in MCI, to expand the scope of cooperation in different disciplines, specialties and geographical areas, and to encourage multi-centre, high-quality clinical research, so as to form a rich and stable cooperative network of researchers and research institutions in this field as soon as possible. The second is to build international or domestic academic exchange platforms, create opportunities for cooperation through increased exchanges, encourage more researchers to participate in research in this field, and continue to make deeper and more detailed and solid research results, so as to strengthen the core group of researchers in this field. Multi-centre, large sample and high quality clinical research is the future trend.

Strengthening cooperation between different countries, institutions and scholars to achieve complementary advantages, information sharing and resource sharing will make the research content and findings more in-depth and scientific and help create more high quality evidence.

4.2. Differences in research hotspots in the English and Chinese literature on acupuncture and moxibustion for MCI

Keywords reflect the focus of literature, with high frequency keywords representing research hotspots and trends to some extent (Zhou et al., 2022). Visual analysis of keywords in Chinese literature showed a focus on needling, treatment, electric acupuncture, nimodipine and cognitive training, with a primary emphasis on clinical research on acupuncture for MCI. In contrast, analysis of keywords in the English literature showed apoptosis, inflammation, and oxidative stress as core keywords, indicating a focus on research results in the mechanism of action category. Future publications should focus on the publication of clinical research findings in international journals.

Analysis of the keyword timeline and emergence revealed that needling, treatment, electric acupuncture, cognitive training were popular areas of research, while nimodipine were relatively popular areas of research. Studies in the Chinese literature from 2007 to 2010 focus on needling and electroacupuncture for MCI, with studies after 2011 refining acupuncture and moxibustion for MCI and the use of medication in combination therapy. In 2020, studies began to focus on acupuncture and moxibustion with cognitive training therapy for cognitive impairment, which remains a hot research topic. Emergent analysis of Keywords in English literature suggests a shift in focus from clinical randomized controlled trials to transgenic mouse animal studies between 1994 and 2022. Researchers shifting their research focus to animal studies based on clinical studies to explore and confirm structural pathological changes in brain function, mechanistic exploration, and associated factors in the treatment of MCI with acupuncture through animal studies, but there is still a lack of evidence from high-quality clinical studies.

4.3. International hotspots and trends in the mechanism of action of acupuncture and moxibustion for MCI

Keyword analysis suggests that acupuncture and moxibustion mechanisms in the treatment of MCI is a current research hotspot (Zhang et al., 2013; Petersen, 2016). Analysis of the published literature on acupuncture and moxibustion mechanisms in treating MCI (Li et al., 2021; Yin et al., 2023)suggests that acupuncture and moxibustion is closely related to the neurotoxic mechanism of Aβ, the mechanism of oxygen free radical damage, the mechanism of cytokine-induced inflammatory response, and the mechanism of cerebral blood perfusion (Yang et al., 2021). Acupuncture can improve cognition by modulating neurotransmitter levels, neuroinflammatory response, and regulating the expression of related genes and brain proteins.

Some studies have shown that electroacupuncture at the "GV20" and "ST36" acupoints can inhibit the inflammatory response mediated by the NLRP3/Caspase-1 signaling pathway, reduce the serum levels of IL-1β, IL-6, IL-18, and TNF-α, down-regulate the protein expression levels of NLRP3, ASC, Caspase-1, GSDM-D, IL-1β, and IL-18 in hippocampal tissues, thereby inhibiting the scorching of hippocampal neuronal cells and effectively improving the learning and memory abilities of SAMP8 mice (Li, 2022). Electroacupuncture at the Baihui and Renyu acupoints was found to inhibit the hyperphosphorylation of Tau protein, as evidenced by the observed downregulation of p-38MAPK and p-tau Thrl81 protein expressions in the hippocampus of rats (Zhang et al., 2015). The "Sancai Puzzle" moxibustion method can also improve the cognitive function and reduce the levels of serum Aβ1-42, Tau, and P-tau in a MCI patients, thus inhibiting the phosphorylation level and improving their learning and memory ability (Wang et al., 2021). Acupuncture and moxibustion treatment for MCI can have a calming effect on the brain by maintaining cognitive function and increasing the connection between the heart, kidneys, and brain. Cholinergic neurons are crucial for the hippocampal loop of learning and memory in the brain, and their damage leads to dementia. Acupuncture and moxibustion can improve cognitive impairment by improving the abnormal state of the cholinesterase system and regulating synaptic plasticity in the hippocampus (Yan et al., 2015; Song et al., 2017). Henan Sun found that the plasma Ach levels in the observation group were significantly higher than those in the control group before and after treatment, and the MMSE and HDS scale scores were positively correlated with plasma Ach levels (Sun et al., 2018).

“Inflammation", a keyword that emerged in 2020 and continues to be used today, is currently the main focus of research in acupuncture and moxibustion for MCI. The pathogenesis of MCI and AD is multifaceted, with the presence of neuroinflammation thought to be one of the driving factors that affect the progression of normal ageing to MCI and MCI to AD. Chronic inflammation of the central nervous system is considered a key factor in the progression of neurodegenerative diseases. Age-related neuroinflammation is characterized by increased levels of pro-inflammatory cytokines, such as interleukin (IL)-1β, as suggested by Norden and Godbout (2013). Acupuncture and moxibustion can improve learning and memory by modulating TNF-α, IL-1β, and IL-6 in the frontal cortex and reducing the damage to brain tissue caused by inflammatory factors. This inflammatory state may result from the activation of glial cells and astrocytes in the ageing brain (Feng et al., 2018) which coincides with an increase in systemic inflammation, that may trigger neuroinflammation, further impairing cognitive abilities (Anderson, 2022).

4.4. Study limitations

The study had limitations as it only searched two databases, CNKI and WOS, and may not have included all relevant studies in this field of acupuncture for MCI. Thus, the results of the analysis have some limitations, but they can still reflect the current research status and future trends in this field to a certain extent. It is hoped that subsequent analysis will cover a wider range of literature and provide more Comprehensive references and evidence for future studies on the hotspots and trends of acupuncture and moxibustion for MCI.

5. Conclusion

In summary, this article presents a visualization and analysis of relevant literature in the field of acupuncture and moxibustion for MCI in the past 30 years using CiteSpace and VOSviewer software. The study suggests that acupuncture and moxibustion for mild cognitive impairment is becoming increasingly popular each year, that acupuncture and moxibustion in conjunction with cognitive training may help improve cognitive function, and that "inflammation" is at the forefront of acupuncture and moxibustion research for MCI. Follow-up studies are needed to strengthen effective communication and cooperation among institutions, especially international cooperation, to conduct more high-quality studies on acupuncture and moxibustion for MCI and improve the output and translation of scientific results.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

WY and XL contributed to conception and design of the study. ZL organized the database. CL performed the statistical analysis. XZ wrote the first draft of the manuscript. ML and YL wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Anderson, R. C. (2022). Can probiotics mitigate age-related neuroinflammation leading to improved cognitive outcomes? *Front. Nutr.* 9:1012076. doi: 10.3389/fnut.2022.1012076

Chen, D., Zhang, G., Wang, J., Chen, S., Wang, J., Nieand, H., et al. (2021). Mapping trends in moyamoya angiopathy research: a 10-year bibliometric and visualization-based analyses of the web of science core collection (WoSCC). *Front. Neurol.* 12:637310. doi: 10.3389/fneur.2021.637310

Cheng, H., Zhang, X., Sun, T., and Huang, G. (2006). Quantitative analysis of ontology research papers based on Web of Science. *MLIT* 1, 46–50.

Feng, Q., Shang, H., Liu, M., Lin, Z., Qiu, L., Zang, Y., et al. (2018). Electrical on learning and memory ability and inflammatory factors in brain tissue in rats with vascular cognitive impairment. *CTCMIM* 25, 39–43.

Gao, M., Gao, D., Sun, H., Cheng, X., An, L., and Qiao, M. (2021). Trends in research related to premenstrual syndrome and premenstrual dysphoric disorder from 1945 to 2018: a bibliometric analysis. *Front. Public Health* 9:596128. doi: 10.3389/fpubh.2021.596128

Jin, Y., Hu, F., and Zhu, J. (2022). Exploration of acupuncture therapy in the treatment of mild cognitive impairment based on the brain-gut axis theory. *Front. Hum. Neurosci.* 16:891411. doi: 10.3389/fnhum.2022.891411

Jongsiriyanyong, S., and Limpawattana, P. (2018). Mild cognitive impairment in clinical practice: a review article. *Am. J. Alzheimers Dis. Other Demen.* 33, 500–507. doi: 10.1177/1533317518791401

Kuang, X., Fan, W., Hu, J., Wu, L., Yi, W., Lu, L., et al. (2021). Acupuncture for post-stroke cognitive impairment: a systematic review and meta-analysis. *Acupunct. Herb. Med.* 39, 577–588. doi: 10.1177/09645284211009542

Li, J., Mao, Y., Ouyang, J., and Zheng, S. (2022). A Review of urban microclimate research based on citeSpace and VOSviewer analysis. *Int. J. Environ. Res. Public Health.* 19:4741. doi: 10.3390/ijerph19084741

Li, M., Wang, H., Wang, W., and Wang, Z. (2021). Progress in the neurobiological mechanisms of acupuncture to improve cognitive impairment after sleep deprivation. *TSJA* 40, 1162–1166.

Li, X. (2022). *Study on the mechanism of the improvement of mild cognitive impairment in SAMP8 mice through the pyroptosis signaling pathway in NLRP3/Caspase-1 cells.* Harbin: Heilongjiang University of Traditional Chinese Medicine. doi: 10.27127/d.cnki.ghlzu.2022.000004

Liu, Y., Chen, F., Qin, P., Zhao, L., Li, X., Han, J., et al. (2023). Acupuncture treatment vs. cognitive rehabilitation for post-stroke cognitive impairment: a systematic review and meta-analysis of randomized controlled trials. *Front. Neurol.* 14:1035125. doi: 10.3389/fneur.2023.1035125

Norden, D. M., and Godbout, J. P. (2013). Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol. Appl. Neurobiol.* 39, 19–34. doi: 10.1111/j.1365-2990.2012.01306.x

Petersen, R. C. (2016). Mild cognitive impairment. *Continuum* 22, 404–418. doi: 10.1212/CON.0000000000000313

Qi, B., Jin, S., Qianand, H., and Zou, Y. (2020). Bibliometric analysis of chronic traumatic encephalopathy research from 1999 to 2019. *Int. J. Environ. Res. Public Health* 17:5411. doi: 10.3390/ijerph17155411

Song, C. M., Huang, J., Lin, B., Yang, M., Zhang, X., Liu, W., et al. (2017). Effect of electroacupuncture at Baihui and ting on learning and memory ability and synaptic ultrastructure of rats in CA1 region of hippocampus after cerebral ischemia and reperfusion. *RTPC* 23, 750–755.

Su, M., and Wang, S. (2021). rogress in acupuncture treatment of mild cognitive impairment. *TCM Inform.* 38, 77–81.

Sun, H., Zhu, M., and Zhang, W. (2018). The effect of acupuncture on Baihui and Yongquan point in treating Alzheimer's disease on plasma acetylcholine and β -amyloid protein. *TWTCM* 13, 2855–2857+2861.

Tangalos, E. G., and Petersen, R. C. (2018). Mild cognitive impairment in geriatrics. *Clin. Geriatr. Med.* 34, 563–589. doi: 10.1016/j.cger.2018.06.005

van Eck, N. J., and Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics.* 84, 523–538. doi: 10.1007/s11192-009-0146-3

Wang, H., Li, S., Hu, Q., Yu, H., and Zhang, H. (2021). Effect of moxibustion on memory function and related serum protein markers in amnestic mild cognitive impairment. *Needle-prick Study* 46, 794–799.

Wang, X., Zou, H. O., and Sun, J. (2022). [international research status and trend of community palliative care:a study based on cite space]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 44, 450–457. doi: 10.3881/j.issn.1000-503X.14524

Wei, N., Pan, J., Zhang, Y., Xu, M., and Huang, S. (2021). Visualization analysis based on the CiteSpace vascular depression study. *Mod. J. Integr. Tradit. Chin. West. Med.* 19, 3877–3885.

Yan, Q., Dai, T., Pan, Y., and Chen, C. (2015). Effects of acupuncture points on learning and memory and expression of hippocampal choline acetyltransferase in rats with vascular dementia. *TCJG* 35, 6712–6714.

Yan, W., Zheng, K., Weng, L., Chen, C., Kiartivich, S., Jiang, X., et al. (2020). Bibliometric evaluation of 2000-2019 publications on functional near-infrared spectroscopy. *Neuroimage* 220:117121. doi: 10.1016/j.neuroimage.2020.117121

Yang, Y., Yang, S., Ma, X., Yu, M., and Pan, J. (2021). Acupuncture and moxibustion treatment of mild cognitive impairment based on data mining. *J. Phys. Conf. Ser.* 1881:022066.

Yin, Z., Li, Y., Jiang, C., Xia, M., Chen, Z., Zhang, X., et al. (2023). Acupuncture for mild cognitive impairment: a systematic review with meta-analysis and trial sequential analysis&#13. *Front. Neurol.* 13:1091125. doi: 10.3389/fneur.2022.1091125

Yuan, H. W., Liu, Y. X., Zhang, H., Liu, Y., Liand, X. L., and Ni, J. X. (2022). [Tongdu Xingshen acupuncture and moxibustion combined with cognitive training in treatment of post-stroke mild cognitive impairment: a randomized controlled trial]. *ZGZJ* 42, 839–843. doi: 10.13703/j.0255-2930.20210811-0005

Zhang, H., Zhao, L., Yang, S., Chen, Z., Li, Y., Peng, X., et al. (2013). Clinical observation on effect of scalp electroacupuncture for mild cognitive impairment. *J. Tradit. Chin. Med.* 33, 46–50. doi: 10.1016/s0254-6272(13)60099-0

Zhang, M., Xu, G., Wang, W., Luo, C., Lu, R., and Meng, D. (2015). Regulation of the p38MAPK pathway by electroacupuncture reduces phosphorylated tau expression in the hippocampus of AD rats. *J. Nanjing Univ. Tradit. Chin. Med.* 31, 261–264.

Zhou, F., Zhou, L., Yu, L., Xu, S., and Yang, Y. (2022). Visualization analysis of the current status of theaflavin studies based on CiteSpace. *Tea Sci.* 42, 131–139.

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Management of auricular transcutaneous neuromodulation and electro-acupuncture of the vagus nerve for chronic migraine: a systematic review

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Background: Migraine is a type of primary headache that is accompanied by symptoms such as nausea, vomiting, or sensitivity to light and sound.

Objective: The aim of this study was to conduct a systematic review on the effectiveness of non-invasive neuromodulation, auricular transcutaneous vagus nerve stimulation (at-VNS), and electro-ear acupuncture of the vagus nerve in patients with migraine headaches.

Methods: Six databases were searched from inception to 15 June 2022 for clinical trials, in which at least one group received any form of non-invasive neuromodulation of the vagus nerve for managing migraine with outcomes collected on pain intensity and related disability. Data, including participants, interventions, blinding strategy, outcomes, and results, were extracted by two reviewers. The methodological quality was assessed with the PEDro scale, ROB, and Oxford scale.

Results: The search identified 1,117 publications with nine trials eligible for inclusion in the review. The methodological quality scores ranged from 6 to 8 (mean: 7.3, SD: 0.8) points. Low-quality evidence suggests some positive clinical effects for the treatment of chronic migraine with 1 Hz with at-VNS and ear-electro-acupuncture compared with the control group at post-treatment. Some of the studies provided evidence of the relationship between chronic migraine and a possible positive effect as a treatment with at-VNS and the neurophysiological effects using fMRI. Six of the studies provided evidence using fMRI of the relationship between chronic migraine and a possible positive effect as a treatment with at-VNS and the neurophysiological effects. Regarding all included studies, the level of evidence with the Oxford scale was level 1 (11.17%), six studies were graded as level 2 (66.66%), and two studies were graded as level 3 (22.2%). With the PEDro score, five studies got a low methodological score <5 and only four got a score superior to 5, being highly methodological quality studies. For ROB, most of the studies were high risk and only a few of them received a low risk of bias. The pain intensity, migraine attacks, frequency, and duration were measured by three studies with positive results at post-treatment. And only 7% reported adverse events using at-VNS. All studies reported results at a post-treatment period in their respective main outcomes. And all studies with fMRI provided strong evidence of the relationship between the Locus Coeruleus, Frontal Cortex, and other superior brain areas with the auricular branch of the Vagus nerve with at-VNS.

Conclusion: Some positive effects regarding the effect of non-invasive neuromodulation, auricular transcutaneous vagus nerve stimulation (at-VNS), and electro-ear acupuncture of the vagus nerve on migraine is reported in the current literature, but there are not enough data to obtain strong conclusions.

Systematic review registration: This systematic review was registered in the PROSPERO database (registration number: CRD42021265126).

KEYWORDS

systematic review, migraine, non-invasive neuromodulation, vagus nerve, electro-acupuncture

Introduction

Migraine is a type of primary headache that causes disability, reduces quality of life, and affects more than 1 billion people worldwide each year. The worldwide prevalence of migraine is around 11.6% (Stovner and Andree, 2010; Vetvik and MacGregor, 2017; Woldeamanuel and Cowan, 2017), with the majority of patients being under 50 years of age, and it affects more females than males (Stovner and Andree, 2010). Globally, migraines are the second-most frequent cause of disability, responsible for 16.3% of neurological symptoms and a significant impact on daily living activities (Amiri et al., 2022).

While the actual etiology of migraines is currently unknown, one hypothesis that may explain their development is the convergence of cervical and trigeminal afferents in the trigeminal–cervical nucleus (GBD 2016 Headache Collaborators, 2018; Huang et al., 2020). There are indicators of neurogenic inflammation associated with this primary headache and a hypersensitive immune system related to central sensitization (Grassini and Nordin, 2017). There is evidence for the association of headache pain with alterations in the brainstem nuclei and cortical regions, while migraine headaches involve increased sensory processing within the peripheral and central trigemino-vascular pathways and the relationship between them (Song et al., 2022).

It is likely that the main cause of migraine is the imbalance between excitatory and inhibitory cortical–subcortical neurotransmission. This abnormal interaction between neurons has been termed the phenomenon of "Cortical Diffuse Depression (CSD)", involving the vascular system and the release of inflammatory mediators modulating neuronal activity (de Tommaso et al., 2021).

Recently, the existence of a trigeminovagal complex has been reported as the basis for connections between the trigeminal and vagus systems, as well as its possible connection with CSD, as a possible relationship of this cranial pair with primary headaches. These findings have been confirmed with functional MRI in humans, and vagus nerve stimulation can modulate the trigeminal autonomic reflex through a complex network which includes the hypothalamus, the trigeminal spinal nuclei, the left pontine nucleus, and the parahippocampal gyrus (Henssen et al., 2019a; Möller et al., 2020).

Traditionally, abortive and prophylactic medications are first-line treatments for migraine therapy, with most migraineurs treating their headaches at the onset of symptoms. The principal treatment options are the 5-HT_{1F} receptor, non-steroidal anti-inflammatories (NSAIDs), Calcitonin gene-related peptide (CGRP), and gepants, but these types of treatments may cause side effects with long-term use, such as gastric ulcer disease and chronic kidney diseases, which are not well tolerated and may increase the risk of medication overuse, headaches, allodynia, and dependence (Blech and Starling, 2020). The limitations of current pharmacological therapies have highlighted the need to explore alternative or integrative treatments for migraine.

One potential non-pharmacological approach to the treatment of migraine patients is auricular transcutaneous vagus nerve stimulation (at-VNS), which is commonly used in clinical practice for treating migraine, cluster headache, depression, epilepsy, and other disorders, such as atrial fibrillation, prosocial behavior, associative memory, schizophrenia, or pain (Usichenko et al., 2017; Badran et al., 2018; Kaniusas et al., 2019; Yap et al., 2020). At-VNS is a non-invasive and inexpensive therapy that involves stimulating the auricular branch of the vagus nerve (ABVN) at the outer parts of the ear, conferring autonomic benefits (Badran et al., 2018). One of the main differences between VNS and at-VNS is that patients do not require general anesthesia for its implantation, thus making at-VNS safer than VNS (Usichenko et al., 2017; Badran et al., 2018; Kaniusas et al., 2019; Blech and Starling, 2020; Yap et al., 2020).

The former method is also more expensive and riskier than at-VNS, with costs ranging from USD 30,000 to USD 50,000 (Usichenko et al., 2017; Badran et al., 2018; Blech and Starling, 2020; Yap et al., 2020). Magnetic resonance imaging (MRI) has recently shown anatomical paths for performing at-VNS, with examples including through the neck and auricula, and acupuncture points in the ear, as they are a connection between the nervous system and the external parts of the body (Rong et al., 2012; Badran et al., 2018; Kaniusas et al., 2019; Zhang et al., 2019). Although the physiological effects of at-VNS on the brain have not yet been fully elucidated, and studies are not homogeneous in their results due to the high risk of bias and unclear parameters, stimulation intensity, pulse width, waveform, or frequency and acupuncture points selection, there still exists a path between the auricular and neck branches to the superior brain areas related to at-VNS (Badran et al., 2018; Zhang et al., 2019). On the other hand, at-VNS and electro-acupuncture have analgesic effects with low frequency in various pain models in humans and rodents. Additionally, the common parameters of frequency are between 1 and 20 Hz, and have a high relevance as a treatment for migraineurs using at-VNS (Ellrich, 2006; Feng et al., 2022; Sacca et al., 2022). Other studies obtained findings about a neurophysiological effect between at-VNS and the auricular branch of the vagus nerve related to superior brain areas with the parameters 500 µs ad 25 Hz, which connects cortical effects that may provide findings for future research. However, it is necessary to understand the possible changes with the proper stimulation. In recent years, findings about the relationship between parasympathetic innervation of the vagus nerve and superior areas of the brainstem, such as the locus coeruleus, nucleus tractus solitarious, and trigeminal spinal tract, have been identified. This provides strong evidence regarding the main role of at-VNS for the management of the pathophysiology of migraine (Simon and Blake, 2017; Henssen et al., 2019b; Zhang et al., 2019). Findings have been made regarding four core areas, namely autonomic nervous system function, inhibition of cortical spreading depression (CSD), neurotransmitter regulation, and nociceptive modulation, as a complex mechanism for the treatment of migraines (Silberstein, 2020).

The growing literature and findings encourage the use of at-VNS as an effective technique for the treatment of migraine, and may have the same mechanisms as auricular acupuncture, applied in the auricular region of the vagus nerve (Usichenko et al., 2017; Hamer and Bauer, 2019).

Currently, there are no systematic reviews of non-invasive auricular vagus nerve electrical neuromodulation (at-VNS) and auricular electro-acupuncture in the treatment of chronic migraine, so the purpose of the following systematic review is to assess the efficacy and quality of studies of at-VNS and electro-acupuncture in the auricular region of the vagus nerve for the treatment of chronic migraine (He et al., 2012; Tobaldini et al., 2019).

Methods

A systematic review following the PRISMA statement (Page et al., 2021) was conducted. This systematic review was registered in the PROSPERO database (registration number: CRD42021265126).

Search strategy

The electronic databases CINALH, MEDLINE, PUBMED, PEDro, and EMBASE were searched by a reviewer up to June of 2022. Search strategies for each database were based on PICO, and eligible studies were included with a population suffering from migraine (acute or chronic) who had been treated with n-NVS, auricular Transcutaneous Vagus Nerve Stimulation (at-VNS), t-VNS, ear-electro-acupuncture, or non-invasive treatments compared with a control group, sham, or other intervention. The studies which were included were RCTs, controlled clinical trials, clinical trials, and pilot studies. The used MeSH terms and free terms included:

"Vagus Nerve", "Auricular Vagus Nerve Stimulation", "Transcutaneous Vagus Nerve Stimulation", "electro-acupuncture", "ear-electro-acupuncture", "migraine", and "headache". The concepts were combined with the "AND" or "OR" operators. Additionally, the combination of Mesh terms was ("auricular vagus nerve stimulation" OR "auricular t-VNS" OR "non-invasive vagus nerve stimulation" OR "electro-acupuncture" OR "ear-electro-acupuncture") AND ("migraine" OR "migraine" OR "headache" OR "headache").

PICO question

This systematic review was conducted to answer the following clinical question: "Is non-invasive neuromodulation effective for the management of chronic migraine?"

Population: Adults with Chronic migraine older than 18 years of age.

Intervention: Application of auricular transcutaneous vagus nerve stimulation technique and ear electro-acupuncture.

Comparator: Acceptable comparators were any type of placebo (e.g., turning-off device), sham, or no intervention of pain, duration of symptoms, and/or adverse events, healthy subjects or those with another type of pathology.

Outcomes: The primary outcomes measured were intensity and frequency of migraine attacks.

The studies have to fully answer all these questions to be part of the acceptable or suitable studies to be included in the systematic review.

Study selection

This systematic review was limited to randomized controlled trials, clinical trials, and controlled trials. Studies were excluded if they were a series of cases, case reports, retrospective and prospective cohorts, notes to editors, chapters of books, trials on animals, or articles with only manual acupuncture as a treatment group. The intervention groups were at-VNS, n-VNS, or ear-electro-acupuncture, and the control group should receive alternative interventions to be able to compare effects (e.g., different places for intervention treatment, turn-off devices, and different treatments) and at least one outcome should be reported at the conclusion of the intervention in chronic migraine.

Data extraction and quality assessment

Data extraction was performed by two authors and the data were compiled into a standardized data extraction form in an Excel spreadsheet. Data included simple size, diagnosis, inclusion/exclusion criteria, duration of symptoms, intervention type (location, technique, and duration), main outcomes, time to outcome, and adverse events. In case of discrepancy between authors, an agreement should be achieved. If no achievement is reached, a third author should be in charge of reaching a consensus.

The methodological quality of the trials was evaluated with the PEDro (Physiotherapy Evidence Database) scale and the ROB-2 Cochrane tool independently by two authors. The RoB-2 tool includes the following items: selection bias (randomization sequence generation, allocation concealment), performance bias (blinding participants, blinding therapists), detection bias (blinding outcome assessor), attrition bias (incomplete outcome data), reporting bias (source of funding bias/selecting outcome reporting), and other bias (sample size). Each item was classified as low risk, high risk, or unclear according to the Cochrane Collaboration tool (Higgins et al., 2011). In all cases, the answer "Yes" indicates a low risk of bias, and the answer "No" indicates high risk of bias. If insufficient details are reported of what occurred during the trial, or the entry was not relevant to the study (particularly for assessing blinding and incomplete outcome data, when the outcome being assessed by the entry has not been measured in the study), the answer was "unclear" risk of bias.

The PEDro scale is based on 11 criteria, of which 10 contribute to the score, representing methodological quality. The first item is not included (but should always be fulfilled) in the score, as it relates to the external validity of the study. The PEDro scale has been shown to have fair-to-good inter-rater reliability (ICC 0.55, 95% CI 0.41–0.72). The PEDro score assessed the following items: random allocation, concealed allocation, between-groups similarity at baseline, participant blinding, therapist blinding, assessor blinding, dropout, intention-to-treat statistical analysis, between-group statistical comparison, point measures, and variability data (Luo et al., 2020). A PEDro score equal to or >5 out of 10 points determined a high-methodological-quality trial. Higher scores indicated higher methodological quality (total score from 0 to 10) (Maher et al., 2003). Trials with a PEDro score ≥5 points were considered to be of high quality. Disagreements between reviewers were resolved by a third reviewer.

Quality of evidence was rated according to the Oxford Center for Evidence-Based Medicine. Levels of Evidence evaluated included: level 1, randomized trials or systematic reviews of randomized trials; level 2, randomized trial or (exceptionally) observational study with dramatic effect; level 3, non-randomized controlled cohort/follow-up study; level 4, case series, case–control study, or historically controlled studies; and level 5, mechanism-based reasoning. Level 1 represented the likely strongest evidence, and level 5 represented the likely weakest evidence (Marx et al., 2015).

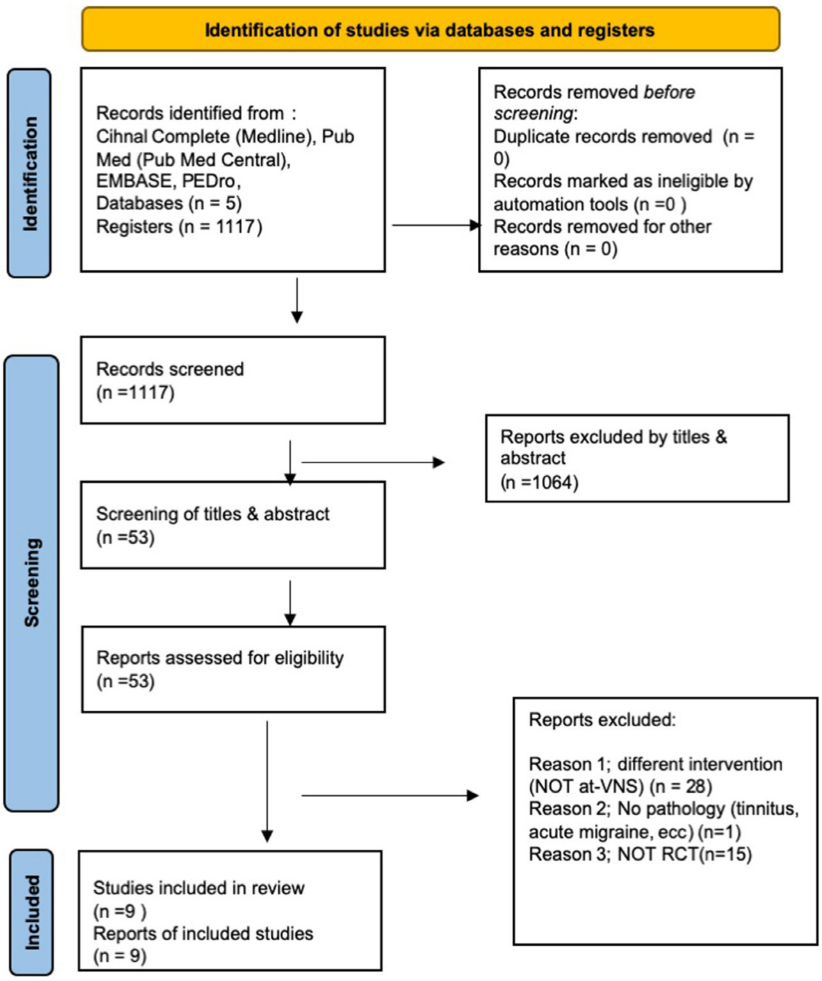

FIGURE 1
Flow diagram of study selection. RCT, randomized controlled trial.

Results

Study selection and characteristics

The data search yielded a total of 1,117 articles, including duplicates. We excluded many articles based on title and abstract ($n = 1064$), and eleven ($n = 11$) because of repetition of the potential eligible articles. Forty-two ($n = 42$) articles were included for abstract/full-text review, of which thirty-three were excluded because of other pathologies or areas of application or another type of pathology such as cluster headache, headache, acute migraine, tinnitus, cervical area of treatment ($n = 33$). Finally, only nine were included in the systematic review (Vijayalakshmi et al., 2014; Yang et al., 2014; Straube et al., 2015; Zhang et al., 2019, 2021; Luo et al., 2020; Cao et al., 2021; Sacca et al., 2022; Wei et al., 2022). Figure 1 shows the flow diagram. The strategy of each database is represented in Table 1.

Table 2 summarizes the studies included in the review. The total sample size consisted of 333 participants (mean age: 34.78, SD: 4.95, 41.74% women). The duration of migraine-associated symptoms was 14.61 (SD: 7.17 years) and the frequency of attacks per month was 10.8 (SD: 5.5).

Outcomes

We extracted the following outcomes: the Visual Analogic Scale (VAS) (Vijayalakshmi et al., 2014; Zhang et al., 2019, 2021; Luo et al., 2020; Cao et al., 2021); the frequency and duration of migraine attacks (Yang et al., 2014; Straube et al., 2015; Zhang et al., 2019, 2021; Luo et al., 2020; Cao et al., 2021); Quality-of-life Questionnaire (QoL) (Vijayalakshmi et al., 2014; Cao et al., 2021); Self-rating Depression Scale (SDS); Self-rating Anxiety Scale (SAS); Migraine-Specific Quality of Life (MSQ) (Yang et al., 2014; Straube et al., 2015; Zhang et al., 2019, 2021; Luo et al., 2020; Cao et al., 2021); Migraine Disability Assessment (MIDAS) (Vijayalakshmi et al., 2014; Straube et al., 2015) and the Headache Impact Test (HIT-6) (Straube et al., 2015); WHO Quality-of-Life BREF (Biomedical Research and Education Foundation) (Vijayalakshmi et al., 2014); HRSD-17 and HRSA-14 (Wei et al., 2022); Migraine-Specific Quality-of-Life Questionnaire; and fMRI scan (Sacca et al., 2022).

There were different post-treatment follow-up periods for respective outcomes. Zhang et al. (2019) assessed the pain intensity and frequency of migraine attacks using MSQ, SDS, and SAS at post-treatment after just a single session. Cao et al. (2021) performed all measurements, namely migraine duration, migraine attacks, average of pain intensity with VAS, and MSQ at post-treatment for all outcomes. Straube et al. (2015) assessed chronic migraine-related disability with the HIT-6 and MIDAS at 14 days, 28 days, and 56 days after starting the treatment, as well as at the end of the study 12 weeks afterwards. Luo et al. (2020) took all measurements at post-treatment (one week). Additionally, Vijayalakshmi et al. (2014) and Yang et al. (2014) took all measurements at post-treatment only.

TABLE 1 Search strategy.

Database	Search number	Search strategy	Number
PubMed	#1	("auricular vagus nerve stimulation"[Mesh])	439
	#2	("auricular t-VNS" OR "noninvasive vagus nerve stimulation" OR "electro-acupuncture" OR "ear-electro-acupuncture")	992
	#3	#1 OR #2	3
	#4	"Migraine"[MeSH]	46,525
	#5	OR headache	117,159
	#6	#4 OR #5	26,314
	#7	#3 AND #6	29
CINAHL (MEDLINE)	#1	("auricular vagus nerve stimulation" OR "auricular t-VNS" OR "non invasive vagus nerve stimulation" OR "electro-acupuncture" OR "ear-electro-acupuncture") AND ("migraine" OR "headache")	21
EMBASE	#1	("auricular vagus nerve stimulation" [Mesh])	676
	#2	("auricular t-VNS" OR "non invasive vagus nerve stimulation" OR "electro-acupuncture" OR "ear-electro-acupuncture")	9,655
	#3	#1 OR #2	9,941
	#4	"Migraine" ([Mesh])	87,466
	#5	OR Headache	345,227
	#6	#4 OR #5	381.197
	#7	#3 AND #6	741
PEDro	#1	Migraine ([Mesh])	325
	#2	Non invasive vagus nerve stimulation ([Mesh])	4
	#3	Noninvasive vagus nerve stimulation AND migraine	2
	#4	#1 AND #2 AND #3	2

Interventions

For the intervention group, all studies investigated at-VNS or ear electro-acupuncture. The at-VNS devices were placed for most of the studies in the left side of the cymba concha (Straube et al., 2015; Zhang et al., 2019; Cao et al., 2021), left cymba concha, and at the concha of the outer ear (Zhang et al., 2021; Sacca et al., 2022), and there were three studies that chose acupuncture points,

TABLE 2 Summary of all included studies.

References	Intervention(s)	Sample size	Age (years)	Intervention duration (sessions/weeks)	Area of treatment	Comparison and outcome measure	Results	Adverse effects	Duration migraine	Evidence level*
Migraine										
Cao et al. (2021), China	G1; taVNS = NR; 20-Hz. G2; taVNS = NR 1 Hz;	$N = 24$	31.33 ± 1.55	taVNS stimulation lasted about 8 min. Unique session	Left concha (cymba and cavum)	VAS, MSQ, SDS, SAS, number migraines fMRI G1 vs. G2	G2; taVNS (1Hz) improved FC in PAG, bilateral MCC, right precuneus/posterior cingulate cortex, left MFG, left cuneus, left insula, ACC. Number migraines better with 1Hz ta-VNS. NS VAS, MSQ, SDS, SAS	None	8.68 ± 1.47 y.	2
Wei et al. (2022), China	G1; EA G2; healthy people	$N = 30$	32.285 ± 6.41	2Hz, 1 mA for 8 min until the end of fMRI scan	GB 8	HRSD-17 & HRSA-14	G1 could improve (GB8) FC between the right insula subregions and parietal lobe, namely, the right dAI and right postcentral gyrus, and the right PI and left precuneus	None	16.20 ± 8.19	3
Sacca et al. (2022), USA	G1; taVNS =; 1-Hz. G2; taVNS = 20 Hz	$N = 20$	31.33 ± 1.55 y	taVNS stimulation 1Hz, 8 minutes, 4 mA, 2 session with fMRI scan	Left concha (cymba and cavum)	Migraine duration, number of migraine attacks, VAS, Migraine Specific Quality of Life Questionnaire, fMRI	G1 Improved NTS/LC–occipital cortex sFC and a decrease of NTS-thalamus sFC, greater LC precuneus and LC–inferior parietal cortex sFC than G2. G1 decreased NTS–postcentral gyrus dFC than G2. G2 compared with baseline increase of the LC–anterior cingulate cortex (ACC) sFC.	None		2

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TABLE 2 (Continued)

References	Intervention(s)	Sample size	Age (years)	Intervention duration (sessions/weeks)	Area of treatment	Comparison and outcome measure	Results	Adverse effects	Duration migraine	Evidence level*
Zhang et al. (2021), China	G1; taVNS = 33, 1Hz, 0.2ms G2; Sham group = 26	$N = 70$	NR	30 min of 12 treatment sessions in total during the 4 week treatment	Left cymba concha	VAS MSQ Migraine attack times SAS fMRI G1 vs. G2	G1 SF number of migraine days (p = 0.024) G1 SF (p = 0.008), migraine attack times (p = 0.015) NS MSQ, SAS, SDS fMRI G1; FC, occipital' thalamic seed and the bilateral PoG reduction of the migraine days (p = 0.016) G2; NS	None	4.0 (1.9) days 4.0 (3.2) days	1
Luo et al. (2020), China	G1; taVNS 1Hz, 0.2ms. G2s; tVNS Sham.	$N = 27$	29.85 ± 8.09	Unique session of MRI scan, total of six 20-min fMRI runs and 8 min ta-VNS	CO11 and CO14, left cymba concha	VAS, MSQ, SAS, SDS, fMRI G1 vs. G2	G1; FC Improved, left amygdala, left MFG, right SMA, left dorsolateral superior frontal gyrus, bilateral paracentral lobules, bilateral postcingulum gyrus, and right frontal superior medial gyrus. Left FC and right SMA in frequency/time in migraine in 4 weeks.	None	NR; NR	2
Zhang et al. (2021), China	G1; ta-VNS, 1Hz, 0.2ms. G2; Sham ta-VNS	$N = 26$	32.50 ± 7.57	MRI session of 30 minutes	Left cymba concha	VAS, MSQ, SDS, SAS, fMRI G1 vs. G2	G1 greater deactivation at the bilateral LC. rsFC the right temporoparietal junction and left secondary somatosensory cortex (S2) SF increased vs. G2	None	7.15 ± 2.87 Y. 3.23 ± 1.58 mo	3
Straube et al. (2015), Germany	G1; taVNS 25 Hz, 250 µs, cycle: 30s on, 30 s off G2; ta-VNS 1 Hz; 250 µs, cycle: 30s on, 30 s off	$N = 46$ $N = 24$ $N = 22$	41,55 ± 11,95	4 h per day during 12 weeks	Concha of the outer ear	Pain Imtensity (NRS), MIDAS, HIT-6, BDI G1 vs. G2	G2 SF headache days, (p = 0.035). HIT-6 & MIDAS SF G1 & G2	Only 3 of 46 patients (7%) dropped out due to side effects of t-VNS.	20.4 ± 12.1 years 27.1 ± 13.0 years	2

(Continued)

TABLE 2 (Continued)

References	Intervention(s)	Sample size	Age (years)	Intervention duration (sessions/weeks)	Area of treatment	Comparison and outcome measure	Results	Adverse effects	Duration migraine	Evidence level*
Yang et al. (2014), China	G1; AG; G2; SAG; G3; MG	$N = 30$	33.28 ± 8.03	30 min of unique session 30 min Sham acupucnture 40 min rest	TE8, TE19, GB33	VAS PET-CT	G1 & G2 VAS SF ($P < 0.05$) MG NS($P = 0.047$) AG vs. MG middle frontal gyrus, postcentral gyrus, the precuneus, parahippocampus, cerebellum and middle cingulate cortex (MCC), and decreased in the left hemisphere of (MTC) SAG vs. MG posterior cingulate cortex (PCC), insula, inferior temporal gyrus, MTC, superior temporal gyrus, postcentral gyrus, fusiform gyrus, inferior parietal lobe, superior parietal lobe, supramarginal gyrus, middle occipital lobe, angular and precuneus cerebellum, parahippocampus	None	NR NR	2
Vijayalakshmi et al. (2014), India	G1; Electro acupuncture; G2; Drug therapy	$N = 60$ $N = 30$ each group	NR	10 sessions for 30 days (0.5 mA; 10-20 Hz) flunarizine 20 mg OD and tab. paracetamol 500 mg SOS for 30 days	DU 20, P.6, St.36, GB.41, GB.14, EM, LI.4, LI.10, ST.44, Ear points: Ear shenmen and Ear stomach (16)	MIDAS WHO QOL BREF	G1 SF ($P = 0.005–0.000$) in all outcomes.	None	NR NR	2

VAS, visual analog scale; MSQ, Migraine Specific Quality of Life; SDS, Self-rating Depression Scale; SAS, Self-rating Anxiety Scale; NVS, transcutaneous auricular-Nerve Vagus stimulation; fMRI, functional Magnetic Resonance Image; AG, Electro-acupuncture group; SAG, Sham Acupunture Group; MG, Migraineur Wait-List control Group; WHO QOL BREF.

*Levels of Evidence based on the Quality Rating Scheme for Studies and Other Evidence modified from the Oxford Centre for Evidence-Based Medicine for rating of individual studies; available online at https://www.cebm.net/2016/05/ocebm-levels-of-evidence/. RCT.

NR, Not reported; LC, locus coeruleus; rsFC, Resting state functional connectivity; SF, Significance; EA, Electro-acupuncture; PCC, Posterior cingulate cortex; MTC, Middle Temporal Cortex; SMA, supplementary motor area; MFG, middle frontal gyrus; MFC, Middle Frontal Cortex.

namely TE 19 (Yang et al., 2014), ST16 (Vijayalakshmi et al., 2014) and CO11, CO14 (Luo et al., 2020), and GB8 (Wei et al., 2022). Accordingly, the area of stimulation was the auricular branch of the vagal nerve.

The parameters of electrical stimulation were poorly described, with a pulse width ranging from 150 to 200 ms. The intensity of the current was the strongest stimulation tolerable for the subjects and a frequency ranging from 1 to 20–25 Hz. The number and time of sessions were also different among studies, most of which included short periods of time between application during the migraine attack, usually 8 min (Straube et al., 2015; Cao et al., 2021; Sacca et al., 2022), whereas one study specifically applied 12 sessions of 30 min each (Luo et al., 2020), a daily application of 4 h during 12 weeks of the study, and 1 during the MRI scan session of 30 min to check the at-VNS effectivity (Cao et al., 2021). Wei et al. (2022) used a device with 2Hz and 1 mA with a continuous wave for 8 min during the fMRI scan at the GB8 acupuncture point. Sacca et al. (2022) used a device with 1 Hz in 8 min and 4 mA in the left concha (cymba and cavum) during 2 fMRI.

The usual sham at-VNS in most of the studies was the placement of the electrode in another anatomical body area, e.g., left tail of the helix (Zhang et al., 2019; Cao et al., 2021), or using a sham device without stimulation (Straube et al., 2015), at-VNS with different parameters (Straube et al., 2015; Zhang et al., 2019; Cao et al., 2021; Sacca et al., 2022), or other acupuncture points further from the ear (Vijayalakshmi et al., 2014; Yang et al., 2014), with non-vagal fibers (Luo et al., 2020) or healthy subjects (Wei et al., 2022).

Methodological quality, risk of bias, and quality of evidence

The methodological quality scores ranged from 6 to 8 (mean: 7.3, SD: 0.8) out of a maximum of 10 points (Table 3). Four studies (Straube et al., 2015; Luo et al., 2020; Cao et al., 2021; Zhang et al., 2021) obtained a high methodological quality (≥5 points) and five obtained a low methodological quality (Vijayalakshmi et al., 2014; Yang et al., 2014; Sacca et al., 2022; Wei et al., 2022). The ROB-2 Cochrane tool identified a low risk of bias for two studies (Zhang et al., 2021), one with some concerns (Cao et al., 2021), and four studies presented a high risk of bias (Vijayalakshmi et al., 2014; Yang et al., 2014; Straube et al., 2015; Luo et al., 2020; Wei et al., 2022) (Figure 2). The risks of bias identified were the following: random sequence generation (D1): 44.4% low risk, 22.2% some concerns, and 33.3% high risk; allocation concealment (D2): 55.5% low risk, 33.3% some concerns, and 11.1% for high risk; blinding of participants and researchers (D3): 66.6% for low risk, 22.2% for some concerns, and 11.1% for high risk; blinding of outcome assessment (D4): 33.3% low risk, 33.3% for some concerns and for high risk; incomplete outcome data (D5): 55.5% low risk, 33.3% some concerns, and 11.1% high risk, (D6): any of them reported the sample size calculation with a high risk of bias (100%), (D7). Some of the studies reported the clinical trial registration number, giving them a low risk of bias (55,5%) but some did not report the prospective registration, which is now a requirement of the revised Declaration of Helsinki, giving them a high risk of bias (44.4%) (Figure 3). Based on the Oxford grading of evidence, one study was graded as level 1 (11.17%) (Zhang et al., 2021), six studies were graded as level 2 (66.66%) (Vijayalakshmi et al., 2014; Yang et al., 2014; Straube et al., 2015; Luo et al., 2020; Cao et al., 2021; Sacca et al., 2022), and two studies were graded as level 3 (22.2%) (Zhang et al., 2019; Wei et al., 2022). Study characteristics are detailed in Table 2.

Summary of results

We found low-quality evidence showing some positive effects on the intensity and frequency of migraine attacks. There was one protocol that compared two at-VNS groups (pulse width: 250 μs; frequency: 1 or 25 Hz; duty cycle: 30 s on/30 s off, 4 hour/day for 12 weeks) and found positive effects in the reduction of days with migraine and also HIT-6 and MIDAS after 12 weeks with at-VNS at 1 Hz, compared with the other group with 25Hz (Straube et al., 2015). Meanwhile, a completely different protocol employed two groups with different frequencies and time of at-VNS application, compared 20 Hz and 1 Hz of frequency of at-VNS with a width of 0.2 ms for 8min, and observed that at-VNS with 1 Hz might be beneficial for reducing chronic migraine pain as an alternative and safe treatment. Additionally, pre-treatment and post-treatment (4 week) VAS, MSQ, SDS, and SAS were assessed. Positive results were only seen in VAS and number of migraine attacks (Cao et al., 2021). On the other hand, three groups were compared: one undergoing electro-acupuncture with TENS (frequency of stimulation was 100 Hz, and the intensity of the electrical stimulus varied from 0.1 to 1.0 mA for 30 min) and two as a control, one with a sham acupuncture point and another from a migraineur wait-list control group in a unique session (Yang et al., 2014). This protocol had more differences in parameters, and compared two groups: one undergoing electro-acupuncture with TENS (wave pulse and a current of 0.5 mA; an output of 6–9 volts would be delivered at 10–20 Hz for 20 min) for 10 sessions, and another with drug therapy (flunarizine 20 mg OD along with tab paracetamol 500 mg SOS), both for 30 days (Vijayalakshmi et al., 2014). Only one differed in days of application at acupuncture points using at-VNS at 1 Hz and 0.2 ms of intensity in the left cymba concha, and a sham at-VNS in 7 days of stimulation of 8 min each. A decrease in migraine pain intensity, but not in the other outcomes, was identified (Luo et al., 2020).

There were four studies where the parameters in their protocols were similar enough to be able to compare the results obtained in each one. One of them used at-VNS, with 1 Hz and 0.2 ms of duration for 30 min each session during 12 sessions in total in the left cymba concha, and a sham at-VNS group where the electrodes were placed in the left tail of the helix. They discovered a positive effect on migraine days, pain intensity, and migraine attack times compared with the sham group, without difference for MSQ, SAS, and SDS (Zhang et al., 2021). The same author published another study with the parameters of 1 Hz, width 0.2 ms, and an intensity of 1.5–3 mA, and a sham at-VNS group (non-stimulation), during 8 min in one session. The study suggests some positive effects on pain and MSQ and a relation between the brain

TABLE 3 Score of randomized clinical trials with PEDro scale.

	1	2	3	4	5	6	7	8	9	10	Total
Migraine											
Wei et al. (2022)	N	N	Y	N	N	N	N	N	Y	Y	3/10
Sacca et al. (2022)	Y	N	Y	N	N	N	N	N	Y	Y	4/10
Cao et al. (2021)	Y	N	Y	Y	N	N	Y	Y	Y	Y	7/10
Zhang et al. (2021)	Y	N	Y	Y	N	Y	N	Y	Y	Y	7/10
Luo et al. (2020)	Y	N	Y	Y	N	N	Y	N	Y	Y	6/10
Zhang et al. (2019)	N	Y	Y	Y	N	N	N	Y	Y	N	5/10
Straube et al. (2015)	Y	N	Y	Y	N	N	N	Y	Y	Y	6/10
Yang et al. (2014)	Y	N	N	Y	N	N	N	N	N	Y	3/10
Vijayalakshmi et al. (2014)	Y	N	N	N	N	N	N	N	N	N	2/10

1: Random Allocation of Participants; 2: Concealed Allocation; 3: Similarity Between Groups at Baseline; 4: Participant Blinding; 5: Therapist Blinding; 6: Assessor Blinding; 7: Fewer than 15% Dropouts; 8: Intention- to-Treat Analysis; 9: Between- Group Statistical Comparisons; 10: Point Measures and Variability Data.
Y, Yes, If the criterion is satisfied.
N, No, If the criterion is not satisfied.

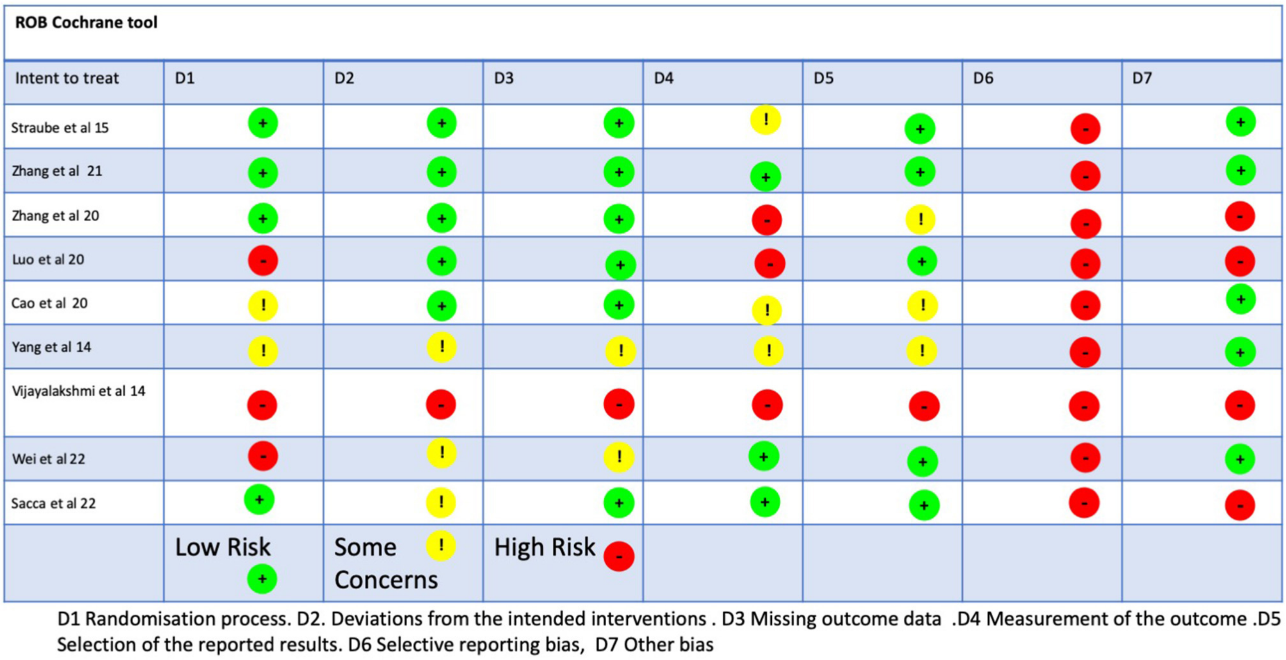

FIGURE 2
ROB2 tool.

system and the modulation of pain (Zhang et al., 2019). One study compared similar outcomes within three groups: Electro-Acupuncture Group (AG), Sham Acupuncture Group (SAG), and Migraineur Wait-List Control Group (MG). For AG, the acupuncture points were the following: Shaoyang meridians, Luxi (TE19), San Yangluo (TE8), and Xi Yangguan (GB33). They used a TENS device (Electrodes) of Han's acupoint nerve stimulator (HANS; model LH 200A; TENS, Nanjing, China) with the following parameters: frequency of stimulation was 100 Hz and the intensity of the electricity stimulus varied from 0.1 to1.0 mA for 30 min. The SAG was designed to choose non-acupuncture points, and the points were the following: anterior border of the insertion of the deltoid muscle, ST 36, and ulnar side of the arm. There was only one application, and two outcomes were measured, with the VAS and PET-CT being measured pre- and post-intervention. The results obtained were the differences in each group's AG ($P < 0.05$) and SAG ($P < 0.05$), but not between them, and there was no difference in MG ($P = 0.047$). Additionally, the last study (Sacca et al., 2022) used two groups of at-VNS— 1 and 20 Hz, respectively—with two fMRI scans. The outcomes used in this

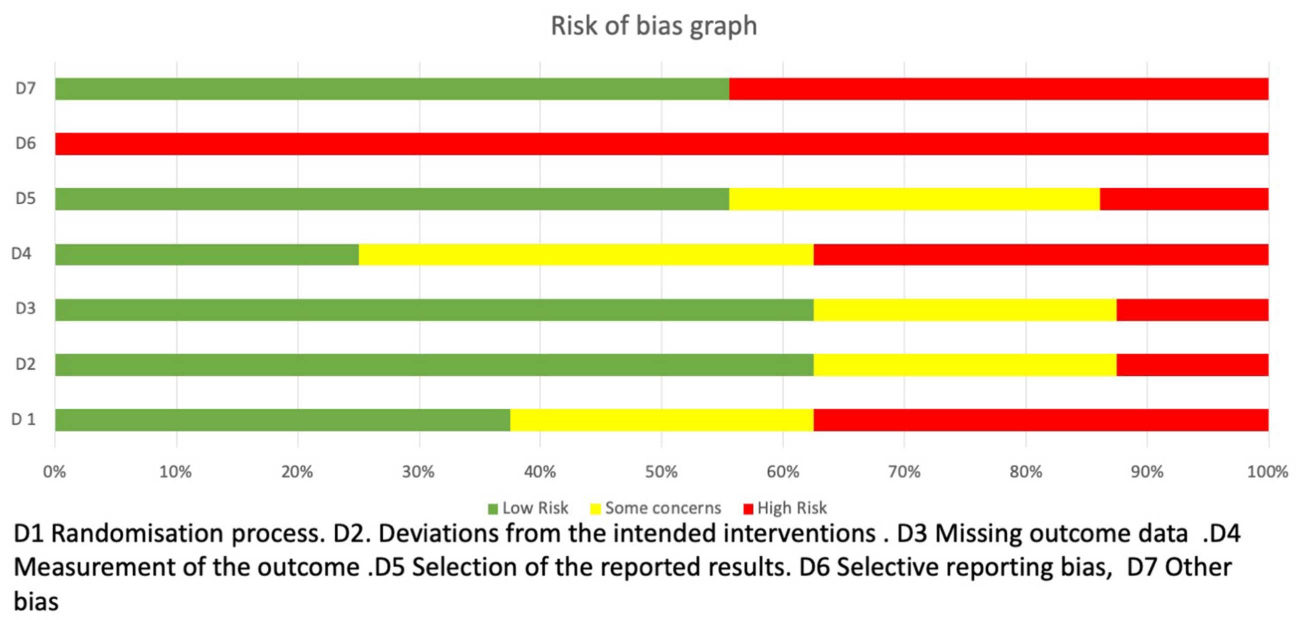

FIGURE 3
Graph of the ROB2.

study were the migraine duration (in years), number of migraine attacks in the last four weeks, the pain intensity measure from 0 to 100, Migraine-Specific Quality of Life Questionnaire, and fMRI scan. The results obtained were the following: with 1 Hz at-VNS, there was an increase in NTS (Nucleus of the Solitary Tract)/LC (locus coeruleus)–occipital cortex sFC (static) and a decrease in NTS-thalamus compared with 20 Hz, which was an increase in the LC–anterior cingulate cortex (ACC) both compared at baseline. Moreover, 1 Hz at-VNS greater LC-precuneus and LC–inferior parietal cortex sFC than 20 Hz and 20 Hz stimulation produced an increased LC-ACC and LC–super temporal gyrus/insula sFC in comparison with 1 Hz in static FC. In Dynamic FC 1-Hz, taVNS decreased NTS–postcentral gyrus dFC (less variability), and 20-Hz taVNS decreased dFC (Dynamic FC) of the LC–superior temporal gyrus and the LC–occipital cortex. The conclusion of these results was the relationship between the number of migraine attacks in the past four weeks and the NTS-thalamus sFC during pre-taVNS resting state. As a result, the parameters are vital to obtain an effective treatment for people who suffer from migraine.

One study used drug therapy (Group D) as a comparator group vs. the intervention group who received electro-acupuncture (Group A). Group A had 10 sessions of treatment in a 30-day period, and if there were any attacks, the patient could take a 500 mg tablet of paracetamol. Group D took two tablets—one of flunarizine 20 mg and one of paracetamol 500 mg for 30 days. The outcomes were WHO, QOL, and BREF and were measured pre- and post-treatment. The results showed that patients with migraine headaches had a lower quality of life and higher disability scores, and only two study groups showed a significant change. We could determine that the electro-acupuncture group showed better pain relief than the drug therapy group ($P = 0.005–0.000$) (Vijayalakshmi et al., 2014).

Pain intensity, migraine attacks, frequency, and duration

There was no difference in four studies regarding pain intensity with VAS score, the number of migraine attacks, and frequency after the stimulation using at-VNS with 1 Hz (Zhang et al., 2019; Luo et al., 2020; Cao et al., 2021; Sacca et al., 2022).

There were positive findings in two studies regarding migraine days and migraine attacks using at-VNS 1 Hz compared with the sham group, and only one study with positive findings in pain intensity using the VAS score (Yang et al., 2014; Straube et al., 2015; Zhang et al., 2021).

Imagining condition and analysis

Functional magnetic resonance imaging (fMRI) was applied in all studies to measure neuronal activity and brain structure in migraine patients treated by at-VNS and electro-acupuncture. Six studies evaluated the FC (Zhang et al., 2019, 2021; Luo et al., 2020; Cao et al., 2021; Sacca et al., 2022; Wei et al., 2022), one of which employed the sFC and dFC (Sacca et al., 2022), while the others employed the rsFC (Zhang et al., 2019) and FC (Cao et al., 2021; Zhang et al., 2021; Sacca et al., 2022).

Brain data image

Brain imaging data were reported in seven studies as shown in Table 2.

There were three studies that employed similar protocols of frequency with 1 Hz in the intervention group, but differed in number of sessions and time of the at-VNS application. As a result, there were some similarities in the stimulated brain areas, but there were also differences, one of which being that the fRMI signal was increased in the bilateral putamen, right caudate, right pallidum/anterior insula, right thalamus, and left frontal operculum, along with a decrease in the bilateral precuneus/posterior cingulate cortex (PCC)/hippocampus/precentral gyrus/medial prefrontal gyrus (mPFC)/anterior cingulate cortex (ACC), bilateral LC, left SN, right RN/PBN, left posterior insula, and bilateral superior/middle frontal gyrus during real at-VNS, compared to the baseline. The comparison between at-VNS and the sham group showed an important deactivation in the at-VNS group at the bilateral LC. The rsFC provided LC rsFC with the right temporoparietal junction, and the left secondary somatosensory cortex (S2) significantly increased compared to the sham group (Zhang et al., 2019). Meanwhile, in another study with a similar protocol of at-VNS with 1 Hz and 8 minutes of intervention, the fRMI scan indicated that FC decreased during at-VNS between the left amygdala and left middle frontal gyrus (MFG), left dorso lateral superior frontal gyrus, right supplementary motor area (SMA), bilateral paracentral lobules, bilateral postcingulum gyrus, and right frontal superior medial gyrus, as did the FC of the right amygdala and left MFG (Luo et al., 2020). Additionally, the last study reported the following related to at-VNS with 1 Hz: the fMRI signal was increased along with the connectivity between the motor-related thalamus subregion and anterior cingulate cortex/medial prefrontal cortex, and there was a decrease in connectivity between the occipital cortex-related thalamus subregion and postcentral gyrus/precuneus (Zhang et al., 2021).

On the other hand, there were two studies that employed two groups using different frequencies, but the last one used two sessions instead of a unique session. The study with one session reported, using an fMRI scan, that an increased signal in the following brain areas produced a significant increasing functional connectivity between the PAG and the bilateral middle cingulate cortex (MCC): right precuneus, left middle frontal gyrus (MFG), and left cuneus with 1 Hz. Additionally, comparing 1 vs. 20 Hz, 1 Hz at-VNS increased PAG connectivity with the MCC, right precuneus/posterior cingulate cortex, left insula, and anterior cingulated cortex (ACC) (Cao et al., 2021). In addition, another study consisting of two sessions reported that in sFC and Fc, comparing 1 Hz and 20 Hz at-VNS showed that 1 Hz at-VNS produced a greater LC–precuneus and LC–inferior parietal cortex sFC than 20 Hz. Additionally, dFC 1-Hz at-VNS decreased NTS–postcentral gyrus dFC (less variability), while 20-Hz at-VNS decreased dFC of the LC–superior temporal gyrus and the LC–occipital cortex (Sacca et al., 2022).

There was only one study which compared healthy people with migraine patients and provided a comparison between electro-acupuncture at GB8 compared with the sham group using an fRMI scan, and there was an increase in FC between the PI (posterior insula) and the left precuneus in the electrical acupuncture group at the baseline, as compared with the sham group and intervention group post-intervention. There was a decreased FC between dAI and the right postcentral gyrus found in the baseline intervention group, compared to the sham group and post-intervention group. Additionally, increased FC between the PI and left precuneus was found in the baseline intervention group, compared to the sham group and post-intervention group. The correlation analysis showed that the FC value of the right postcentral gyrus in the baseline intervention group was negatively correlated with the scores of the Hamilton Rating Scale for Depression and Hamilton Rating Scale for Anxiety. The FC value of the left precuneus in the baseline intervention group was positively correlated with the visual analog scale score (Wei et al., 2022).

A study unique in its protocol employed three groups in order to avoid risk of bias and better mask the true effect of at-VNS than others, and reported the scan imaging with an increase in the PET signal in the middle frontal gyrus, postcentral gyrus, precuneus, parahippocampus, cerebellum, and middle cingulate cortex (MCC), and a decrease in the left hemisphere of the Middle Temporal Cortex (MTC) in the acupuncture group compared with the migraine group. Additionally, an increase in the PCC, insula, inferior temporal gyrus, MTC, superior temporal gyrus, postcentral gyrus, fusiform gyrus, inferior parietal lobe, superior parietal lobe, supramarginal gyrus, middle occipital lobe, angular, and precuneus, and a decrease in cerebellum and parahippocampus vs. the sham group was observed (Yang et al., 2014).

The fMRI Scan outcome provides us with some strong evidence regarding the effectiveness, effect, and relationship between Auricular Vagus Nerve Branches (AVNB); using at-VNS or electro-acupuncture with 1 Hz at the left concha had some positive effects in the Frontal Cortex (FC) of the brain and some other areas, such as the periaqueductal gray (PAG), bilateral MCC, right precuneus/posterior cingulate cortex, left MFG, left cuneus, left insula, and ACC (Cao et al., 2021). The at-VNS at GB8 within 1 Hz could improve the right insula subregions and parietal lobe, specifically the right dAI and right postcentral gyrus, as well as the right PI and left precuneus (Martelletti et al., 2018). Another study, in its protocol, also used at-VNS within 1 Hz at the left cymba concha to provide fMRI evidence of the effect in the occipital thalamic seed and the bilateral PoG (Zhang et al., 2021). The signal in the fMRI FC Improved, left amygdala, left MFG, right SMA, left dorsolateral superior frontal gyrus, bilateral paracentral lobules, bilateral post-cingulum gyrus, right frontal superior medial gyrus, and left FC and right SMA with 1 Hz in 8 min of at-VNS stimulation frequency/time in migraine over 4 weeks (Luo et al., 2020). The last study protocol employing at-VNS with 1 Hz in the left cymba concha had better results in fMRI than sham at-VNS, with a greater deactivation at the bilateral LC, rsFC, right temporoparietal junction, and left secondary somatosensory cortex (Zhang et al., 2019). There was only one protocol with three groups using electro-acupuncture in TE8, TE19, and GB33, which obtained better results compared with the migraine group and sham group. The PET-CT proved that the signal was increased in the middle frontal gyrus, postcentral gyrus, the precuneus, parahippocampus, cerebellum, and middle cingulate cortex (MCC), and decreased in the left hemisphere of the Middle Temporal Cortex (MTC) (Yang et al., 2014).

We divided all therapies/interventions and their efficacy into different groups for each study, as shown in Table 4.

The first group was at-VNS vs. another group of at-VNS, but with different parameters to compare which frequency was

TABLE 4 Summary of the efficacy of the interventions.

References	Intervention(s)	Intervention duration (sessions/weeks)	Efficacy
at-VNS vs. at-VNS (different parameters of frequency)			
Cao et al. (2021), China	G1; taVNS = 20-Hz. G2; taVNS = 1 Hz continuous wave (width: ∼ 0.2 ms), stimulation of 8 min Intensity ∼4 mA	Unique session	ta-VNS 1 HZ was superior in terms of the number of migraine attacks and functional brain connectivity.
Wei et al. (2022), China	G1; electrical acupuncture with low-frequency pulse therapy instrument G2; healthy people 2 Hz, 1 mA for 8 min	Unique session	G1 (electrical acupuncture) had some influence in brain connectivity with a therapeutic role.
Sacca et al. (2022), USA	G1; taVNS = 1 Hz. G2; taVNS = 20 Hz 8 minutes, 4 mA,	2 sessions	G1 (1 Hz) improves more than G2 (20 Hz) in migraine attacks. Both improved functional brain connectivity.
Straube et al. (2015), Germany	G1; taVNS 25 Hz, 250 µs, cycle: 30 s on, 30 s off G2; ta-VNS 1 Hz 250 µs, cycle: 30s on, 30 s off	4 h per day over 12 weeks	G2; t-VNS at 1 Hz was safe and effective and after 12 weeks showed a reduction of migraine.
Real at-VNS vs. sham at-VNS therapies			
Zhang et al. (2021), China	G1 ta-VNS 1 Hz with the duration of 0.2 ms. Stimulation was continuously applied for 30 min. Intensity 1.5–5 mA G2; Sham group = another location	30 min of 12 treatment sessions in total during the 4-week treatment	G1; relieved the symptoms of headache as well as modulated the thalamocortical circuits in migraine patients
Luo et al. (2020), China	G1; taVNS 1 Hz, 0.2 ms. intensity below the pain threshold (vagal afferent fibers) G2; taVNS Sham. (no vagal afferent fibers)	Unique session of MRI scan, total of 6 20 min fMRI runs and 8 min ta-VNS	G1; FC Improved, left amygdala, left MFG, right SMA, left dorsolateral superior frontal gyrus, bilateral paracentral lobules, bilateral postcingulum gyrus, and right frontal superior medial gyrus. Left FC and right SMA in frequency/time in migraine in 4 weeks.
Zhang et al. (2021), China	G1; taVNS = (frequency: 1 Hz; width: 0.2 ms). Stimulation intensity was adjusted to approximately 1.5–3 mA) G2; Sham group = another location	Unique session of fMRI with at-VNS and sham at-VNS	G1; 1 Hz can significantly modulate activity/connectivity of brain regions and pain modulation system in migraine.
Electro-acupuncture (auricular branch) vs. another technique			
Yang et al. (2014), China	G1; AG G2; SAG 100 Hz, for 30 min, 1,0 mA G3; MG	30 min of unique session	Acupuncture stimulation at both sub-specific acupoints evokes central mechanism of acupuncture analgesia by neuroimaging measurement.
Vijayalakshmi et al. (2014), India	G1; Electro acupuncture 10-20 Hz, 0.5 mA; an output of 6-9 volts for 20 min G2; Drug therapy; flunarizine 20 mg OD and tab. paracetamol 500 mg SOS	10 sessions for 30 days	G1 improved in QOL and MIDAS.

VAS, visual analog scale; MSQ, Migraine Specific Quality of Life; SDS, Self-rating Depression Scale; SAS, Self-rating Anxiety Scale; NVS, transcutaneous auricular-Nerve Vagus stimulation; fMRI, functional Magnetic Resonance Image; AG, Electro-acupuncture group; SAG, Sham Acupunture Group; MG, Migraineur Wait-List control Group; WHO QOL BREF.

Levels of Evidence based on the Quality Rating Scheme for Studies and Other Evidence modified from the Oxford Centre for Evidence-Based Medicine for rating of individual studies; available online at https://www.cebm.net/2016/05/ocebm-levels-of-evidence/.RCT.

NR, Not reported; LC, locus coeruleus; rsFC, Resting state functional connectivity; SF, Significance; EA, Electro-acupuncture; PCC, MTC, Middle Temporal Cortex; SMA, supplementary motor area; MFG, middle frontal gyrus; MFC, Middle Frontal Cortex.

more effective. Additionally, the efficacy was improved in the at-VNS group to 1 Hz, while the pain of migraine attacks was not; a relationship between brain connectivity and the peripheral branch of the vagus nerve in the auricula with superior areas of the brain was shown, such as MCC, right precuneus, left middle frontal gyrus (MFG), left cuneus, PAG, and AC.

The next group was at-VNS compared with sham group studies, where the sham group was the stimulation or vagal fever of the vagus nerve compared to no stimulation. Additionally, the efficacy that we could extract clearly showed that at-VNS had a greater effect on pain relief and migraine attacks compared with the sham group and the brain activity and connectivity in pain modulation with higher brain areas, such as the left amygdala and left middle frontal gyrus (MFG), left dorso lateral superior frontal gyrus, right supplementary motor area (SMA), bilateral paracentral lobules, bilateral postcingulum gyrus, and right frontal superior medial gyrus, as did the FC of the right amygdala and left MFG.

The last group was at-VNS compared with another treatment or technique, such as drug therapy or manual acupuncture. The results showed that at-VNS was superior compared to drug therapy alone and manual acupuncture or acupuncture in distal points.

Adverse events

There was only one study of the seven which showed side effects; 7% of patients were reported to have dropped out due to the side effects of t-VNS (Straube et al., 2015). The other six studies reported no adverse events or side effects of neuromodulation (t-VNS, at-VNS, ear-electro-acupuncture) (Vijayalakshmi et al., 2014; Yang et al., 2014; Zhang et al., 2019, 2021; Luo et al., 2020; Cao et al., 2021; Wei et al., 2022) (Table 2).

Discussion

The aim of this systematic review was to determine the effects of at-VNS for managing chronic migraine-associated symptoms. The results suggest that application of at-VNS may have some positive effects at post-treatment on the frequency and intensity of chronic migraine attacks with 1 Hz of application, as compared with the control group. However, because each study exhibits some differences, more studies are required in order to obtain a good protocol with the exact parameters for finding the best treatment option for these patients.

This study reinforces that our theory is an effective and low-cost treatment option (Platzbecker et al., 2020) compared with pharmaceutical treatments which are available but expensive (Martelletti et al., 2018).

Even the latest publications on acupuncture alone for treating migraine have shown the same results as the current review, thus demonstrating that follow-up for this pathology must be applied effectively to determine the presence of a positive or long-term effect (Naguit et al., 2022). Another finding was the small number of studies of high relevance included in the final screening. Moreover, for at-VNS, we excluded two studies which applied it in the neck area; in the auricula application area, only eight studies were found.

Previous systematic reviews included neuromodulations such as transcranial magnetic stimulation (TMS), non-invasive vagal nerve stimulation (nVNS), non-painful remote electrical stimulation (NRES), and external trigeminal nerve stimulation (e-TNS) (Clark et al., 2022; Naguit et al., 2022), as well as transcranial direct current stimulation (tDCS) (Moisset et al., 2020). All reviews suggest a potential positive but small effect for the treatment of migraine (Martelletti et al., 2018; Cai et al., 2021; Moreno-Ajona et al., 2022). One difference between previous reviews and the current one is that most previous reviews focused on acute migraine, whereas our review focused on chronic migraine (Martelletti et al., 2018). Only one other review also targeted chronic migraine, but used tDCS treatment, which is very different from at-VNS (Cai et al., 2021). The outcomes in two studies were similar in the measurement of pain intensity and number of migraine attacks or duration. As a conclusion for the clinical results, there are many different n-VNS for the treatment of migraine; whether acute or chronic, there are positive effects of the different n-VNS applications which must be taken into consideration to ensure a safe treatment choice (Moisset et al., 2020; Cai et al., 2021; Clark et al., 2022; Naguit et al., 2022).

Some publications have investigated the common pathways of tinnitus and migraine, and the underlying mechanisms as to how n-VNS could work through the vagus nerve for proper management of them. They were focused on applying stimulus to the peripheral nervous system (PNS) through the central nervous system (CNS) to higher brain areas, such as the hippocampus, amygdala, anterior cingulate cortex, and hypothalamus (Moisset et al., 2020; Cai et al., 2021; Clark et al., 2022; Naguit et al., 2022).

Meanwhile, this publication specifically focuses on the auricula area to treat chronic migraine through the vagus nerve. One of the publications on n-VNS referred to neck application, which differs from our work and shows different results. The network could represent a relationship between the peripheral nervous system and the CNS related to the gate control as a primary pain relief, as well as with the calcitonin gene-related peptide (CGRP) and its role in the descending pain modulatory system (DPMS) (Marx et al., 2015; Straube et al., 2015; Zhang et al., 2019, 2021; Luo et al., 2020). Additionally, in some studies, at-VNS had effects on the peripheral nervous system which, through the central nervous system, reached higher brain areas, such as the anterior cingulate cortex (ACC), periaqueductal gray (PAG), prefrontal cortex (PFC), cingulate gyrus, supplementary motor area (SMA), amygdala, and thalamus (Martelletti et al., 2018; Moisset et al., 2020; Cai et al., 2021; Clark et al., 2022; Moreno-Ajona et al., 2022; Naguit et al., 2022). Based on the recent literature and the results of this systemic review, there are no clear mechanisms regarding the role of the n-VNS and at-VNS using acupuncture points to treat chronic migraine and their relationship with the peripheral vagus nerve branches and the ANS. However, there are positive results that encourage more studies with better methodology to be conducted in order to extract strong conclusions to clarify the pathways and the relationship that exists between them.

On the other hand, the results from the studies included in this and another review which used fMRI scanning provide a possible relationship between the at-VNS and positive effects in migraine patients and the brainstem. As a result, it could be linked to an influence in the brainstem, such as in the dorsoposterior insula, low medullary brainstem, medial thalamic, ACC, posterior insula, lower medullary brainstem, and medial thalamic/ACC deactivation with the use of at-VNS/n-VNS. These findings provide a convergence of preliminary evidence supporting the relationship between peripheral areas of the branch of the vagus nerve and superior areas of the brain previously demonstrated (Moulton et al., 2008; Lerman et al., 2019). Neuroanatomical findings support the evidence of the relationship between NTS and the following sites: parabrachial area, locus coeruleus, dorsal raphe, periaqueductal gray, thalamus, amygdala, insula, nucleus accumbens, and bed nucleus of the stria terminalis through the left cymba. Moreover, anti-noception is well referenced with the stimulation of the brain areas such as periaqueductal gray, dorsal raphe, and locus coeruleus, each of which activates descending inhibitory pathways to the spinal cord dorsal horn (Frangos and Komisaruk, 2017), even though the anti-depressive and anti-convulsion have similar effects in some similar areas, such as the amygdala, accumben, and hippocampus, and dorsal raphe and locus coeruleus, respectively. Then, based on our included studies—which show abnormalities of these areas compared to those in healthy humans—there are enough findings that support the relationship between AVNB (ear, concha) with the brainstem (ACC, NTS, LC, etc.) in migrainous and healthy people. However, more clinical trials are required to support the evidence for the best parameters of treatment with at-VNS/n-VNS.

Additionally, there are findings that support the relationship between the superior areas of the brainstem and acupuncture points using fMRI. The areas of the brain which were involved were the following: PAG, ACC, left PCC, insula, limbic/paralimbic, and precuneus (Lerman et al., 2019). Moreover, there is further evidence providing results related to other pathologies, such as low back pain, tinnitus, and the connectivity of the network between the brain and peripheral acupuncture points (Cheng et al., 2020). The areas of stimulation in the brain for low back pain were: PFC (prefrontal cortex), insula, cerebellum, SI (secondary somatosensory cortex), and ACC. For tinnitus, similar areas were involved, such as the right MTG (middle temporal gyrus). As we highlighted previously, there is a relationship between peripheral branch nerves and acupuncture points linked to superior brain areas with a change using at-VNS/n-VNS through fMRI.

Recommendations and future studies

Future research is required to clarify some important points, not least the effectiveness of the technique. Additionally, for future systematic reviews, studies must be published with high-quality research, such as true randomized controlled trials with adequate control groups, to be able to compare the data derived from them. In terms of quality assessment, they have to follow methodology scales, such as high PEDro or GRADE scores. If they are able to reach those, then future meta-analyses will receive high scores in GRADE as well. Additionally, a recent publication regarding CONSORT included more items, reinforcing the need to improve the research quality of future studies. At the same time, the inclusion criteria should include similar protocols of treatments with parameters such as frequency, intensity, time, number of sessions of treatment, and follow up(s) of the same or similar main outcomes. In conclusion, this systematic review summarizes the main points to improve the quality of future studies with scales and homogeneity in the study design to be able to extract conclusions with similarities and low risk of bias (Butcher et al., 2022).

Additionally, it is important, as a recommendation for future research, that portable devices for at-VNS are promoted. This would be an important advancement in terms of self-treatment at any place and any time for the onset of migraine. It would be really helpful for giving patients who suffer from these sudden attacks the possibility of management. A report was previously published regarding the cost and effectiveness and the role of sequence strategies in migraine attacks. The conclusions were that a portable and effective device could change the quality of life of these patients, providing a low-cost option for most of them in terms of treating the onset of a sudden attack (Mwamburi et al., 2018). If this is compared with traditional methods, the low cost, ease of use, improvement of quality of life and social engagement, and the effectiveness of treatment are the main points underlying the advantages of new models vs. traditional ones. It is well reported that new models, such as NEMOS® or gammacore®, are safer and more tolerable than traditional ones, such as with a surgical implant because of AEs (Ben-Menachem et al., 2015; Mwamburi et al., 2018).

Limitations

We should recognize some limitations of the current review. First, most studies had some concerns regarding their methodological quality (Martelletti et al., 2018), even if the results were slightly positive, and low pain intensity increased the risk of bias and affected quality. For these reasons, we cannot obtain any firm conclusion regarding the effectiveness of at-VNS and ear-electro-acupuncture for managing chronic migraine.

The inconsistency of the follow-up periods and the difference between study protocols, as well as the small number of trials available for systematic review, did not permit us to perform a meta-analysis. Furthermore, the study protocols regarding the same parameters, times of application, measurements, and electro-acupuncture points must be similar, even including the same pathology if there is an acute or chronic migraine, for the same reason previously highlighted for strong conclusions, while also following the guidelines and standards from STRICTA (STandards for Reporting Interventions in Clinical Trials of Acupuncture) (MacPherson et al., 2010).

Conclusions

The current systematic review found low-quality evidence supporting the idea that at-NVS or ear electro-acupuncture may have some positive effects in the treatment of chronic migraine post-treatment in terms of reducing the frequency and intensity of migraine attacks. Additionally, positive effects were shown in fMRI scans and the relationship between peripheral vagus nerve branches with the superior brainstem. The small number of RCTs and the heterogeneity in the data did not permit us to pool data.

Author contributions

JM-J contributed to the conception and design of this study, takes responsibility for the integrity of the work as a whole, and resolved any disagreements between both reviewers. CF-d-l-P, JP-G, and FG-E contributed to the interpretation of the data. Article drafts were written by DF-H and JM-J and critically revised by all authors. JP-G and FG-E contributed to realizing the suggestions of the discussion. The literature search was made by DF-H and FG-E. The study selection and the quality of the assessment was carried out by CF-d-l-P and DF-H. The final version of the article was approved by all authors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Amiri, P., Kazeminasab, S., Nejadghaderi, S. A., Mohammadinasab, R., Pourfathi, H., Araj-Khodaei, M., et al. (2022). Migraine: a review on its history, global epidemiology, risk factors, and comorbidities. *Front. Neurol.* 12, 800605. doi: 10.3389/fneur.2021.800605

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.*11, 492–500. doi: 10.1016/j.brs.2017.12.009

Ben-Menachem, E., Revesz, D., and Simon, B. J. (2015). Surgically implanted and non-invasive vagus nerve stimulation: a review of efficacy, safety and tolerability. *Eur. J. Neurol.* 22, 1260–1268. doi: 10.1111/ene.12629

Blech, B., and Starling, A. J. (2020). Noninvasive neuromodulation in migraine. *Curr. Pain Headache Rep*. 24, 78. doi: 10.1007/s11916-020-00914-3

Butcher, N. J., Monsour, A., Mew, E. J., Chan, A.-W., Moher, D., Mayo-Wilson, E., et al. (2022). Guidelines for reporting outcomes in trial reports: the CONSORT-outcomes 2022 extension. *JAMA.* 328, 2252–2264. doi: 10.1001/jama.2022.21022

Cai, G., Xia, Z., Charvet, L., Xiao, F., Datta, A., Androulakis, X. M. A., et al. (2021). Systematic review and meta-analysis on the efficacy of repeated transcranial direct current stimulation for migraine. *J. Pain Res*. 14, 1171–1183. doi: 10.2147/JPR.S295704

Cao, J., Zhang, Y., Li, H., Yan, Z., Liu, X., Hou, X., et al. (2021). Different modulation effects of 1 hz and 20 hz transcutaneous auricular vagus nerve stimulation on the functional connectivity of the periaqueductal gray in patients with migraine. *J. Translational Med.* 19, 354. doi: 10.1186/s12967-021-03024-9

Cheng, S., Xu, G., Zhou, J., Qu, Y., Li, Z., He, Z., et al. (2020). A multimodal meta-analysis of structural and functional changes in the brain of tinnitus. *Front. Human Neurosci.* 14, 28. doi: 10.3389/fnhum.2020.00028

Clark, O., Mahjoub, A., Osman, N., Surmava, A. M., Jan, S., Lagman-Bartolome, A. M., et al. (2022). Non-invasive neuromodulation in the acute treatment of migraine: a systematic review and meta-analysis of randomized controlled trials. *Neurol Sci.* 43, 153–165. doi: 10.1007/s10072-021-05664-7

de Tommaso, M., Vecchio, E., Quitadamo, S. G., Coppola, G., Di Renzo, A., Parisi, V., et al. (2021). Pain-related brain connectivity changes in migraine: a narrative review and proof of concept about possible novel treatments interference. *Brain Sci.* 11, 234. doi: 10.3390/brainsci11020234

Ellrich, J. (2006). Long-term depression of orofacial somatosensory processing. *Suppl Clin Neurophysiol.* 58, 195–208. doi: 10.1016/S1567-424X(09)70069-8

Feng, M., Zhang, Y., Wen, Z., Hou, X., Ye, Y., Fu, C., et al. (2022). Early fractional amplitude of low frequency fluctuation can predict the efficacy of transcutaneous auricular vagus nerve stimulation treatment for migraine without aura. *Front. Mol. Neurosci.* 15, 778139. doi: 10.3389/fnmol.2022.778139

Frangos, E., and Komisaruk, B. R. (2017). Access to vagal projections via cutaneous electrical stimulation of the neck: fMRI evidence in healthy humans. *Brain Stimulat.* 10, 19–27. doi: 10.1016/j.brs.2016.10.008

GBD 2016 Headache Collaborators (2018). Global, regional, and national burden of migraine and tension-type headache, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 17, 954–976. doi: 10.1016/S1474-4422(18)30322-3

Grassini, S., and Nordin, S. (2017). Comorbidity in migraine with functional somatic syndromes, psychiatric disorders and inflammatory diseases: a matter of central sensitization? *Behav Med*. 43, 91–99. doi: 10.1080/08964289.2015.1086721

Hamer, H. M., and Bauer, S. (2019). Lessons learned from transcutaneous vagus nerve stimulation (tVNS). *Epilepsy Res*. 153, 83–84. doi: 10.1016/j.eplepsyres.2019.02.015

He, W., Wang, X., Shi, H., Shang, H., Li, L., Jing, X., et al. (2012). Auricular acupuncture and vagal regulation. *Evid Based Complement Alternat Med*. 2012, 786839. doi: 10.1155/2012/786839

Henssen, D. J. H. A., Derks, B., van Doorn, M., Verhoogt, N., Van Cappellen van Walsum, A.-M., Staats, P., et al. (2019b). Vagus nerve stimulation for primary headache disorders: An anatomical review to explain a clinical phenomenon. *Cephalalgia*. 39, 1180–94. doi: 10.1177/0333102419833076

Henssen, D. J. H. A., Derks, B., van Doorn, M., Verhoogt, N. C., Staats, P., Vissers, K., et al. (2019a). Visualizing the trigeminovagal complex in the human medulla by combining ex-vivo ultra-high resolution structural MRI and polarized light imaging microscopy. *Sci. Rep.* 9, 11305. doi: 10.1038/s41598-019-47855-5

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The Cochrane Collaboration' s tool for assessing risk of bias in randomised trials.*BMJ*. 343, d5928. doi: 10.1136/bmj.d5928

Huang, T., Wang, S., and Kheradmand, A. (2020). Vestibular migraine: an update on current understanding and future directions. *Cephalalgia*. 40, 107–121. doi: 10.1177/0333102419869317

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019). Current directions in the auricular vagus nerve stimulation I – a physiological perspective. *Front. Neurosci.* 13, 1–23. doi: 10.3389/fnins.2019.00854

Lerman, I., Davis, B., Huang, M., Huang, C., Sorkin, L., Proudfoot, J., et al. (2019). Noninvasive vagus nerve stimulation alters neural response and physiological autonomic tone to noxious thermal challenge. *PloS ONE.* 14, e0201212. doi: 10.1371/journal.pone.0201212

Luo, W., Zhang, Y., Yan, Z., Liu, X., Hou, X., Chen, W., et al. (2020). The instant effects of continuous transcutaneous auricular vagus nerve stimulation at acupoints on the functional connectivity of amygdala in migraine without aura: a preliminary study. *Neural Plasticity.* 2020, 1–13. doi: 10.1155/2020/8870589

MacPherson, H., Altman, D. G., Hammerschlag, R., Youping, L., Taixiang, W., White, A., et al. (2010). Revision group. revised standards for reporting interventions in clinical trials of acupuncture (STRICTA): extending the CONSORT statement. *PLoS Med.* 7, e1000261. doi: 10.1371/journal.pmed.1000261

Maher, C. G., Sherrington, C., Herbert, R. D., Moseley, A. M., and Elkins, M. (2003). Reliability of the PEDro scale for rating quality of randomized controlled trials. *Physical Therapy*. 83, 713–721. doi: 10.1093/ptj/83.8.713

Martelletti, P., Barbanti, P., Grazzi, L., Pierangeli, G., Rainero, I., Geppetti, P., et al. (2018). Consistent effects of non-invasive vagus nerve stimulation (nVNS) for the acute treatment of migraine: additional findings from the randomized, sham-controlled, double-blind PRESTO trial. *J. Headache Pain.* 19, 101. doi: 10.1186/s10194-018-0949-9

Marx, R. G., Wilson, S. M., and Swiontkowski, M. F. (2015). Updating the assignment of levels of evidence. *J. Bone. Joint Surg. Am.* 97, 1–2. doi: 10.2106/JBJS.N.01112

Moisset, X., Pereira, B., Ciampi, D., Andrade, D., Fontaine, D., Lantéri-Minet, M., et al. (2020). Neuromodulation techniques for acute and preventive migraine treatment: a systematic review and meta-analysis of randomized controlled trials. *J. Headache Pain.* 21, 142. doi: 10.1186/s10194-020-01204-4

Möller, M., Mehnert, J., Schroeder, C. F., and May, A. (2020). Noninvasive vagus nerve stimulation and the trigeminal autonomic reflex: an fMRI study. *Neurology.* 94, e1085–e1093. doi: 10.1212/WNL.0000000000008865

Moreno-Ajona, D., Hoffmann, J., and Akerman, S. (2022). Devices for episodic migraine: past, present, and future. *Curr. Pain Headache Rep.* 26, 259–265. doi: 10.1007/s11916-022-01024-y

Moulton, E. A., Burstein, R., Tully, S., Hargreaves, R., Becerra, L., Borsook, D., et al. (2008). Interictal dysfunction of a brainstem descending modulatory center in migraine patients. *PloS ONE.* 3, e3799. doi: 10.1371/journal.pone.0003799

Mwamburi, M., Tenaglia, A. T., and Leibler, E. J. (2018). Cost-effectiveness of noninvasive vagus nerve stimulation for acute treatment of episodic migraine and role in treatment sequence strategies. *Am. J. Managed Care.* 24, S527–S533.

Naguit, N., Laeeq, S., Jakkoju, R., Reghefaoui, T., Zahoor, H., Yook, J. H., et al. (2022). Is acupuncture safe and effective treatment for migraine? a systematic review of randomized controlled trials. *Cureus.* (2022) 14, e20888. doi: 10.7759/cureus.20888

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 372, n71. doi: 10.1136/bmj.n71

Platzbecker, K., Timm, F. P., Ashina, S., Houle, T. T., and Eikermann, M. (2020). Migraine treatment and the risk of postoperative, pain-related hospital readmissions in migraine patients. *Cephalalgia.* 40, 1622–1632. doi: 10.1177/0333102420949857

Rong, P. J., Fang, J. L., Wang, L. P., Meng, H., Liu, J., Ma, Y., et al. (2012). Transcutaneous vagus nerve stimulation for the treatment of depression: a study protocol for a double blinded randomized clinical trial. *BMC Complement. Altern. Med.* 12, 255. doi: 10.1186/1472-6882-12-255

Sacca, V., Zhang, Y., Cao, J., Li, H., Yan, Z., Ye, Y., et al. (2022). evaluation of the modulation effects evoked by different transcutaneous auricular vagus nerve stimulation frequencies along the central vagus nerve pathway in migraines: a functional magnetic resonance imaging study. *Neuromodulation*. 26, 620–628. doi: 10.1016/j.neurom.2022.08.459

Silberstein, S. D, Yuan, H, Najib, U, Ailani, J, de Morais, A. L, Mathew, P. G, et al. (2020). Non-invasive vagus nerve stimulation for primary headache: a clinical update. *Cephalalgia*. 40, 1370–1384. doi: 10.1177/0333102420941864

Simon, B., and Blake, J. (2017). Mechanism of action of non-invasive cervical vagus nerve stimulation for the treatment of primary headaches. *Am J Manag Care*. 23, S312–6.

Song, Y., Li, T., Ma, C., Liu, H., Liang, F., Yang, Y., et al. (2022). Comparative efficacy of acupuncture-related therapy for migraine: a systematic review and network meta-analysis. *Front. Neurol.* 13, 1010410. doi: 10.3389/fneur.2022.1010410

Stovner, L. J., and Andree, C. (2010). Prevalence of headache in Europe: a review for the Eurolight project. *J. Headache Pain*. 11, 289–299. doi: 10.1007/s10194-010-0217-0

Straube, A., Ellrich, J., Eren, O., Blum, B., and Ruscheweyh, R. (2015). Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (Auricular t-VNS): a randomized, monocentric clinical trial. *J Headache and Pain.* 16, 543. doi: 10.1186/s10194-015-0543-3

Tobaldini, E., Toschi-Dias, E., Appratto, D., Vicenzi, M., Sandrone, G., Cogliati, C., et al. (2019). Cardiac and peripheral autonomic responses to orthostatic stress during transcutaneous vagus nerve stimulation in healthy subjects. *J. Clin. Med*. 8, 496. doi: 10.3390/jcm8040496

Usichenko, T., Hacker, H., and Lotze, M. (2017). Transcutaneous auricular vagal nerve stimulation (taVNS) might be a mechanism behind the analgesic effects of auricular acupuncture. *Brain Stimulation.* 10, 1042–1044. doi: 10.1016/j.brs.2017.07.013

Vetvik, K. G., and MacGregor, E. A. (2017). Sex differences in the epidemiology, clinical features, and pathophysiology of migraine. *Lancet Neurol.* 16, 76–87. doi: 10.1016/S1474-4422(16)30293-9

Vijayalakshmi, I., Shankar, N., Saxena, A., and Bhatia, M. S. (2014). Comparison of effectiveness of acupuncture therapy and conventional drug therapy on psychological profile of migraine patients. *Indian J Physiol Pharmacol.* (2014) 58, 69–76.

Wei, X. Y., Luo, S. L., Chen, H., Liu, S. S., Gong, Z. G., Zhan, S. H., et al. (2022). Functional connectivity changes during migraine treatment with electroacupuncture at Shuaigu (GB8). *J. Integrat. Med.* 20, 237–243. doi: 10.1016/j.joim.2022.01.009

Woldeamanuel, Y. W., and Cowan, R. P. (2017). Migraine affects 1 in 10 people worldwide featuring recent rise: a systematic review and meta-analysis of communitybased studies involving 6 million participants. *J. Neurol. Sci*. 372, 307–315. doi: 10.1016/j.jns.2016.11.071

Yang, M., Yang, J., Zeng, F., Liu, P., Lai, Z., Deng, S., et al. (2014). Electroacupuncture stimulation at sub-specific acupoint and non-acupoint induced distinct brain glucose metabolism change in migraineurs: a PET-CT study. *J. Transl. Med.* 12, 351. doi: 10.1186/s12967-014-0351-6

Yap, J. Y. Y., Keatch, C., Lambert, E., Woods, W., Stoddart, P. R., Kameneva, T., et al. (2020). Critical review of transcutaneous vagus nerve stimulation: challenges for translation to clinical practice. *Front. Neurosci.* 14, 284. doi: 10.3389/fnins.2020.00284

Zhang, Y., Huang, Y., Li, H., Yan, Z., Zhang, Y., Liu, X., et al. (2021). Transcutaneous auricular vagus nerve stimulation (TaVNS) for migraine: an FMRI study. *Regional Anesthesia Pain Med.* 46, 145–150. doi: 10.1136/rapm-2020-102088

Zhang, Y., Liu, J., Li, H., Yan, Z., Liu, X., Cao, J., et al. (2019). Transcutaneous auricular vagus nerve stimulation at 1 Hz modulates locus coeruleus activity and resting state functional connectivity in patients with migraine: an fMRI study. *NeuroImage Clin.* 24, 1–8. doi: 10.1016/j.nicl.2019.101971

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Fire acupuncture for anti-LGI1 antibody autoimmune encephalitis: a case report

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Autoimmune encephalitis, a class of encephalitis, is clinically characterized by multifocal or diffuse brain injury, including aberrant mental behavior, convulsions, and near-event memory impairment. In this article, we describe a female patient with autoimmune encephalitis who tested positive for leucine-rich glioma inactivated 1 (LGI1) antibodies and had hippocampal inflammatory edema in the lesion area. During the first 3months of her illness, the patient primarily experienced memory loss, the onset of rigid twitching in her extremities that lasted for 1min while in remission, and incontinence. After gamma globulin administration, methylprednisolone shock, and other symptomatic therapies during hospitalization, the patient's psychiatric symptoms and seizures improved considerably; however, she did not fully recover her memory. After receiving fire acupuncture for 6months, the patient's understanding, orientation, and calculation skills improved considerably. Her memory and mental state were also improved at the follow-up visit. In this case, the use of fire acupuncture for the treatment of autoimmune encephalitis resulted in favorable outcomes with important benefits for conditions affecting the central nervous system; however, more convincing data are required to support the effectiveness of this treatment method.

KEYWORDS
fire acupuncture, autoimmune diseases, cognitive dysfunction, glucocorticoids, inflammation

1. Introduction

Autoimmune encephalitis is a novel inflammatory disease of the central nervous system mediated by antibodies directed against neurotransmitter receptors or neuronal surface proteins. The clinical syndrome is complex, and its manifestations vary depending on the type of antibody involved; however, it mainly presents with acute or subacute onset of cognitive impairment, epileptic seizures, psychobehavioral abnormalities, and a wide variety of movement disorders (Dutra et al., 2018).

Anti-leucine-rich glioma inactivated 1 (LGI1) antibody-positive encephalitis accounts for approximately 30% of limbic encephalitis-associated antibodies and is typically characterized by faciobrachial dystonic seizures, cognitive decline, hyponatremia, anti-LGI1 antibody-positive serum or cerebrospinal fluid, and abnormal magnetic resonance imaging (MRI) signals in the medial temporal lobe or hippocampus (Linnoila et al., 2014; Plantone, 2018).

The treatment regimens for autoimmune encephalitis are based on the treatment principles for other life-threatening autoimmune diseases. Various therapies, including corticosteroids, intravenous immunoglobulin, plasma exchange, rituximab, and cyclophosphamide, are currently in use (Newman et al., 2016). However, there are no specific treatment options for certain antibody-mediated types of encephalitis.

2. Case description

The treatment of autoimmune encephalitis with acupuncture has not yet been documented. Here, we report a case of autoimmune encephalitis (anti-LGI1 antibody encephalitis) treated with fire needles.

On November 12, 2021, a 44-year-old female patient was admitted to the hospital with memory loss for 3 months and convulsions for 2 days. She presented with symptoms such as limb stiffness, incontinence, and consciousness impairment that lasted for 1 min and then resolved. Positron electron tomography showed swelling of the left hippocampus with slightly decreased density and increased glucose metabolism, cranial MRI showed abnormal signals in the bilateral anterior temporal lobes with left medial temporal lobe swelling (Figure 1), and blood-biochemical tests showed a blood sodium concentration of 106 mmol/L. The EEG showed fully guided short-to-long-range diffuse 2.0–4.5 Hz slow-wave activity of medium-high amplitudes with high amplitudes during interictal, awake, and sleep periods and higher amplitudes in the frontal and temporal regions. Anti-LGI1 antibodies were present in serum and lumbar puncture cerebrospinal fluid. Based on the patient's clinical presentation, LGI1 autoimmune encephalitis was diagnosed. The patient was treated with 20 g gamma globulin intravenously for 5 days, combined with methylprednisolone shock therapy (methylprednisolone sodium succinate 1,000 mg/d intravenously for 3 days, then 500 mg/d intravenously for 3 days, after which the dose was reduced to 40–80 mg/d intravenously for 2 weeks), along with levetiracetam as an antiepileptic drug, pantoprazole sodium enteric solution tablets for gastric protection, and potassium chloride extended-release tablets for potassium supplementation. The patient was discharged after 20 days of hospitalization with substantial symptom improvement. After discharge, glucocorticoid treatment was further tapered (60 mg of prednisone acetate once a day, 5 mg every 2 weeks until it was stopped). Although the disease was controlled in the acute phase, the patient reported distant and recent memory losses, resulting in loss of the patient's ability to perform daily activities, sleep with dreaminess and easy awakening, rapid weight gain after taking hormonal drugs, menstrual disorders, dry eyes, and depressed mood. Although these symptoms can be improved by oral administration of sleeping pills, antidepressants, progestins, and other drugs, these treatment options can only alleviate the symptoms but do not address the root cause of the problem. As the patient turned down these options, she visited a traditional Chinese medicine (TCM) clinic on December 23, 2021.

The acupuncturist, with more than 30 years of experience, arranged for her to receive fire acupuncture treatment while continuing to take prednisone. During the treatment, a 0.4 × 25 mm fine fire needle was selected and held in the left hand with an alcohol lamp, while the right hand held the needle in a pen grip, with the tip and part of the needle body inserted into the flame. The acupuncture points were selected according to the functional brain regions classified by Western medicine, with projection points of the prefrontal, temporal, parietal, and occipital regions of the head on the scalp. For example, the Baihui (GV20), Sishencong (EX-HN1), and Tongtian (BL7) points of the parietal lobe, the Shenting (GV24) point of the prefrontal lobe, the Shuaigu (GB8) point of the temporal lobe, and the Fengchi (GB20) point of the occipital lobe were selected (Figure 2). The operator must be strictly disinfected before the operation of the fire needle; operation process to "red" "accurate" "fast," the operation site after the needle prick mild itching or small red swelling, hand scratching is strictly prohibited, keep needle hole clean, dry, so as to avoid infection of the needle hole, if there is redness, swelling, heat and pain and other inflammatory reactions, available fire needle local puncture or oral anti-inflammatory drugs. Treatment with fire needles is prohibited for patients with blood clotting disorders, and patients with diabetes mellitus should use fire needles with caution (Huang et al., 2013). After treatment, the head was kept warm and observed for half an hour in the consultation room before the patient left the clinic. She underwent acupuncture treatment twice a week, and the same acupuncture point was not repeatedly used within a week; instead, the treatment points were adjusted according to the patient's condition in a timely manner.

In fact, although the patient was out of life-threatening conditions during her hospitalization and no longer suffered from loss of consciousness, grand mal seizures, and incontinence, the patient's distant and recent memory was severely lost and her calculation, orientation, and comprehension were severely affected when she came to the TCM clinic, so she was not considered to have entered the clinical recovery period, but rather the clinical treatment period. During the first 3 months of treatment with fire acupuncture, the patient reported a gradual recovery of recent memory and an improvement in calculation and comprehension. The EEG of the hospital also showed that "during the interictal period, a small amount of slow wave activity intermittently appeared in the left frontal and temporal areas during sleep, with a medium amplitude of 1.9–3.0 HZ, compared with the EEG at the beginning of the attack, the slow wave activity was significantly reduced," and entered the clinical recovery period. The patient continued the fire acupuncture treatment for another 2 months, and all of the patient's cognitive functions recovered significantly, and the patient's weight increased due to taking hormone drugs for several months, and her emotional impatience and menstrual disorder also gradually returned to normal. After complete discontinuation of hormones, another month of fire acupuncture treatment alone, the patient's condition was more stable than before, no abnormalities, reaching a stable stage of clinical recovery, and follow-up observation was recommended. To date, the patient has been followed up for 10 months without recurrence. Cranial MRI was reviewed for abnormal signaling of both anterior temporal lobes with a slight swelling of the medial left temporal lobe (Figure 3). Repeat EEG showed no obvious electrical abnormalities during the interictal period.

Abbreviations: LGI1, Leucine-rich glioma inactivated 1; MRI, Magnetic resonance imaging; TCM, Traditional Chinese medicine.

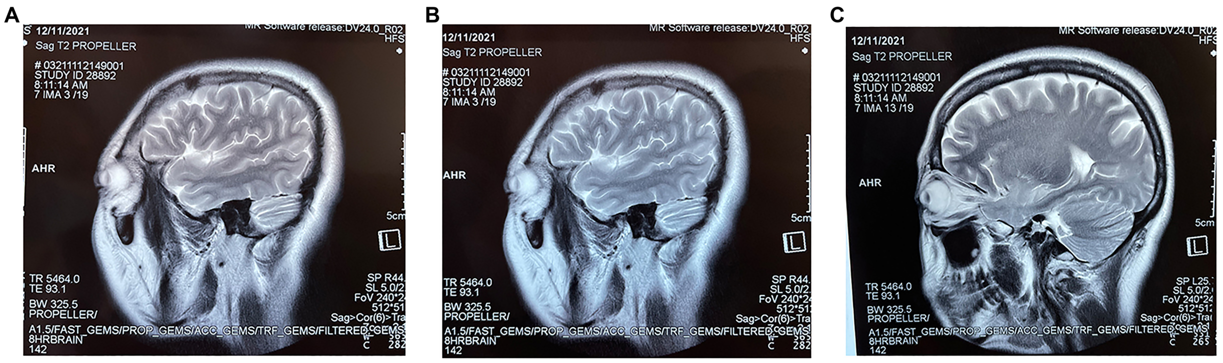

FIGURE 1
(A–C) are three different sections of the sagittal position of the first MRI at the patient's admission.

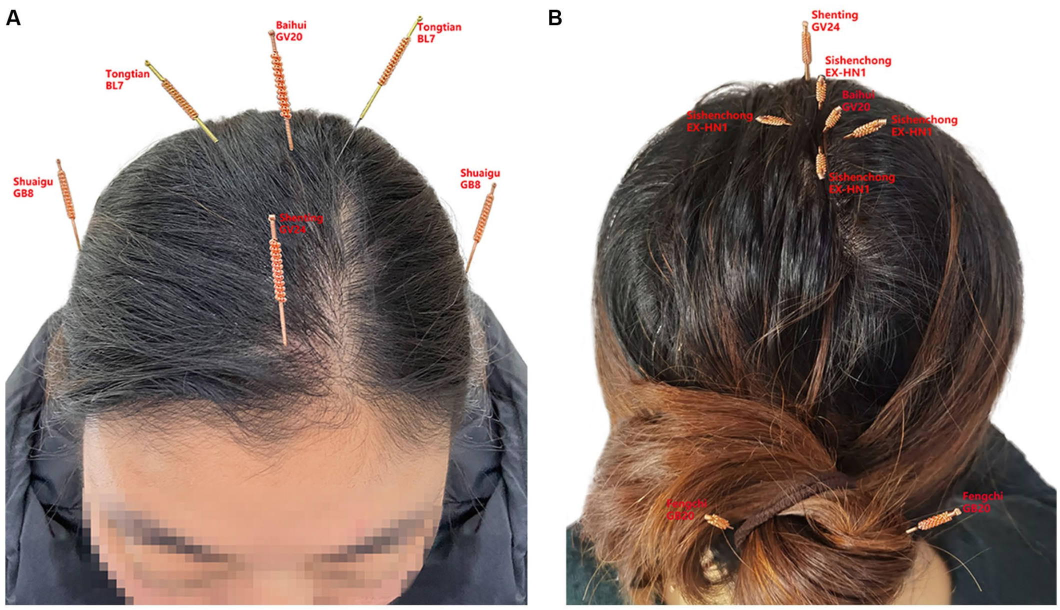

FIGURE 2
(A) represents the front of the head, **(B)** represents the back of the head, and the small labels inside represent the acupuncture points of the head, Baihui (GV20), Shenting (GV24), Sishen cong (EX-HN1), Tongtian (BL7), Shuaigu (GB8), and Fengchi (GB20).

3. Discussion

In this case, conventional hormonal medications did not significantly improve the patient's memory. After six courses of acupuncture treatment, memory recovery was successfully achieved, and the quality of life was also improved considerably by the effective alleviation of the side effects of hormonal treatment and maintenance of disease remission after the withdrawal of late hormonal medications, suggesting the effectiveness of acupuncture for the treatment of autoimmune encephalitis.

LGI1 is a secreted protein that is mainly distributed in the hippocampus and temporal cortex. It is expressed in neuronal axons and at the initiation of glutamatergic synapses (Hivert et al., 2019). LGI1 is involved in brain development, neuronal excitation, and synaptic transmission. In addition, some types of anti-LGI1 antibody encephalitis cause hippocampal atrophy and microstructural damage (Finke et al., 2017; Szots et al., 2017), which may be partly due to complement-dependent cytotoxic effects and consequential reduction in the number of neurons (Bien et al., 2012; Bauer and Bien, 2016). Recent studies have shown that monoclonal antibodies against LGI1 increase cellular excitability and glutamatergic synaptic transmission velocity in hippocampal CA3 neurons, which may contribute to the pathogenicity of LGI1 antibodies (Kornau et al., 2020; Ramberger et al., 2020).

Previous studies have demonstrated that the hippocampal CA1 region is associated with cognitive function and contains various neurons important for information processing. The CA3 area is rich in glutamate receptors, specifically NMDA receptors, which are critical for the formation of associative memories (Doron et al., 2022). In addition, they play an important role in encoding new spatial information in short-term memory (Rolls, 2022). Memory, cognitive abilities, and emotion regulation are all regulated by the hippocampus, a key region of the limbic system (Zeidman and Maguire, 2016). The patient in this case had left hippocampal swelling, resulting in severe memory loss and cognitive dysfunction.

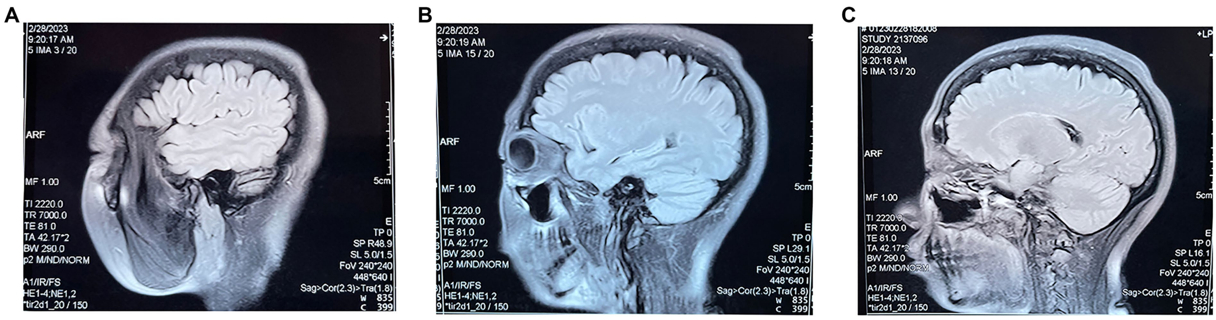

FIGURE 3
(A–C) are three different sections of the sagittal position of the MRI re-examined 10 months after the patient's fire needle treatment.

Based on replicable randomized controlled trials and functional fMRI studies, the understanding of acupuncture has been shifted to a less mysterious and more quantifiable science. MRI studies have demonstrated how the stimulation of certain acupoints results in the activation of corresponding brain areas (Zeidman and Maguire, 2016). The exact mechanism of action of acupuncture is not fully understood. However, it is thought to stimulate the central nervous system by releasing specific neurotransmitters and hormones. It has been hypothesized that the specific pathways, neurotransmitters, and hormones involved differ in different symptoms and disease states (Cai and Shen, 2018; Guo and Ma, 2019; Yu et al., 2020). Based on the TCM theory, both conventional and fire acupuncture play a therapeutic role by regulating the balance of qi and blood in the body, and as a whole, help relieve and cure diseases. The amount of stimulation of the body during the fire acupuncture process is much greater than that of milli-needle; secondly, the patient's attention during the fire acupuncture operation is much greater than that of milli-needle; again, the intensity of excitation foci formed in the cerebral cortex by fire acupuncture is much greater than that of milli-needle stimulation. Fire acupuncture has a dual role of acupuncture therapy and moxibustion therapy, not only providing the stimulating effect of the needles but also the stimulating effect of the heat. Through warm stimulation of the acupuncture points, fire acupuncture aims to warm the meridians and regulate the function of internal organs (Yue et al., 2019). Modern research has found that fire acupuncture can improve blood circulation and increase metabolism. Infrared thermograms of fire acupuncture sites demonstrated that the temperature of lesions treated by fire acupuncture increased significantly, suggesting improved local blood circulation and metabolism (Xing et al., 2019). Fire acupuncture can eliminate local tissue congestion, edema, exudation, and adhesions, improve local tissue calcification, ischemia, contracture, and other pathological changes, promote lesion liquefaction and necrosis, stimulate the benign regulatory mechanisms of the body, and enhance immune function (Wan et al., 2022). Previous studies have shown that fire acupuncture significantly increases brain-derived neurotrophic factor expression, promotes endogenous neural stem cell proliferation and differentiation into neurons, inhibits neuronal apoptosis, reduces inflammation through autophagy, and promotes the recovery of motor neuron function (Qiu et al., 2021).

Head fire acupuncture is a modern acupuncture technique that combines the acupuncture methods of Chinese medicine and the functions of the brain areas, using needles to stimulate different locations (points, zones, or areas) of the scalp. The effects of head stimulation on cerebral cortex function may be achieved by stimulating specific anatomical structures. Stimulation of the skin, fascia, muscles, and periosteum of the head can activate the functional areas of the cerebral cortex through the midbrain, thalamus, and brainstem, and the influence of different anatomical structures on brain function is regular and variable (Kim et al., 2020). Therefore, during treatment with fire needles, the angle and depth of acupuncture should be adjusted according to the patient's reaction under the needle to achieve better treatment results.

Several studies suggest that acupuncture stimulation increases cyclic-AMP response binding protein activity and reduces neuronal cell loss in the hippocampal region (Yun et al., 2017; Xie et al., 2021), which can improve memory impairment in various disorders, including Alzheimer's disease, Parkinson's disease, and stroke (Wattanathorn and Sutalangka, 2014; Page et al., 2021). It has also been demonstrated that acupuncture is beneficial for post-stroke rehabilitation, including aphasia, insomnia, neuroplasticity, depression, and cognitive impairment in diseases including Parkinson's disease, spinal cord injury, and stroke (Dou et al., 2016; Widrin, 2018; Xiao et al., 2018; Xiong et al., 2020; Li et al., 2021). Previous studies have revealed that acupuncture reorganizes motor-related networks, including the primary motor network, sensorimotor network, default mode network, and language-related brain areas, including the frontal, temporal, parietal, and occipital lobes of the inferior frontal gyrus, and cognition-related brain regions (Li S. et al., 2017; Li Y. et al., 2017; Lee et al., 2019; Zhang et al., 2021). In addition, some studies have shown that acupuncture can stimulate bilateral regions, regulate the whole-brain network, and enhance functional connections. This suggests that acupuncture can specifically regulate bilateral homeostasis in the brain and the entire brain network and functional connections (Li et al., 2015).

Acupuncture also has positive effects on autoimmune diseases. It regulates immunity by stimulating the central nervous system and peripheral neuroendocrine immune mechanisms. Neuromediators, hormones, cytokines, and other substances that constitute the continuum have a broad regulatory spectrum of immune activity to safeguard all known immunopathological responses, including a range of pathological links that contribute to autoimmune diseases (Dantzer, 2018). Moreover, with the help of the peripheral components of the neuroendocrine-immune system, the pathological links of the main etiology of the disease can be successfully regulated (Wang et al., 2020).

Previous studies have also shown that acupuncture regulates immunity by stimulating natural killer cell activity, regulating Th1/Th2 balance, reducing apoptosis, and increasing lymphokine-induced killer cytotoxic activity. Acupuncture also regulates the biochemical synergy between electroacupuncture and neurotrophic factors and the mobilization of corticosterone, endorphins, and adrenocorticotropic hormone. Acupuncture has both pro-and anti-inflammatory effects and improves the function of all immune cells. Increased release of endogenous opioid peptides is a crucial step in the activation of the immune system that can be achieved through acupuncture (Cabioğlu and Cetin, 2008; Takahashi et al., 2009; Kim and Bae, 2010; Torres-Rosas et al., 2014). Although there is no clear record of acupuncture treatment for autoimmune encephalitis, acupuncture has a large number of evidence-based reports for autoimmune diseases, such as autoimmune encephalomyelitis, multiple sclerosis, rheumatoid arthritis, and Guillain-Barré syndrome (Lee et al., 2016; Criado et al., 2017; Xu et al., 2018; Li et al., 2022).

Previous studies have shown that acupuncture improves neurological and autoimmune diseases. However, medical records and randomized controlled trials on acupuncture for autoimmune encephalitis are lacking. TCM focuses on the overall concept, and acupuncture treatment is based on the patient's inquisition and dialectical therapy; however, from the perspective of evidence-based medicine, acupuncture has enough evidence to support its effectiveness in the treatment of autoimmune and central nervous system diseases.

In this case report, we combined acupuncture with the brain function division of Western medicine to select acupuncture points on the patient's head for fire acupuncture treatment to improve symptoms. The Baihui acupoint (GV20) is located at the top of the head and is a common acupuncture point for relieving dizziness, headache, and anxiety, due to the regulatory effect of acupuncture GV20 on the endocrine system, immune system, and nervous system (Deng et al., 2016). Baihui (GV20) and Shenting (GV24) are often used as acupoint combinations to treat nervous system diseases (Zhan et al., 2016; Li S. et al., 2017; Li Y. et al., 2017). The Sishencong is located 1 inch on the left and right sides of the Baihui point. It has a calming and strengthening effect on the mind and plays a major role in promoting sleep, nourishing the spirit, and enhancing memory.

Mild focal seizures and faciobrachial dystonic seizures in anti-LGI1 antibody encephalitis mostly precede memory impairment seizures. Later in the disease course, 63% of patients have tonic-clonic seizures. The initial MRI shows high T74 signals in the hippocampus of 2% of all patients. These lesions regularly evolve into medial temporal lobe sclerosis (44%). However, 80% of patients have a significant response to immunotherapy, leading to early seizure response and slow cognitive recovery (van Sonderen et al., 2016). Although the incidence of anti-LGI1 antibody encephalitis is low and can be controlled by early clinical diagnosis and treatment, its prognosis is not objective, with memory impairment and spatial disorientation (van Sonderen et al., 2017). The recurrence rate of anti-LGI1 antibody encephalitis is 16.2%, and the median time between the first onset and first recurrence is 5 months (Qiao et al., 2021). At more than 2 years of follow-up, the majority of surviving patients report still having mild cognitive impairment, and 86% of patients are affected by persistent amnesia during their disease course. Relapses are common, occurring even 8 years after the initial disease. The two-year morbidity and mortality rates are 19% (van Sonderen et al., 2016). Therefore, timely follow-up after discontinuation of long-term immunotherapy is required to prevent disease progression or recurrence. Western medicine has only a few effective measures for preventing disease recurrence, and symptomatic treatment is an advantage of Western medicine. We performed fire acupuncture treatment for another 1 month after the patient discontinued hormonal drugs and followed up for 1 year, and the patient's condition considerably improved. Acupuncture may have improved this patient's condition; however, more cases and randomized controlled trials are needed to confirm that acupuncture can prevent or reduce recurrence, which is also consistent with the concept of Chinese medicine, "not treating one's own disease before it occurs."

4. Conclusion

Our case demonstrates that scalp fire acupuncture can improve memory and cognitive function in autoimmune encephalitis, attenuate the side effects of hormonal drugs, improve patients' quality of life, and, most importantly, reduce disease recurrence. Scalp fire needling may be an effective, cost-effective, and safe adjuvant treatment; however, further studies with larger sample sizes are needed to investigate its mechanism and efficacy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YL contributed to the conception and design of the study and drafted the manuscript. YG contributed to drafting the text and preparing figures. X-mH, X-lW, J-pL, K-zY, and Y-YL translated, revised, and proofread the manuscript. X-yG, JZ, and LZ collected case data. X-dZ, JW, and Q-gL participated in the acupuncture intervention. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Bauer, J., and Bien, C. G. (2016). Neuropathology of autoimmune encephalitides. *Handb. Clin. Neurol.* 133, 107–120. doi: 10.1016/B978-0-444-63432-0.00007-4

Bien, C. G., Vincent, A., Barnett, M. H., Becker, A. J., Blümcke, I., Graus, F., et al. (2012). Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* 135, 1622–1638. doi: 10.1093/brain/aws082

Cabioğlu, M. T., and Cetin, B. E. (2008). Acupuncture and immunomodulation. *Am. J. Chin. Med.* 36, 25–36. doi: 10.1142/S0192415X08005552

Cai, W., and Shen, W. D. (2018). Anti-apoptotic mechanisms of acupuncture in neurological diseases: a review. *Am. J. Chin. Med.* 46, 515–535. doi: 10.1142/S0192415X1850026X

Criado, M. B., Santos, M. J., Machado, J., Gonçalves, A. M., and Greten, H. J. (2017). Effects of acupuncture on gait of patients with multiple sclerosis. *J. Altern. Complement. Med.* 23, 852–857. doi: 10.1089/acm.2016.0355

Dantzer, R. (2018). Neuroimmune interactions: From the brain to the immune system and vice versa. *Physiol. Rev.* 98, 477–504. doi: 10.1152/physrev.00039.2016

Deng, D., Liao, H., Duan, G., Liu, Y., He, Q., Liu, H., et al. (2016). Modulation of the default mode network in first-episode, drug-naïve major depressive disorder via acupuncture at Baihui (GV20) acupoint. *Front. Hum. Neurosci.* 10:230. doi: 10.3389/fnhum.2016.00230

Doron, A., Rubin, A., Benmelech-Chovav, A., Benaim, N., Carmi, T., Refaeli, R., et al. (2022). Hippocampal astrocytes encode reward location. *Nature* 609, 772–778. doi: 10.1038/s41586-022-05146-6

Dou, J.-M., Huang, C.-Y., Ge, Y.-J., Ji, Y.-Y., Jiang, Z.-D., Wan, Z.-Y., et al. (2016). Analysis of the clinical value of modified scalp acupuncture in rehabilitation treatment of stroke. *World Latest Inf.* 57:175. doi: 10.3969/j.issn.1671-3141.2016.57.139

Dutra, L. A., Abrantes, F., Toso, F. F., Pedroso, J. L., Barsottini, O. G. P., and Hoftberger, R. (2018). Autoimmune encephalitis: a review of diagnosis and treatment. *Arq. Neuro Psiquiatr.* 76, 41–49. doi: 10.1590/0004-282X20170176

Finke, C., Prüss, H., Heine, J., Reuter, S., Kopp, U. A., Wegner, F., et al. (2017). Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurol.* 74, 50–59. doi: 10.1001/jamaneurol.2016.4226

Guo, X., and Ma, T. (2019). Effects of acupuncture on neurological disease in clinical- and animal-based research. *Front. Integr. Neurosci.* 13:47. doi: 10.3389/fnint.2019.00047

Hivert, B., Marien, L., Agbam, K. N., and Faivre-Sarrailh, C. (2019). ADAM22 and ADAM23 modulate the targeting of the Kv1 channel associated protein LGI1 to the axon initial segment. *J. Cell Sci.* 132:jcs219774. doi: 10.1242/jcs.219774

Huang, C. J., Huang, Y. J., and Chen, C. Y. (2013). The developmental origin of fire acupuncture therapy. *Zhongguo Zhen Jiu* 33, 455–458. Chinese. doi: 10.13703/j.0255-2930.2013.05.022

Kim, S. K., and Bae, H. (2010). Acupuncture and immune modulation. *Auton. Neurosci.* 157, 38–41. doi: 10.1016/j.autneu.2010.03.010

Kim, H., Mawla, I., Lee, J., Gerber, J., Walker, K., Kim, J., et al. (2020). Reduced tactile acuity in chronic low back pain is linked with structural neuroplasticity in primary somatosensory cortex and is modulated by acupuncture therapy. *NeuroImage* 217:116899. doi: 10.1016/j.neuroimage.2020.116899

Kornau, H. C., Kreye, J., Stumpf, A., Fukata, Y., Parthier, D., Sammons, R. P., et al. (2020). Human cerebrospinal fluid monoclonal LGI1 autoantibodies increase neuronal excitability. *Ann. Neurol.* 87, 405–418. doi: 10.1002/ana.25666

Lee, M. J., Jang, M., Choi, J., Lee, G., Min, H. J., Chung, W. S., et al. (2016). Bee venom acupuncture alleviates experimental autoimmune encephalomyelitis by upregulating regulatory T cells and suppressing Th1 and Th17 responses. *Mol. Neurobiol.* 53, 1419–1445. doi: 10.1007/s12035-014-9012-2

Lee, J. H., Kyeong, S., Kang, H., and Kim, D. H. (2019). Structural and functional connectivity correlates with motor impairment in chronic supratentorial stroke: a multimodal magnetic resonance imaging study. *Neuroreport* 30, 526–531. doi: 10.1097/WNR.0000000000001247

Li, N., Guo, Y., Gong, Y., Zhang, Y., Fan, W., Yao, K., et al. (2021). The anti-inflammatory actions and mechanisms of acupuncture from acupoint to target organs via neuro-immune regulation. *J. Inflam. Res.* 14, 7191–7224. doi: 10.2147/JIR.S341581

Li, M. K., Li, Y. J., Zhang, G. F., Chen, J. Q., Zhang, J. P., Qi, J., et al. (2015). Acupuncture for ischemic stroke: cerebellar activation may be a central mechanism following Deqi. *Neural Regen. Res.* 10, 1997–2003. doi: 10.4103/1673-5374.172318

Li, S., Tan, J., Zhang, H., Huang, G., Deng, D., and Jiang, Q. (2017). Discussion on rules of acupoint selection for vascular dementia. *Zhongguo Zhen Jiu* 37, 785–790. Chinese. doi: 10.13703/j.0255-2930.2017.07.026

Li, Y., Wang, Y., Liao, C., Huang, W., and Wu, P. (2017). Longitudinal brain functional connectivity changes of the cortical motor-related network in subcortical stroke patients with acupuncture treatment. *Neural Plast.* 2017:5816263. doi: 10.1155/2017/5816263

Li, J., Xu, D., Liu, Y., Cao, Y., He, J., and Liao, M. (2022). Acupuncture treatment of Guillain-Barré syndrome after using immune checkpoint inhibitors: A case report. *Front. Neurol.* 13:908282. doi: 10.3389/fneur.2022.908282

Linnoila, J. J., Rosenfeld, M. R., and Dalmau, J. (2014). Neuronal surface antibody-mediated autoimmune encephalitis. *Semin. Neurol.* 34, 458–466. doi: 10.1055/s-0034-1390394

Newman, M. P., Blum, S., Wong, R. C., Scott, J. G., Prain, K., Wilson, R. J., et al. (2016). Autoimmune encephalitis. Intern. *Med. J.* 46, 148–157. doi: 10.1111/imj.12974

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372:n71. doi: 10.1136/bmj.n71

Plantone, D. (2018). Striatum involvement in LGI1 limbic encephalitis. *Clin. Psychopharmacol. Neurosci.* 16, 508–509. doi: 10.9758/cpn.2018.16.4.508

Qiao, S., Wu, H. K., Liu, L. L., Wang, M. L., Zhang, R. R., Han, T., et al. (2021). Clinical features and long-term outcomes of anti-leucine-rich glioma-inactivated 1 encephalitis: a multi-center study. *Neuropsychiatr. Dis. Treat.* 17, 203–212. doi: 10.2147/NDT.S292343

Qiu, X., Gao, Y., Zhang, Z., Cheng, S., and Zhang, S. (2021). Fire acupuncture versus conventional acupuncture to treat spasticity after stroke: A systematic review and meta-analysis. *PLoS One* 16:e0249313. doi: 10.1152/physrev.00039.2016

Ramberger, M., Berretta, A., Tan, J. M. M., Sun, B., Michael, S., Yeo, T., et al. (2020). Distinctive binding properties of human monoclonal LGI1 autoantibodies determine pathogenic mechanisms. *Brain* 143, 1731–1745. doi: 10.1093/brain/awaa104

Rolls, E. T. (2022). The hippocampus, ventromedial prefrontal cortex, and episodic and semantic memory. *Prog. Neurobiol.* 217:102334. doi: 10.1016/j.pneurobio.2022.102334

Szots, M., Blaabjerg, M., Orsi, G., Iversen, P., Kondziella, D., Madsen, C. G., et al. (2017). Global brain atrophy and metabolic dysfunction in LGI1 encephalitis: a prospective multimodal MRI study. *J. Neurol. Sci.* 376, 159–165. doi: 10.1016/j.jns.2017.03.020

Takahashi, T., Sumino, H., Kanda, T., and Yamaguchi, N. (2009). Acupuncture modifies immune cells. *J. Exp. Clin. Med.* 1, 17–22. doi: 10.1016/S1878-3317(09)60006-1

Torres-Rosas, R., Yehia, G., Peña, G., Mishra, P., del Rocio Thompson-Bonilla, M., Moreno-Eutimio, M. A., et al. (2014). Dopamine mediates vagal modulation of the immune system by electroacupuncture. *Nat. Med.* 20, 291–295. doi: 10.1038/nm.3479

van Sonderen, A., Roelen, D. L., Stoop, J. A., Verdijk, R. M., Haasnoot, G. W., Thijs, R. D., et al. (2017). Anti-LGI1 encephalitis is strongly associated with HLA-DR7 and HLA-DRB4. *Ann. Neurol.* 81, 193–198. doi: 10.1002/ana.24858

van Sonderen, A., Thijs, R. D., Coenders, E. C., Jiskoot, L. C., Sanchez, E., de Bruijn, M. A., et al. (2016). Anti-LGI1 encephalitis: Clinical syndrome and long-term follow-up. *Neurology* 87, 1449–1456. doi: 10.1212/WNL.0000000000003173

Wan, R., Fan, Y., Zhao, A., Xing, Y., Huang, X., Zhou, L., et al. (2022). Comparison of efficacy of acupuncture-related therapy in the treatment of rheumatoid arthritis: A network meta-analysis of randomized controlled trials. *Front. Immunol.* 13:829409. doi: 10.3389/fimmu.2022.829409

Wang, Y. F., Chen, X., Sha, L., Kendrick, K. M., Lee, L. T. O., and Cheng, C. H. K. (2020). Editorial: Neuroendocrine research in health and disease. *Front. Neurosci.* 14:176. doi: 10.3389/fnins.2020.00176

Wattanathorn, J., and Sutalangka, C. (2014). Laser acupuncture at HT7 acupoint improves cognitive deficit, neuronal loss, oxidative stress, and functions of cholinergic and dopaminergic systems in animal model of Parkinson's disease. *Evid. Based Complement. Alternat. Med.* 2014:937601. doi: 10.1155/2014/937601

Widrin, C. (2018). Scalp acupuncture for the treatment of motor function in acute spinal cord injury: a case report. *J. Acupunct. Meridian Stud.* 11, 74–76. doi: 10.1016/j.jams.2018.01.002

Xiao, L. Y., Wang, X. R., Yang, Y., Yang, J. W., Cao, Y., Ma, S. M., et al. (2018). Applications of acupuncture therapy in modulating plasticity of central nervous system. *Neuromodulation* 21, 762–776. doi: 10.1111/ner.12724

Xie, L., Liu, Y., Zhang, N., Li, C., Sandhu, A. F., Williams, G. 3rd, et al. (2021). Electroacupuncture improves M2 microglia polarization and glia anti-inflammation of hippocampus in Alzheimer's disease. *Front. Neurosci.* 15:689629. doi: 10.3389/fnins.2021.689629

Xing, M., Yan, X., Sun, X., Wang, S., Zhou, M., Zhu, B., et al. (2019). Fire needle therapy for moderate-severe acne: A PRISMA systematic review and meta-analysis of randomized controlled trials. *Complement. Ther. Med.* 44, 253–260. doi: 10.1016/j.ctim.2019.04.009

Xiong, J., Zhang, Z., Ma, Y., Li, Z., Zhou, F., Qiao, N., et al. (2020). The effect of combined scalp acupuncture and cognitive training in patients with stroke on cognitive and motor functions. *NeuroRehabilitation* 46, 75–82. doi: 10.3233/NRE-192942

Xu, Y., Hong, S. H., Zhao, X., Wang, S., Xu, Z., Ding, S., et al. (2018). Acupuncture alleviates rheumatoid arthritis by immune-network modulation. *Am. J. Chin. Med.* 46, 997–1019. doi: 10.1142/S0192415X18500520

Yu, C. C., Du, Y. J., Wang, S. Q., Liu, L. B., Shen, F., Wang, L., et al. (2020). Experimental evidence of the benefits of acupuncture for Alzheimer's disease: an updated review. *Front. Neurosci.* 14:549772. doi: 10.3389/fnins.2020.549772

Yue, X. Y., Feng, Z. Q., Yu, X. Y., Hu, J. M., He, X. J., and Shu, S. (2019). Fire-needle acupuncture for upper limb spastic paralysis after stroke: Study protocol for a randomized controlled trial. *J Integr Med.* 17, 167–172. doi: 10.1016/j.joim.2019.03.002

Yun, Y. C., Jang, D., Yoon, S. B., Kim, D., Choi, D. H., Kwon, O. S., et al. (2017). Laser acupuncture exerts neuroprotective effects via regulation of Creb, Bdnf, Bcl-2, and Bax gene expressions in the hippocampus. *Evid. Based Complement. Alternat. Med.* 2017:7181637. doi: 10.1155/2017/7181637

Zeidman, P., and Maguire, E. A. (2016). Anterior hippocampus: the anatomy of perception, imagination and episodic memory. *Nat. Rev. Neurosci.* 17, 173–182. doi: 10.1038/nrn.2015.24

Zhan, J., Pan, R., Guo, Y., Zhan, L., He, M., Wang, Q., et al. (2016). Acupuncture at Baihui (GV 20) and Shenting (GV 24) combined with basic treatment and regular rehabilitation for post-stroke cognitive impairment: a randomized controlled trial. *Zhongguo Zhen Jiu* 36, 803–806. Chinese. doi: 10.13703/j.0255-2930.2016.08.007

Zhang, J., Lu, C., Wu, X., Nie, D., and Yu, H. (2021). Neuroplasticity of acupuncture for stroke: an evidence-based review of MRI. *Neural Plast.* 2021:2662585. doi: 10.1155/2021/2662585

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Case report: Cheek acupuncture exhibits an immediate effect in relieving severe pain associated with nerve compression or damage of central nervous system and its potential mechanism of action

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Peripheral nerve compression or permanent damage of central nervous system (CNS) can trigger severe neuralgia to patients. Analgesic medicine or even surgery to remove nerve compression is commonly used for pain relief. But these treatments either are ineffective, have side-effect or can cause subsequent complications. Acupuncture, a technique that has been widely used in China and other Asian countries for thousands of years, is an alternative to relieve pain, although the mechanism of action is not fully understood. In this study, two patients who had symptoms of severe neuralgia associated with peripheral nerve compression or permanent damage/dysfunction of CNS and analgesic medicines are ineffective, underwent cheek acupuncture, a new technique established recent years by the author with the features of painless, standardization, simplicity, and precision. An immediate analgesic effect of the cheek acupuncture was observed without any side effects, and clinical remission was achieved after several sessions of treatments. It suggests that this new approach is an efficient alternative for pain relief induced by nerve impairment. The authors proposed a biological holographic model of triplet homunculi existing at the level of the local cheek, spinal cord, and cerebral cortex, to explain the immediate and accurate analgesic effect of the cheek acupuncture. These homunculi have the same structure, and synchronized sensations and actions that are mediated by afferent and efferent neurons, as the integrated human body. Therefore, the nociception and needling signals are sensed, transmitted, analyzed, and manipulated cooperatively and simultaneously among these homunculi with the subsequent pain relief in the body.

KEYWORDS

pain, central nerve system, nerve compression, cheek acupuncture, mechanism of action

Highlights

Relieving severe pain resulting from peripheral nerve compression or permanent damage/dysfunction of the CNS is a major challenge for public health. Analgesic medicines or even surgery are commonly used for pain relief; however, these methods either are ineffective, have side effects or can cause subsequent complications. Thus, it is essential to pursue an alternative approach to relieve the pain associated with nerve impairment. Acupuncture has been applied for thousands of years in China for pain relief or other diseases. But more than 300 acupoints of traditional acupuncture make it complicated to learn and manipulate. This study reports that cheek acupuncture, a new technique established by the author, has immediate and accurate analgesic effect on the pain associated with a disorder of the nervous system, and is almost painless during needling, when analgesic medicines are ineffective. This technique contains only 16 standard acupoints on each cheek, whose orientation is precisely determined based on the anatomic structure of the skull. Therefore, cheek acupuncture represents an efficient strategy to relieve the pain induced by the impairment of the nervous system. We propose a biological holographic model of triplet homunculi located on the cheek, spinal cord, and CNS to interpret the immediate and accurate analgesia of this technique.

Introduction

Pain is "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al., 2020), which is experienced by everyone in their lifetime. Based on the duration or suffering time, pain can be divided into acute and chronic pain. Acute pain can be considered as the normal physical response of the body to a stimulus and is relieved once the body recovers (Ashburn and Staats, 1999; Carr and Goudas, 1999), while chronic pain is defined as pain lasting for more than three to six months (Treede et al., 2019). It is estimated that almost half of the patients pursuing for medical care each year in the USA are due to pain, and that one in four Americans suffers from chronic or recurrent pain (Turk and Melzack, 2011). Similarly, chronic pain is diagnosed in nearly 20% of Europeans based on a survey among 16 countries (Breivik et al., 2006). Pain severely affects the quality of life and the ability to work of patients and can even cause psychological disorders. Therefore, pain and its treatment are major challenges in clinical medicine and public health.

Nerve pain (neuropathic pain or neuralgia) is a particular type of pain with an electric shooting-, stabling- or burning-like sensation. Peripheral nerve compression/pinching or damage of the CNS is one of the common causes of this disease. Various disorders can cause the compression or damage of nerves, including accidents and trauma, infection/inflammation, bone fractures, herniated discs, surgical complications, tumors, cerebral hemorrhage, and ischemic stroke, etc (Bendszus and Stoll, 2005). Nerve compression or pinching can last for several days to a couple of years. But severe and persistent compression of peripheral nerve or CNS damage can eventually lead to severe pain, irreversible muscle loss, and permanent nerve damage with subsequent dysfunction of the tissues or organs.

Despite surgical decompression of compressed nerves or improvement of blood supply in the brain of patient with stroke, prescription and non-prescription analgesics, including non-steroidal anti-inflammatory drugs (NSAIDs), steroids, opioids, paracetamol, COX-2 inhibitors, and more, have been widely used to relieve pain (Breivik et al., 2006). However, long-term use of these medicines can cause side effects such as addiction and resistance. Additionally, the pharmacological strategy is often ineffective, particularly for severe neuralgia, leading to sustained or recurrent pain in many cases (Breivik et al., 2009). For these reasons, more and more patients seek non-medication treatments such as massage and physical therapy to relieve pain. Among these alternatives, acupuncture has been widely used for thousands of years in China and other Asian countries for pain relief and other diseases. In 1979, a symposium on acupuncture organized by the World Health Organization (WHO) recommended 43 diseases suitable for acupuncture therapy (World Health Organization, 1979). Since then, the analgesic effect of this technique has been approved by several national institutes of health (NIH), such as the NIH of the US in 1997 (National Institutes of Health, 1997), and more clinical trials on acupuncture have been conducted worldwide for hundreds of diseases or conditions including pain relief (World Health Organization, 2002).

Microneedling therapy belongs to one of the acupuncture methods, involving the needling of a special organ's local surface or a specific location on the body to treat diseases initially occur in another part of the body. The principle of facial acupuncture, the first microneedling system, was described thousands years ago in "The Yellow Emperor's Classic of Internal Medicine (also referred to as Huangdi Neijing)—Miraculous Pivot: Chapter of Five Colors." Nowadays, various microneedling systems have been established and widely used in China and other countries, including scalp acupuncture, ear acupuncture, abdominal acupuncture, wrist-ankle acupuncture, and cheek acupuncture, etc (Xu et al., 2019). In particular, cheek acupuncture developed by Wang (2017), has been confirmed to effectively and immediately relieve pain associated with a range of diseases (Wang et al., 2000; Ren et al., 2014; Wang, 2017; He and Li, 2018; Cai et al., 2020; Ding et al., 2020; Sun et al., 2022).

In this study, we report on cheek acupuncture used to alleviate severe neuralgia caused by peripheral nerve compression or permeant damage/dysfunction of CNS in patients with an immediate analgesic effect. The potential mechanism of action of this new technique is also discussed.

Case description

Case 1

In April 2020, a 57-year-old female patient initially experienced a sudden onset of a right-sided limb movement disorder and a lack of fluency in speech. The cephalometric CT scan at a local hospital showed thalamic hemorrhages. After conservative treatment, her speech function recovered, but she still felt poor physical strength and had an abnormal gait. She was then admitted to Beijing United Rehabilitation Hospital for further rehabilitation in March 2021. Two months after hospitalization, the patient developed moderate-to-severe pain [visual analog scale (VAS) 6–9] on the right side of her body, including the face, arm, trunk, and limbs, with no apparent cause. The pain was persistent and tightness-like, accompanied by paroxysmal pinprick-like pain occurring 30–50 times per day. The patient felt a painful wandering mass on the right plantar aspect of her foot and was unable to put weight on the right foot due to the pain. She also experienced paroxysmal knife-like pain (VAS 9) in the right upper arm, and moderate-to-severe tearing-like pain (VAS 6–9) in the right axilla and lateral chest wall. The magnetic resonance imaging (MRI) showed a malacic lesion in the left thalamus, and chronic ischemic lesions in the brainstem, bilateral basal ganglia, and periventricular white matter. Multiple white matter lesions in bilateral periventricular and subcortical white matter were observed, and Fazekas 3 was considered (Figure 1).

She was prescribed oral medications, including Gabapentin® (1,200 mg, three times a day), Duloxetine® (60 mg, once a day), Neurotropine® (8 u, twice a day), Tylenol® (1 tablet, as needed), and received traditional acupuncture, herbal medicine, and physical therapy. However, the pain was not alleviated. The patient frequently woke up at night due to pain outbreaks, which severely influenced her mood, sleep, and her ability to cooperate with rehabilitation treatment. Thus, she sought a consultation from the Division of Pain for further treatment.

During the consultation, the patient exhibited slower speech. Her right side of the body was hyperalgesic and could not tolerate cold when wiped with an alcohol swab. The muscle strength of the proximal right limb was grade 4, and the distal limb was grade 4^{-}. The patient's right hand was unable to hold chopsticks or use a pen due to the contracture of the 3rd-5th fingers. Pressing pain was identified in the cervical spinous processes (C2–7), right transverse processes, and coracoid process of the right scapula. Similar symptoms were also detected in the right sternocleidomastoid, trapezius, and pectoralis major muscles. The abdomen was tense with pressing pain in the upper abdomen. The bilateral iliopsoas muscles, upper and lower back, and right hip experienced pressing pain. The patient also reported pressing pain in the posterior leg and on the right plantar.

After the first treatment with cheek acupuncture (Figure 2), the patient experienced significant relaxation on the right side of her body and a 60% reduction in spontaneous pain. The painful mass in the right foot shifted to the heel, decreasing in size and pain level. The patient could tolerate the pain in the right foot triggered by touching the ground. The first four sessions were administered once a week, followed by treatments every two weeks. The symptoms continuously improved during the treatments. Eight sessions later, the spontaneous pain in the right limb and trunk was reduced by 70–80%, and paroxysmal pinprick-like pain episodes occurred less than five times a day, lasting 2–3 seconds each time. There were no more nocturnal eruptive pain episodes. The patient only took Gabapentin 900 mg three times a day. The patient could stand only on right leg. The contracture of the right hand significantly improved, and the patient could perform certain fine activities, such as eating with chopsticks and writing. The walking gait was much better than before. Importantly, the patient's mood and sleep improved significantly, which facilitated rehabilitation training.

Case 2

A 46-year-old female is a full-time mother and has been engaging in physical exercise for 2 h a day before sick, mainly to strengthen her abdominal, back, and gluteal muscles. She experienced on March 8, 2021 with severe pain in the right buttock, radiating to the area between the perineum and anus. The pain in the right gluteal region was moderate to severe, sharp pain (VAS 7–10). The radicular pain in the perineum was burning-like and electric shooting-like. Additionally, the patient experienced a slow onset of urination, anal swelling, and an increase in the frequency of stools (3–4 times a day). Turning over were difficult and lying down was in restricted positions due to the pain, which severely affected her sleep. She required regular walks or extra analgesic medicine at night to alleviate the pain and for sleep. An ultrasound of the superficial hip and a pelvic CT scan conducted in another hospital did not reveal any abnormalities. Blood sedimentation, C-reactive protein, and blood routine parameters were within the normal range. The patient had no significant medical history.

Over the last two weeks, she consulted several hospitals and took various analgesic medicines, including Imrecoxib®, Aescuven®, Mecobalamin®, Tizanidine®, TaiLeNing®, and Pregabalin®, which provided slight relief, but she still experienced moderate to severe pain (VAS 7–9).

The patient came for consultation (March 23, 2021) in our service with a limp. Physical examination revealed a positive Patrick's test, increased tone of the right rectus abdominis muscle, pressing pain on the right pubic tuberosity with evoked right perineal pain. Pressing pain was also detected in the right iliopsoas muscle, with radicular pain extending to the perineal area, as well as in the right lesser trochanter and right gluteal region (between the right sacrum and the right greater trochanter). Increased tone of the right erector spinae muscle and pain sensitization in the right perineal area were also observed. The pudendal neuralgia was considered.

Following cheek acupuncture, the burning-like pain in the right perineal area and the right hip pain disappeared, and the hip pressure pain was relieved by 90%. At the follow-up after treatment, the patient occasionally experienced mild electric shooting-like pain (VAS 2–3) in the right perineal area about 0–1 time per hour, along with mild deep hip pressure pain. On the fifth day after treatment, she discontinued the analgesic medications Imrecoxib®, Aescuven®, Mecobalamin®, Tizanidine®, and TaiLeNing®, resulting in a slight increase in resting pain (VAS 2–3), hip pressure pain (VAS 6), and occasional mild electric shooting-like pain in the perineal area.

On the seventh day (March 30, 2021) after the initial acupuncture, an ultrasound was performed to measure the thickness of the piriformis muscle of the hip before the second session of acupuncture. The ultrasound probe was placed between the sacrum and the greater trochanter of the femur, where two layers of muscles, the gluteus maximus and piriformis muscles, were observed (Figure 3A). The piriformis muscle was identified by rotating the leg internally and externally. Color Doppler was used to determine the position of the inferior gluteal artery, next to which the thickness of the piriformis muscle was measured as 1.18 cm (left side, non-injured) and 2.05 cm (right side, injured) (Figures 3A, B), respectively. Then, cheek acupuncture treatment was performed for 30 mins (Figure 3C). After the treatment, the patient no longer experienced pain in the right perineal region and the right gluteus, and the gluteal pressure pain was relieved by 70%. Ultrasound showed the thickness of the piriformis muscle in right side decreased to 1.31 cm (Figure 3D). Follow-up on the

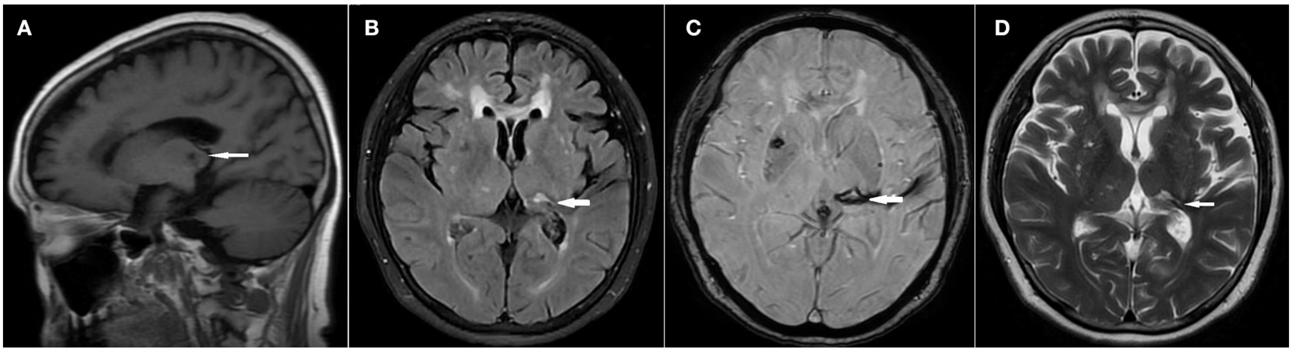

FIGURE 1
MRI without contrast showed ischemic stroke. The arrows indicated lesion of malacic at the left thalamus. **(A)** Saggital T1WI. **(B)** Axial T2 FLAIR. **(C)** 3D SWAN. **(D)** Axial T2WI.

FIGURE 2
The selected acupoints for the first acupuncture treatment. **(A)** Acupoints were used on the cheek of left side. **(B)** Acupoints were selected on the right cheek.

third day after the second acupuncture session showed that the patient was pain-free and able to move without any positional limitations. Mild electric shooting-like pain occurred occasionally in the perineal area.

Acupuncture manipulation

The acupuncturist has a couple of years of experience in cheek acupuncture. Prior to performing acupuncture manipulation, a thorough physical examination of the patients was required to accurately localize the pain in the body. This helped in identifying the specific muscle(s) and soft tissues that were injured, and/or the nerve was compressed or influenced. The corresponding acupoint(s) on the cheek and the appropriate size of acupuncture needles were then selected for the acupuncture procedure. Once the needles were inserted, the analgesic effect was reevaluated through physical examination. If the pain did not fully or only partially alleviate, the depth and direction of needling needed to be adjusted, or additional needles closer to the acupoint were required until complete or significant pain relief was achieved. For each treatment, specific acupoints were selected based on the results of the physical examination. Generally, the needles were left in place for 30 mins during each session, and treatments were performed once every 1–2 weeks depending on the progress of the symptoms. Table 1 presents the standard acupoints of cheek acupuncture, their orientations, applications for various diseases, and acupoints that have been used (all sessions) for two patients in the present study.

Discussion

For neuropathic pain induced by damage to the CNS or peripheral nerve compression, the application of analgesic medicine or even decompression of the compressed nerve by surgery has been utilized. However, these approaches are either ineffective, have side effects, or can lead to subsequent complications. Specifically, in the present study, cheek acupuncture proved to be highly effective in alleviating severe pain resulting from both peripheral nerves compression and permanent damage/dysfunction to the CNS. In contrast, the analgesic medicines had limited effect, as described in the present study. Interestingly, cheek acupuncture provided immediate pain relief post-acupuncture and achieved clinical remission after several sessions. Additionally, cheek acupuncture is nearly painless compared to classical acupuncture, which often cause sensations of soreness, numbness, swelling, and pain at the site of needling. Thus,

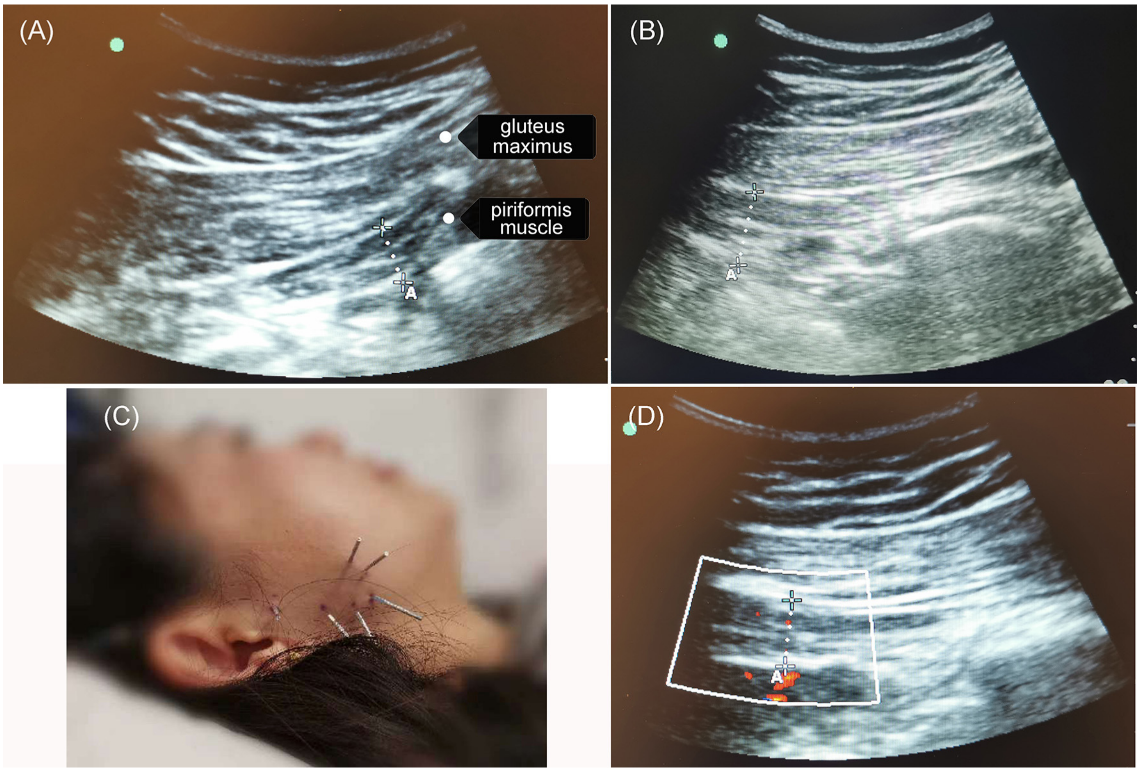

FIGURE 3
The cheek acupuncture reduced the thickness of the piriformis muscle. On day 7 after the first acupuncture session, an ultrasound was performed to measure the thickness of the piriformis muscle of the hip before and after the second session of treatment. **(A)** The piriformis muscle in the left hip (healthy) was 1.18 cm. **(B)** Before the second session of acupuncture, the thickness of the piriformis muscle in the right hip (injured) was 2.05 cm. **(C)** The selected acupoints for acupuncture treatment. **(D)** The piriformis muscle in the right hip (injured) decreased to 1.31 cm post-second treatment.

cheek acupuncture represents an efficient approach for relieving neuropathic pain, although substantial number of cases are still required to confirm its effect on neuralgia. However, the collective data from the cheek acupuncture team, widely distributed and practiced in China and abroad, have demonstrated its remarkable efficacy in relieving pain resulting not only from nerve disorders but also from other diseases (Wang et al., 2000; Ren et al., 2014; Wang, 2017; He and Li, 2018; Cai et al., 2020; Ding et al., 2020; Sun et al., 2022).

The cheek acupuncture technique was established by Dr. Yongzhou Wang. The core of this technique incorporates the Zang-fu viscera, the meridian theories, and the San Jiao (also known as the three energizers) theory of traditional Chinese medicine (TCM) (Wang, 2017). The author developed three major theoretical systems for cheek acupuncture: Da San Jiao, holography, and physical/mental integration. These systems are based on the physical-mental theory of Western medicine, the biological holographic theory developed by Zhang (1985), the anatomical structure of the human body, and the Qi pathway of TCM. Therefore, the indications for cheek acupuncture mainly focus on diseases occurring at these three levels.

In addition to its immediate pain-relieving effect and painless manipulation, the cheek acupuncture system utilizes only 16 acupoints (Wang, 2017), which is significantly fewer than the conventional acupuncture of TCM, which contains more than 300 acupoints. The orientation of these 16 acupoints on the buccal area is precisely determined according to the anatomical structure of the skull. Cheek acupuncture can be applied to relieve a wide range of pains induced by trauma, sports injuries, infection/inflammation, cancer, muscle injuries, post-operation recovery, and nerve compression/damage as described in this study (Wang, 2017). Furthermore, cheek acupuncture not only relieves local pain such as headaches and toothaches but also addresses pain in other parts of the body, including the neck, shoulders, extremities, back, lumbar area, and abdomen, regardless of whether the pain is acute or chronic, although the precise mechanism of action for pain relief by cheek acupuncture remains unclear.

Numerous studies have been conducted to investigate the mechanism through which acupuncture relieves pain [see review (Chen et al., 2020)], particularly in China in1960s and 1970s. The first national conference of acupuncture anesthesia research work held in Shanghai in February 1966 proposed that acupuncture could induce the release of potential molecules to relieve operative pain (Li, 2022). These molecules were later identified as endogenous opiate-like substances (OLS) and endorphin in 1970s by Chinese and American researchers, respectively. Han et al. further demonstrated that OLS in nucleus accumbens, amygdala, habenula, and periaqueductal gray (PAG) played a pivotal role in acupuncture analgesia (Han et al., 1980), and that cholecystokinin octapeptide (CCK8) acted as endogenous anti-opioid substance during acupuncture analgesia (Han et al., 1985). The interaction of endorphin with the opioid receptor, inhibiting

TABLE 1 Standard acupoints of cheek acupuncture, their main applications and acupoints used in the present study for pain relief.

Name	Orientation	Applications	Used for patients in this report
Head point	1 inch above the upper edge of the middle point of the zygomatic arch	Headache, dizziness, toothache, insomnia, stress, anxiety, depression, stroke, etc.	P1
Upper energizer point	The cross of the posterior coronoid of the mandible and the lower edge of the zygomatic arch	Headache, cervical pain, chest pain, chest tightness, breast swelling and pain, tachycardia, arrhythmia, asthma, etc.	P1
Middle energizer point	The middle point of the connecting line between the upper and lower energizer acupoints	Stomach cramp, acute/chronic gastritis, heartburn with acidity,	P1
Lower energizer point	Anterior oblique line of the mandible	Abdominal bloating and pain, colitis, dysmenorrhea, pelvic inflammatory disease, menstrual irregularities, leukorrhea, gynecological disease	P1
Cervical point	Top edge of the root of the zygomatic arch	Neck pain, stiff neck after sleeping, cervical spondylosis, sore throat, dizziness, stress, scalene spasm, tinnitus, etc.	P1
Dorsal point	The cross of the lower edge of the zygomatic arch and the inferior capsule of the temporomandibular joint	Back pain, rhomboid muscle strain, chest tightness, shortness of breath, stomachache, heart palpitations, etc.	P1, P2
Lumbar point	The middle of the connecting line between dorsal and sacral points	Lower back pain, lumbar muscle strain, acute lumbar sprains, sciatica pain, herniated disc, etc	P1, P2
Sacral point	0.5 inch to the anterior & superior angle of the mandible	Sacrospinous muscle strain, lower back pain in women, injuries of sacroiliac ligament, bedwetting, prostatitis, etc.	P1, P2
Shoulder point	The middle point of the temporozygomatic sature	Shoulder pain, frozen shoulder, tendonitis of biceps brachii, synovitis of infra-acromion of scapula, tendonitis of supraspinatus muscle, etc.	P1
Elbow point	The middle point of the connecting line between the lateral canthus and the bottom of the zygomatic bone	Elbow pain, tennis elbow, golf elbow, wrist extensor tendonitis, wrist flexor tendonitis, etc.	
Wrist point	The point of nasolabial folds at the horizon level of the lower edge of nostrils	Wrist pain, injuries of wrist joint, carpal tunnel syndrome, finger pain	
Hand point	The middle of the connecting line between the middle point of the lower edge of nostril and vermilions border	Finger arthritis, tenosynovitis, finger numbness, hand numbness	
Hip point	1 inch of anterior & superior of the angle of the mandible on the masseteric tuberosity	Sciatica pain, wound-induced hip osteoarthritis, injury of piriform muscle, groin pain	P1, P2
Knee point	The middle point of the connecting line between the angle of the mandible and the chengjiang point	Knee pain, superficial fibular nerve pain, arthritis of knee joint, hamstring muscle injury, gastrocnemius muscle spasm, etc.	P1
Ankle point	1/3 proximity of the connecting line between the knee and Chengjiang points	Ankle joint sprain, ankle joint swelling and pain, ankle arthritis, Achilles tendinitis, heel pain	
Foot point	0.5 inch lateral to the Chengjiang point	Gout, metatarsals fascia sprain, plantar fasciitis, heel pain, toe pain	P1

P1, patient #1; P2, patient #2.

the release of tachykinins (key proteins for pain transmission), is one widely accepted explanation for pain relief by acupuncture (Han, 2003; Sprouse-Blum et al., 2010). It has also been proposed that acupuncture-elicited endogenous opioids, serotonin, and norepinephrine released by immune cells at the local position and spinal level mediate persistent pain relief by desensitizing peripheral spinal nociceptor and by reducing the phosphorylation of the spinal N-methyl-D-aspartate receptor, respectively (Zhang et al., 2014; Lin et al., 2022). In line with this hypothesis, naloxone, an opioid antagonist, attenuated the analgesia effect of electro-acupuncture (Pomeranz and Chiu, 1976). Animal studies also have shown that adenosine, derived from rapid degradation of adenosine triphosphate (ATP) released by needle stimulation, may mediate the analgesic effect by binding to adenosine receptor (Tang et al., 2019). Other reports have also demonstrated that cheek acupuncture decreased the levels of 5-hydroxytryptamine (5-HT) and noradrenaline (NE) in patients with operative pain and in a rabbit model of rheumatoid arthritis (RA) respectively (Pu et al., 2018; Sun et al., 2022), indicating the potential involvement of both molecules in pain relief by acupuncture.

In addition to above neurochemical mechanism, the neurophysiological pathway is also considered to mediate pain relief by acupuncture. Similar to the transmission pathway of nociception and heat signals, it is known that the afferent

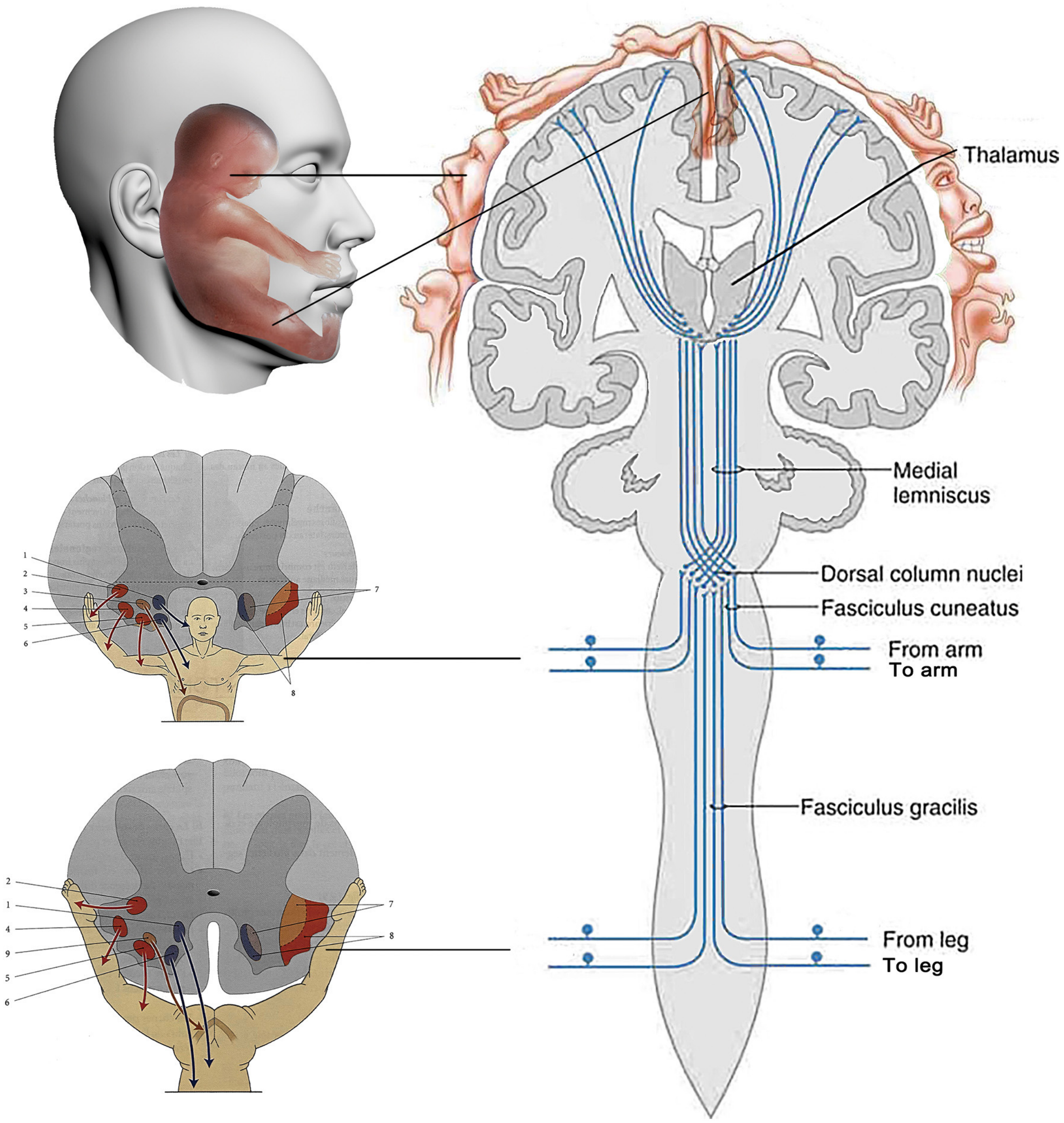

FIGURE 4
The simplified model of the mechanism of action of cheek acupuncture. The existence of biological holographic embryos (homunculi) as triplets at the levels of the local cheek, spinal cord, and cerebral cortex, which are the same as integrated body, is proposed. The simultaneous communication among three homunculi helps the integrated body sense pain signal and respond to needling, as well as transmit feedback and intervention signals, ultimately resulting in pain relief. The following are the labeled components of the model: 1. posterior-medial nucleus; 2. retro-posterolateral nucleus; 3. phrenic nuclei; 4. posterior-lateral nucleus; 5. anterolateral nucleus; 6. anteromedial core; 7. flexor muscle medulla; 8. extensor muscle medulla; 9. perineal core.

signal from needling is transmitted to the spinal cord through the lateral spinothalamic tract, providing a structural basis for the potential interaction between afferent nociception and needling signals. In these models, the signal of needling stimulation is considered to be manipulated alongside the pain signal from the original injury in the body at different levels of the nervous system, including the spinal cord, brainstem, thalamus and cerebral cortex, respectively. Therefore, the afferent nociception to the CNS is down-regulated or the efferent pulse of inhibition from the CNS, activated by the afferent signals, is transmitted, resulting in pain relief. However, these mechanisms of action need to be further confirmed. Nevertheless, neither neurochemical nor neurophysiological mechanisms can fully explain the immediate and precise effect of cheek acupuncture on pain relief.

In the present study, we propose the biological holographic model to explain the immediate and precise analgesic effect of cheek acupuncture. In this model, we assume the existence of biological holographic embryos (homunculi) as triplets at the levels of the local cheek, spinal cord, and cerebral cortex. These homunculi have the same structure, organization, synchronized sensations and actions, as the integrated human body, which are mediated through afferent & efferent neurons. Nociception from local body injury is sequentially sensed, positioned, and analyzed by the homunculus localized at higher levels of the nervous system, including the cerebral cortex. Upon needle insertion, the buccal homunculus senses the needling stimulation via facial and trigeminal nerves distributed on the cheek. It immediately and directly "informs" homunculus in the cerebral cortex through afferent nerve fibers of the facial and trigeminal nerves, where the needling and nociception signals are communicated and analyzed. The feedback signal from the cerebral cortex is then transmitted directly to the homunculus in the buccal area via efferent fibers of the facial and trigeminal nerves. The intervention signal from cerebral cortex "informs" the homunculus in the spinal cord before precisely targeting the original injured site through efferent nerve fibers, resulting in analgesic, anti-inflammatory, tissue repair, and immune modulation effect. All these processes occur almost simultaneously among the homunculi and the integrated body. The simplified model is demonstrated in Figure 4. Our proposed model easily explains the immediate pain relief experienced by the integrated body once the needles are inserted at specific acupoint(s) of the homunculus on the cheek, corresponding to the localization of pain on the integrated body, regardless of whether the pain occurs locally or distantly in the body. Our proposed triplet homunculi model is supported by the evidence from: (1) the biological holographic embryo theory established by Prof. Yiqing Zhang in 1985 (Zhang, 1985); (2) the cloning of Dolly sheep using genomic DNA isolated from somatic cells, where the similar concept of biological holographic embryo was applied (Wilmut et al., 1997); (3) confirmation of homunculus on the cheeks and somatotopic homunculus in the cerebral cortex using precision functional magnetic resonance imaging (fMRI) methods (Wang, 2017; Gordon et al., 2023).

Conclusion

This report introduces a new approach referred to as cheek acupuncture for relieving severe pain in two patients with either peripheral nerve compression or permanent damage/dysfunction to the CNS, when analgesic medicines were ineffective for both individuals. An immediate and accurate analgesia by cheek acupuncture was observed in these two cases, which is consistent with our collective data on pain relief by this technique (Wang et al., 2000; Ren et al., 2014; Wang, 2017; He and Li, 2018; Cai et al., 2020; Ding et al., 2020; Sun et al., 2022). Both patients achieved a clinical remission after 8 and 2 sessions of treatments, respectively. Complete recovery for case 2 and more improvement for case 1 may be achieved if more sessions of cheek acupuncture are provided.

Nevertheless, the features of standard acupoints, simplicity for manipulation, and accurate and immediate analgesia, make cheek acupuncture an efficient approach for relieving the pain suffered from disorders of the nervous system or other diseases. A biological holographic model of triplet homunculi is proposed to explain the mechanism of action of this technique.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving human participants in accordance with local legislation. Written informed consent was obtained from individuals to participate in this study and for the publication of any potentially identifiable images or data included in this article.

Author contributions

The cheek acupuncture was manipulated by LY. YWa and YWu designed the study. The manuscript was written by YWu.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Ashburn, M. A., and Staats, P. S. (1999). Management of chronic pain. *Lancet* 353, 1865–1869. doi: 10.1016/S0140-6736 (99)04088-X

Bendszus, M., and Stoll, G. (2005). Technology insight: visualizing peripheral nerve injury using MRI. *Nat. Clin. Pract. Neurol.* 1, 45–53. doi: 10.1038/ncpneuro0017

Breivik, H., Cherny, N., Collett, B., de Conno, F., Filbet, M., Foubert, A. J., et al. (2009). Cancer-related pain: a pan-European survey of prevalence, treatment, and patient attitudes. *Ann. Oncol.* 20, 1420–1433. doi: 10.1093/annonc/mdp001

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur. J. Pain* 10, 287–333. doi: 10.1016/j.ejpain.2005.06.009

Cai, M., Wu, Z., and Guo, Z. (2020). Clinical study of 30 cases of mastocytosis treated with cheek acupuncture. *Jiangsu J. Tradit. Chinese Med.* 52, 68–70. doi: 10.19844/j.cnki.1672-397X.2020.12.025

Carr, D. B., and Goudas, L. C. (1999). Acute pain. *Lancet.* 353, 2051–2058. doi: 10.1016/S0140-6736 (99)03313-9

Chen, T., Zhang, W., Chu, Y., and Wang, Y. (2020). Acupuncture for pain management: molecular mechanisms of action. *Am. J. Chin. Med.* 48, 793–811. doi: 10.1142/S0192415X20500408

Ding, X., Zhang, B., Ouyang, Q., Xie, Z., Chen, Y., and Lv, X. (2020). Clinical observation of buccal needle treatment for soft tissue injury of low back caused by military training. *Med. J. Nat. Defend. Forces Northwest China* 41, 379–382. doi: 10.16021/j.cnki.1007-8622.2020.06.012

Gordon, E. M., Chauvin, R. J., Van, A. N., Rajesh, A., Nielsen, A., Newbold, D. J., et al. (2023). A somato-cognitive action network alternates with effector regions in motor cortex. *Nature* 617, 351–359. doi: 10.1038/s41586-023-05964-2

Han, J. S. (2003). Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci.* 26, 17–22. doi: 10.1016/S0166-2236 (02)00006-1

Han, J. S., Ding, X. Z., and Fan, S. G. (1985). Is cholecystokinin octapeptide (CCK-8) a candidate for endogenous anti-opioid substrates? *Neuropeptides* 5, 399–402. doi: 10.1016/0143-4179 (85)90038-1

Han, J. S., Tang, J., Ren, M. F., Zhou, Z. F., Fan, S. G., and Qiu, X. C. (1980). Central neurotransmitters and acupuncture analgesia. *Am. J. Chin. Med.* 8, 331–348. doi: 10.1142/S0192415X80000311

He, G., and Li, Y. (2018). Clinical efficacy of cheek acupuncture therapy in treating 90 cases of cervical spondylosis. *Clinic. Res. Pract.* 3, 127–128. doi: 10.19347/j.cnki.2096-1413.201821059

Li, J. W. (2022). *The History of Traditional Chinese Medicine (revised version)*. China: Hainan Press.

Lin, J. G., Kotha, P., and Chen, Y. H. (2022). Understandings of acupuncture application and mechanisms. *Am J Transl Res* 14, 1469–1481.

National Institutes of Health. (1997). Acupuncture. *NIH Consens State*. 15, 1–34.

Pomeranz, B., and Chiu, D. (1976). Naloxone blockade of acupuncture analgesia: endorphin implicated. *Life Sci.* 19, 1757–1762. doi: 10.1016/0024-3205 (76)90084-9

Pu, R. S., Fang, X. L., Jie, W. J., Liu, D. L., and Su, C. H. (2018). Experimentally investigating the effect of buccal acupuncture on analgesic time-effect characteristics and monoamine neurotransmitters. *J. Acupunct. Tuina Sci.* 16, 229–235. doi: 10.1007/s11726-018-1055-x

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., et al. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain* 161, 1976–1982. doi: 10.1097/j.pain.0000000000001939

Ren, C. Z., Fang, X. L., and Du, X. Z. (2014). Combination of cheek acupuncture and physical therapy in treating acute lumbar strain with 56 cases. *Chinese Acupunct. Moxibust.* 34, 245–246.

Sprouse-Blum, A. S., Smith, G., Sugai, D., and Parsa, F. D. (2010). Understanding endorphins and their importance in pain management. *Hawaii. Med. J.* 69, 70–71.

Sun, Y., Zhou, Q., Xiao, J. Y., and Cheng, Z. G. (2022). The effect of buccal acupuncture on the postoperative visceral pain in patients undergone gynecological laparoscopic surgery and its action mechanism. *Jiangxi Med. J.* 57, 584–586. doi: 10.3969/j.issn.1006-2238.2022.06.009

Tang, Y., Yin, H. Y., Liu, J., Rubini, P., and Illes, P. (2019). P2X receptors and acupuncture analgesia. *Brain. Res. Bull.* 151, 144–152. doi: 10.1016/j.brainresbull.2018.10.015

Treede, R. D., Rief, W., Barke, A., Aziz, Q., Bennett, M. I., Benoliel, R., et al. (2019). Chronic pain as a symptom or a disease: the iasp classification of chronic pain for the international classification of diseases (ICD-11). *Pain* 160, 19–27. doi: 10.1097/j.pain.0000000000001384

Turk, D. C., and Melzack, R. (2011). "The measyrement of pain and the assement of peope experiencing pain," in *Handbook of Pain Assessment,* ed. D.C.T.a.R. Melzack. (New York: The guilford Press), 3–18.

Wang, Y. Z. (2017). *Cheeks Acupuncture Therapy.* Beijing, China: People's Health Publishing House Co. Ltd

Wang, Y. Z., Wang, H. D., Fang, X. L., Qu, B. P., Zhao, J. X., and Ma, M. F. (2000). Application of buccal acupuncture in clinical pain relief. *Chinese Acupuncture and Moxibustion S*1, 2.

Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., and Campbell, K. H. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* 385, 810–813. doi: 10.1038/385810a0

World Health Organization. (1979). "Acupuncture," in *World Health*, ed J. Bland (Geneva: World Health Organization), 1–32.

World Health Organization. (2002). *Acupuncture: Review and Analysis of Reports on Controlled Clinical Trials*. Geneva: World Health Organization.

Xu, J., Shi, J., Wang, J., and Jia, C. (2019). Clinical application rules of different micro needle system therapy. *World J. Acupunct. Moxibustion* 29, 3. doi: 10.1016/j.wjam.2019.12.008

Zhang, R., Lao, L., Ren, K., and Berman, B. M. (2014). Mechanisms of acupuncture-electroacupuncture on persistent pain. *Anesthesiology* 120, 482–503. doi: 10.1097/ALN.0000000000000101

Zhang, Y. (1985). *Holographic Biology—The Research on Holographic Biology*. China: Shandong University Press.

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The effects of acupuncture on sleep disorders and its underlying mechanism: a literature review of rodent studies

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Sleep is a set of physiological processes mainly under neurobiological regulation that affect several physiological systems, and sleep disorders are a condition where normal sleep patterns are disturbed. Clinical studies have confirmed the effects of acupuncture on sleep duration and quality. Although many studies have explored the therapeutic effects of acupuncture on sleep disorders, the mechanisms are unclear. We investigated the mechanism of acupuncture efficacy in a rodent model of sleep disorders and evaluated the therapeutic effects of acupuncture treatment. According to our results, sleep disorders are associated with several brain regions and neurotransmitters. Furthermore, this review showed that neurological processes, such as catecholamine and BDNF signaling pathways, can be regulated by acupuncture, which is a crucial aspect of the acupuncture mechanism in sleep disorders.

KEYWORDS

sleep disorder, acupuncture, rodent study, literature review, animal study

1. Introduction

In most humans, sleep accounts for approximately 20–40% of the day. Sleep is a set of physiological processes under neurobiological regulation that affects several physiological systems (Grandner, 2017). Therefore, sufficient sleep is essential for the health of individuals (Kumar, 2008). Sleep disorders are characterized by disturbances in normal sleep patterns (Karna et al., 2023). The six major categories of sleep disorders are insomnia, sleep-disordered breathing, central hypersomnolence disorders, circadian rhythm sleep–wake disorders, parasomnia, and sleep-related movement disorders (Sateia, 2014). Sleep problems are associated with adverse health outcomes such as obesity, cardiovascular diseases, mental health, and neurodegenerative diseases (Hale et al., 2020).

Sleep disorders, such as insomnia or hypersomnia, are frequently observed in neurodegenerative conditions (Malhotra, 2018) including Alzheimer's disease (AD) and Parkinson's disease (PD). In individuals with AD, frequent symptoms of sleep disturbances include reversal of day-night sleep patterns, frequent nighttime awakenings, increased daytime sleep, decreased rapid eye movement sleep, and decreased slow wave sleep (Rose and Lorenz, 2010). Regulation of sleep and wakefulness relies on complex functions of several brain areas and neurotransmitters, many of which have been shown to be affected in patients with PD (Stefani and Hogl, 2020). Accordingly, sleep is a complex and active neural process involving several brain structures, such as the hypothalamus, brain stem, amygdala, thalamus, pineal gland, and basal forebrain (Murillo-Rodriguez et al., 2012). Although diverse methods have been used to treat sleep disorders, they lack efficacy and safety (National Institutes of Health, 2005). However, alternative treatments for sleep maintenance are still being developed.

Acupuncture is a therapy based on the insertion of a needle at a specific point, and clear evidence of its efficacy in Western medicine has been reported (Kaptchuk, 2002). The efficacy of acupuncture in improving sleep duration and quality has been confirmed in clinical studies (Cao et al., 2009). Acupuncture in insomnia treatment improves sleep quality and psychological health (Yin et al., 2017). The therapeutic effects of acupuncture in sleep disorders are closely associated with neurological diseases. In particular, acupuncture in PD affects neurotransmitters and their receptors, resulting in increased dopamine, γ-aminobutyric acid (GABA) inhibition, and decreased glutamate levels (Tamtaji et al., 2019). Moreover, acupuncture improves sleep in PD patients (Aroxa et al., 2017; Li et al., 2022).

Although many studies have explored the therapeutic effects of acupuncture on sleep disorders, the mechanisms underlying these effects on sleep disorders are unclear. We evaluated the therapeutic effects of acupuncture treatment and investigated the mechanism of acupuncture efficacy in a rodent model of sleep disorders.

2. Methods

2.1. Search strategy

We included studies published in English that investigated the effects of acupuncture on sleep disorders in animal models. The literature was retrieved from EMBASE, MEDLINE, PubMed, and the Research Information Service System from inception until April 2023. The keywords for the search were as follows: "(acupuncture OR electroacupuncture) AND (mice OR mouse OR rat OR rats) AND (sleep disorders)." Studies were included based on the following criteria: subjects (animal models of sleep disorders), interventions (acupuncture), and outcomes (electroencephalograms (EEGs) and mechanism). Studies written in languages other than English, those without acute disease models of sleep disorders, or those not needing acupuncture were excluded. Two authors (Lee and Kim) independently extracted the data. The first author, publication year, type of animal, type of sleep disorder and disease model, type of acupuncture, corresponding parameters, and target outcomes were retrieved to evaluate the therapeutic effect of acupuncture on sleep disorders.

2.2. Quality assessment

The risk of bias was assessed using the Systematic Review Center for Laboratory Animal Experimentation's risk of bias (SYRCLE's RoB) Tool (Hooijmans et al., 2014). The SYRCLE RoB tool contains 10 entries related to selection bias (random sequence generation, baseline characteristics, and allocation concealment), performance bias (random housing and blinding), detection bias (random outcome assessment and blinding), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other biases (other sources of bias). Each entry was marked as "Low risk of bias," "High risk of bias" or "Unclear." Two authors (Lee and Kim) independently evaluated the RoB scores of 17 studies. The Review Manager (RevMan) version 5.4 software (The Cochrane Collaboration, 2020) was used to calculate the risk of bias.

3. Results

3.1. Study inclusion and quality assessment

Among the 43 initially identified studies, 22 studies were removed due to not being written in English and not including acupuncture for treatment. Following full-text screening, four studies that did not provide animal models of inappropriate sleep disorders were excluded. A final total of 17 studies were included in the present study. Quality assessments of the 17 studies were performed by two individual assessors. All studies were rated as having a low risk of sequence generation bias because they mentioned that the animals were randomly grouped. The studies started with animals of similar weights that were maintained under similar environmental conditions. The baseline values of the two groups were similar. Four studies were evaluated as having a high risk of bias in the "incomplete outcome data" domain: not given total number of rats used in the experiment or no explanation for variation of differed number of rats in each experiment. All the studies included the results of their experiments; however, we were unable to determine whether these statements were adequate for the conclusion. Two studies had a high risk of other biases: one study provided insufficient information on electroacupuncture (EA), and one study did not mention the depth of the acupuncture needle during treatment. None of the studies provided information regarding allocation concealment. Flow diagram and quality assessment results were summarized in Supplementary data (Supplementary Figures S1, S2).

3.2. Study characteristics

The characteristics of the included studies are summarized in Table 1. Among the 17 studies, all used rats except for one that used mice. EA was the most frequently used intervention. Acupoints located at different sites in the body appear to have multiple uses. Among them, HT7 had the highest number of acupoints.

3.3. Therapeutic effect and its underlying mechanism

We selected 17 studies and highlighted the mechanisms underlying the effects of acupuncture. The mechanisms of each study are summarized in Table 2. Several neurotransmitters and brain sites have been shown to be duplicated in different studies; however, the upregulation of their protein expression levels vary. Therefore, some acupuncture types and acupoints were also duplicated, especially electroacupuncture, which has been shown to be commonly used for treatment of sleep disorders, such as acupoint HT7.

The effect of Mongolian medical warm acupuncture (MMWA) on insomnia is related to the regulation of miR-101a and PAX8. MMWA increased the expression of miR-101a which inhibited PAX8 expression in the hippocampus of a rat model of insomnia The levels of 5-HT, Acetylcholine (ACh), and GABA increased and

TABLE 1 Study characteristics.

Author	Disease	Animal	Intervention	Methods	Acupoint
Bo et al. (2017)	Insomnia	rat	MMWA	15 min, 7 days	Dinghui, Heyi, Xin
A et al. (2019)	Insomnia	rat	MWA	15 min, 7 days	GV20, ST36
Hong et al. (2020)	Insomnia	mouse	MA	1 min, 7 days	DU20, SP6, HT7
Xu et al. (2020)	Insomnia	rat	MMWA	15 min, 7 days	Dinghui, Heyi, Xin
Xie et al. (2021)	Insomnia	rat	EA	2 Hz, 6–7 mA, 15 min	BL 18, ST36
Cao et al. (2022)	Insomnia	rat	EA	2/15 Hz, 1 mA	DU20, SP6, HT7
Yu et al. (2022)	Insomnia	rat	MMWA	15 min, 7 days	Dinghui, Heyi, Xin
Zheng et al. (2017)	Sleep deprivation	rat	MA	40 min	EX-HN 1, HT7, SP6
Li et al. (2018)	Sleep deprivation	rat	EA	2 Hz, 120 s	GV20
Chen et al. (2020)	Sleep deprivation	rat	EA	2/100 Hz, 03 mA	GV20, ST36
Pei et al. (2021)	Sleep deprivation	rat	EA	20 min, 4 days	EX-HN1
Hao et al. (2022)	Sleep deprivation	rat	EA	2 Hz, 1 mA, 15 min/day for 7 days	GV20, GV14
Xi et al. (2023)	Chronic sleep deprivation	rat	EA	2 Hz, 1.5 mA	EX-HN 3, HT7
Yi et al. (2015)	Sleep disruption	rat	EA	10 Hz	GB20
Seo et al. (2021)	Sleep disruption	rat	MA	85 Hz, 1 min	HT7
Seo and Ryu (2022)	Sleep disruption	rat	EA	2 Hz, 20 min	HT7
Li et al. (2011)	Sleep disturbance	rat	EA	2 or 100 Hz	ST36, SP6

EA, electroacupuncture; MA, manual acupuncture; MMWA, Mongolian medical warm acupuncture; N/A, not applicable.

those of dopamine (DA), norepinephrine (NE), and glutamate (Glu) decreased in the hippocampus, hypothalamus, and prefrontal cortex (Bo et al., 2017). Mongolian warm acupuncture (MWA) in a rat model of insomnia improved sleep and the expression levels of Egr1, Btg2, and brain-derived neurotrophic factor (BDNF) in the hypothalamus increased in the MWA treated group (A et al., 2019). Manual acupuncture (MA) stimulation enhanced the sleep disorder phenotype in a rat model of insomnia and had a substantial effect on the recovery of the gut microbiota. Melatonin levels in the pineal gland increased and DA, 5-HT, and NE levels in the serum decreased in the MA treatment group at the DU20, SP6, and HT7 acupoints (Hong et al., 2020). Improvement in sleep was confirmed with MMWA treatment in a rat model of insomnia. Differential levels in the hypothalamus of four proteins related to nerves, including prolargin (PRELP), microtubule-associated protein 1 B (MAP1B), transmembrane protein 41 B (TMEM41B), NMDA receptor synaptonuclear-signaling, and neuronal migration factor (NSMF), are involved in the MMWA treatment of insomnia (Xu et al., 2020). The effect of electroacupuncture treatment (EA) improving sleep disturbance was evaluated in rats with insomnia. The hypothalamic levels of DA, corticotropin-releasing hormone, adrenocorticotropic hormone (ACTH), and cortisol (CORT) increased in the EA group treated at the BL18 and ST36 acupoints. Moreover, EA treatment increased D1R and D2R mRNA levels (Xie et al., 2021). The DU20, HT7, and SP6 acupoints were used for EA treatment in a rat model of insomnia. The EA treatment group showed higher 5-HT levels and lower DA, NE, and EPI levels in the hippocampus and brainstem than the insomnia group. The expression of Bcl-2 was upregulated, whereas Bax, Bad and Caspase-3 were downregulated. EA affects the TrkB, PI3K/Akt, and cAMP/CREB/BDNF pathways (Cao et al., 2022). The anti-insomnia effect of MMWA has been observed in rats with insomnia as MMWA treatment decreased ACh and NE levels and increased 5-HT and GABA levels in the serum. The gut microbiota improved, and cAMP and GABA transporter 1 (GAT-1) levels in the brain stem were reduced (Yu et al., 2022).

The treatment of sleep-deprived rats with acupuncture affected their learning and memory. The expression levels of the presynaptic marker synaptophysin (SYP) and BDNF in the hippocampus of the acupuncture group treated with HT7, EX-HN1, and SP6 were higher than those in the sleep deprivation (SD) group (Zheng et al., 2017). The effects of EA on memory were assessed using an SD rat model. Compared with the SD group, the EA group treated at the GV20 and ST36 acupoints showed higher p-synapsin I, p-CaMK II, and tyrosine hydroxylase levels in the hippocampus (Chen et al., 2020). Compared with the SD group, more neurons were observed in the CA1 and CA2 regions of the hippocampus in the EA group treated at the EX-HN1 acupoint. The expression levels of SYP and the postsynaptic marker, postsynaptic density (PSD) 95, increased in the EA group compared to those in the SD group. Furthermore, EA activates the BDNF/TrkB/Erk pathway (Pei et al., 2021). Two acupoints, GV20 and GV14, were used for EA treatment in a REM sleep deprivation (REMSD) rat model. EA alleviates damage to the synaptic ultrastructure and upregulates dendrite branching and length in the hippocampus in the REMSD rat model. Structural synaptic plasticity in REMSD model rats was attenuated by EA regulation of miR-132-3p and p250GAP. Moreover, EA elevated the expression of Rac1 and Cdc42 in the hippocampus. EA treatment increases the expression of Rac1 and Cdc42, which are connected to miR-132 (Hao et al., 2022). The EA group treated with EX-HN3 and HT7 in a rat model of chronic sleep deprivation reduced DA expression via the VTA-NAc DA pathway (Xi et al., 2023).

TABLE 2 Effect of acupuncture on sleep disorders and its underlying mechanisms.

Author	Disease	Site of sample collection	Mechanism	EEGs
Bo et al. (2017)	Insomnia	Hippocampus, hypothalamus, prefrontal cortex	miR-101a, 5-HT, ACh, GABA ▲ PAX8, DA, NE, Glu ▼	N/A
A et al. (2019)	Insomnia	Hypothalamus	Egr 1, Btg2, BDNF ▲	N/A
Hong et al. (2020)	Insomnia	Pineal gland, serum	melatonin ▲ DA, 5-HT, NE ▼	N/A
Xu et al. (2020)	Insomnia	Hypothalamus	PRELP, MAP1B, TMEM41B, NMDA, NSMF ▲/▼	N/A
Xie et al. (2021)	Insomnia	Hypothalamus	DA, CRH, ACTH, CORT, D1R, D2R mRNA ▲	NREM ▲
Cao et al. (2022)	Insomnia	Hippocampus, brain stem	5-HT, Bcl-2 ▲ DA, NE, EPI, Bax, Bad, Caspase-3 ▼	N/A
Yu et al. (2022)	Insomnia	Brain stem, serum	ACh, NE, 5-HT, GABA ▲ cAMP, GAT-1 ▼	N/A
Zheng et al. (2017)	Sleep deprivation	Hippocampus	SYP, BDNF ▲	N/A
Li et al. (2018)	Sleep deprivation	Cerebral cortex	N/A	α wave frequency ▲ β wave frequency ▼
Chen et al. (2020)	Sleep deprivation	Hippocampus	p-synapsin I, p-CaMK II, tyrosine hydroxylase ▲	N/A
Pei et al. (2021)	Sleep deprivation	Hippocampus	SYP, PSD95, BDNF/TrkB/Erk pathway ▲	N/A
Hao et al. (2022)	Sleep deprivation	Hippocampus	Rac1, Cdc42 ▲	N/A
Xi et al. (2023)	Chronic sleep deprivation	Hippocampus	DA ▼	N/A
Yi et al. (2015)	Sleep disruption	Central nucleus of amygdala	opioid receptors ▲	NREM ▲
Seo et al. (2021)	Sleep disruption	MS-VDB	c-Fos ▼	NREM ▲
Seo and Ryu (2022)	Sleep disruption	Medial septum	mBDNF ▲	REM ▲
Li et al. (2011)	Sleep disturbance	N/A	N/A	NREM, REM, total sleep time ▲

EA, electroacupuncture; MA, manual acupuncture; MMWA, Mongolian medical warm acupuncture; N/A, not applicable.

EA treatment suppresses epilepsy and improves sleep disruption at the GB20 acupoint in rats with epilepsy. NREM sleep reduction is blocked by EA treatment, and the therapeutic effect is mediated by opioid receptors in the central nucleus of the amygdala (CeA) (Yi et al., 2015). The effect of mechanical acupuncture instrument (MAI) treatment was induced by the HT7 acupoint in a rat model of sleep disruption. MAI stimulation reduced c-Fos expression in arousal regions, especially the medial septum/vertical limb of the diagonal band of Broca (MS-VDB) (Seo et al., 2021). EA treatment of HT7 cells alleviates endoplasmic reticulum (ER) stress in the medial septum of rats with sleep disruption. HT7 stimulation increased the expression of mBDNF and regulated ER stress via pTrkB in mBDNF (Seo and Ryu, 2022).

4. Discussion

This study explored the beneficial effects of acupuncture in animal models of sleep disorders and the underlying mechanisms. According to the results of our study, sleep disorders are associated with several brain regions and neurotransmitter levels. Various brain regions and expression levels that represent the acupuncture mechanism described in the main results are shown in Figure 1. Sleep is closely intertwined with physiological processes, typically in the brain (Lin et al., 2014). Previous studies have presented the neuroprotective activity and neurotransmitter regulation of acupuncture therapy (Su et al., 2020). BDNF is a protein that is extensively distributed in the cartilage tissue, bone, endocrine system, and central nervous system (CNS) (Greenberg et al., 2009), and is widely involved in neural plasticity (Park and Poo, 2013; Colucci-D'Amato et al., 2020). Studies have also demonstrated that acupuncture induces advantages in the central nervous system (CNS) through BDNF activation and signaling pathways (Lin et al., 2014). Two studies confirmed the effect of acupuncture on BDNF expression levels (Zheng et al., 2017; A et al., 2019). Moreover, various downstream pathways, such as the TrKB, PI3K/Akt, cAMP/CREB/BDNF (Cao et al., 2022), and BDNF/TrkB/Erk pathways (Pei et al., 2021), were found to be influenced by acupuncture treatment. BDNF appears to be essential for mediating neuroprotective effects and may play a role in neuronal plasticity (Colucci-D'Amato et al., 2020). Therefore, the effects of acupuncture on the regulation of BDNF appear to be important for its role in treating sleep dysfunction.

Changes in neurotransmitter levels such as DA, 5-HT, NE, Glu, and GABA in various brain regions lead to neuropsychiatric,

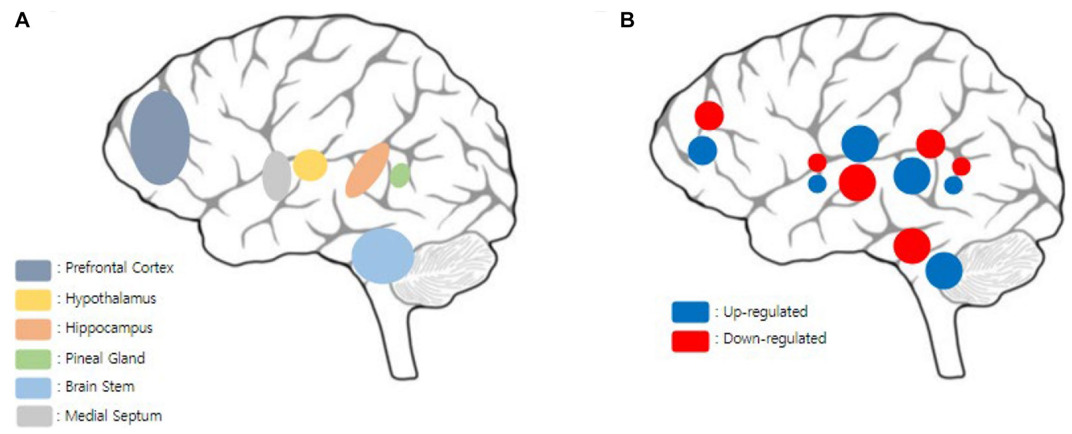

FIGURE 1
Visualization of mechanisms via acupuncture stimulation in multiple brain regions. **(A)** Multiple brain regions that acupuncture mechanisms are involved in this report. Various brain regions were detected such as prefrontal cortex, hypothalamus, hippocampus, pineal gland, brain stem, and medial septum. **(B)** Expression levels such as neurotransmitters, proteins, or genes which are involved in multiple brain regions according to this report. Each color pretends the up/down regulations of expression levels, and the size of the circle pretends the number of catecholamine types analyzed in each brain regions. In the prefrontal cortex, miR-101a, 5-HT, Ach, GABA were up regulated, and PAX8, DA, NE, and Glu down regulated. In, medial septum, mBDNF were up regulated and c-Fos were down regulated. In the hypothalamus, miR-101a, 5-HT, Ach, GABA, Egr 1, Btg 2, BDNF, DA, CRH, ACTH, CORT, D1R and D2R mRNA, were up regulated, and PAX8, DA, NE, Glu, were down regulated. PRELP, MAP1B, TMEM41B, NMDA, NSMF were shown have differential expression levels. In the hippocampus, miR-101a, 5-HT, Ach, GABA, Bcl-2, SYP, BDNF, p-synapsin I, p-CaMK II, tyrosine hydroxylase, PSD95, BDNF/TrkB/Erk pathway, Rac1, Cdc42 were up regulated, and PAX8, DA, NE, Glu, EPI, Bax, Bad, Caspase-3 were down regulated. In the pineal gland, melatonin was up regulated and DA, 5-HT, NE were down regulated. In the brain stem, miR-101a, 5-HT, Ach, GABA, Bcl-2, NE, were up regulated, and PAX8, DA, NE, Glu, EPI, Bax, Bad, Caspase-3, cAMP, GAT-1 were down regulated.

autonomic nervous system, and sleep disorders (Zhao et al., 2021). Because neurological processes are involved in sleep–wake regulation (Scammell, 2015), the interaction of diverse neurotransmitter systems is widely related to sleep dysfunction (Moszczynski and Murray, 2012). Acupuncture therapy, which has beneficial effects on neuroprotection, has been shown to improve serum and hippocampal 5-HT and DA levels in depression model rats (Li et al., 2021). Interestingly, several studies have shown increased levels of 5-HT expression in different sites, such as the serum (Yu et al., 2022), hypothalamus (Bo et al., 2017; Xie et al., 2021), and hippocampus (Bo et al., 2017; Cao et al., 2022) following acupuncture treatment. Among these studies, one showed an improvement in both 5-HT and DA expression levels (Xie et al., 2021). Moreover, according to previous reports, dopaminergic neurons are typically found in the midbrain, particularly in the VTA (Juarez Olguin et al., 2016). In this review, the VTA-NAc DA pathway was demonstrated to be regulated through acupuncture stimulation in one study (Xi et al., 2023). Two studies have reported decreased levels of DA and increased levels of 5-HT (Bo et al., 2017; Cao et al., 2022). In contrast, one study reported downregulated levels of DA and 5-HT (Hong et al., 2020). This review demonstrates that catecholamines are regulated by acupuncture in animal models of sleep disorders.

In this review, various acupoints were used for acupuncture treatment. Among these acupuncture points, HT7 was the most frequently used. According to recent studies, HT7 has been used for neuropsychological disorders such as amnesia, epilepsy, and insomnia (Wattanathorn and Sutalangka, 2014). In this study, stimulation of HT7 mediated memory improvements and ACh and DA levels were enhanced by HT7. However, the acupoints ST36 (Tao et al., 2016), GV20 (Wang et al., 2014), and SP6 (Wu et al., 2015) have also been used to mitigate sleep dysfunction. Interestingly, these acupoints have been known to have neuroprotective effects on various neurological disease animal models. Sleep patterns and insomnia symptoms seem to correlate with brain injury (Korostovtseva, 2021) and an imbalance in neurotransmitters (Levenson et al., 2015; Holst and Landolt, 2018). Therefore, this difference in mechanism suggests a distinct and specific effect of acupuncture on the treated acupoints. Along with the effects of acupuncture therapy, multiple acupoints and their potential roles in various mechanisms may be crucial for establishing a strategy for treating sleep disorders.

Understanding sleep regulation may encourage the management of neurocognitive disorders (Miller, 2015) and chronic diseases (Reis et al., 2018). Although this review had a small sample size, we attempted to explore the potential effect of acupuncture on sleep disorders in neuropsychology and to understand its mechanism in animal models. Accordingly, the present study is meaningful and important in exploring the correlation between acupuncture and neuropsychological disorders in animal models. However, multiple interconnections between acupuncture, sleep problems, and neuroscience still need to be described. We hope that this report will provide a basis for exploring the mechanisms of action of acupuncture in neuropsychological disorders.

Author contributions

SL searched the database and extracted the data. S-NK designed and supervised the study. SL and S-NK analyzed the data and wrote the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

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References

A, G., Li, X., Su, B., Lian, H., Bao, M., Liang, Y., et al. (2019). Effect of Mongolian warm acupuncture on the gene expression profile of rats with insomnia. *Acupunct. Med.* 37, 301–311. doi: 10.1136/acupmed-2016-011243

Aroxa, F. H., Gondim, I. T., Santos, E. L., Coriolano, M. D., Asano, A. G., and Asano, N. M. (2017). Acupuncture as adjuvant therapy for sleep disorders in Parkinson's disease. *J. Acupunct. Meridian Stud.* 10, 33–38. doi: 10.1016/j.jams.2016.12.007

Bo, A., Si, L., Wang, Y., Bao, L., and Yuan, H. (2017). Mechanism of Mongolian medical warm acupuncture in treating insomnia by regulating miR-101a in rats with insomnia. *Exp. Ther. Med.* 14, 289–297. doi: 10.3892/etm.2017.4452

Cao, H., Pan, X., Li, H., and Liu, J. (2009). Acupuncture for treatment of insomnia: a systematic review of randomized controlled trials. *J. Altern. Complement. Med.* 15, 1171–1186. doi: 10.1089/acm.2009.0041

Cao, F., Xu, Y., Zhang, M., Li, X., Chen, Y., Zhi, M., et al. (2022). Baihui (DU20), Shenmen (HT7) and Sanyinjiao (SP6) target the cAMP/CREB/BDNF and PI3K/Akt pathways to reduce central nervous system apoptosis in rats with insomnia. *Heliyon.* 8:e12574. doi: 10.1016/j.heliyon.2022.e12574

Chen, D., Zhang, Y., Wang, C., Wang, X., Shi, J., Zhang, J., et al. (2020). Modulation of hippocampal dopamine and synapse-related proteins by electroacupuncture improves memory deficit caused by sleep deprivation. *Acupunct. Med.* 38, 343–351. doi: 10.1177/0964528420902147

Colucci-D'Amato, L., Speranza, L., Neurotrophic, V. F., and Factor, B. D. N. F. (2020). Physiological functions and therapeutic potential in depression, neurodegeneration and brain Cancer. *Int. J. Mol. Sci.* 21, 1–29. doi: 10.3390/ijms21207777

Grandner, M. A. (2017). Sleep, health, and society. *Sleep Med. Clin.* 12, 1–22. doi: 10.1016/j.jsmc.2016.10.012

Greenberg, M. E., Xu, B., Lu, B., and Hempstead, B. L. (2009). New insights in the biology of BDNF synthesis and release: implications in CNS function. *J. Neurosci.* 29, 12764–12767. doi: 10.1523/JNEUROSCI.3566-09.2009

Hale, L., Troxel, W., and Buysse, D. J. (2020). Sleep health: an opportunity for public health to address health equity. *Annu. Rev. Public Health* 41, 81–99. doi: 10.1146/annurev-publhealth-040119-094412

Hao, L., Wu, Y., Xie, J., and Chen, X. (2022). Electroacupuncture enhances cognitive deficits in a rat model of rapid eye movement sleep deprivation via targeting MiR-132. *Evid. Based Complement. Alternat. Med.* 2022, 7044208–7044214. doi: 10.1155/2022/7044208

Holst, S. C., and Landolt, H. P. (2018). Sleep-Wake Neurochemistry. *Sleep Med. Clin.* 13, 137–146. doi: 10.1016/j.jsmc.2018.03.002

Hong, J., Chen, J., Kan, J., Liu, M., and Yang, D. (2020). Effects of acupuncture treatment in reducing sleep disorder and gut microbiota alterations in PCPA-induced insomnia mice. *Evid. Based Complement. Alternat. Med.* 2020, 3626120–3626114. doi: 10.1155/2020/3626120

Hooijmans, C. R., Rovers, M. M., de Vries, R. B., Leenaars, M., Ritskes-Hoitinga, M., and Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* 14:43. doi: 10.1186/1471-2288-14-43

Juarez Olguin, H., Calderon Guzman, D., Hernandez Garcia, E., and Barragan, M. G. (2016). The role of dopamine and its dysfunction as a consequence of oxidative stress. *Oxidative Med. Cell. Longev.* 2016, 9730467–9730413. doi: 10.1155/2016/9730467

Kaptchuk, T. J. (2002). Acupuncture: theory, efficacy, and practice. *Ann. Intern. Med.* 136, 374–383. doi: 10.7326/0003-4819-136-5-200203050-00010

Karna, B, Sankari, A, and Tatikonda, G. (2023). *Sleep Disorder*. StatPearls. Treasure Island, FL.

Korostovtseva, L. (2021). Ischemic stroke and sleep: the linking genetic factors. *Cardiol. Ther.* 10, 349–375. doi: 10.1007/s40119-021-00231-9

Kumar, V. M. (2008). Sleep and sleep disorders. *Indian J. Chest Dis. Allied Sci.* 50, 129–135.

Levenson, J. C., Kay, D. B., and Buysse, D. J. (2015). The pathophysiology of insomnia. *Chest* 147, 1179–1192. doi: 10.1378/chest.14-1617

Li, P., Huang, W., Yan, Y. N., Cheng, W., Liu, S., Huang, Y., et al. (2021). Acupuncture can play an antidepressant role by regulating the intestinal microbes and neurotransmitters in a rat model of depression. *Med. Sci. Monit.* 27:e929027. doi: 10.12659/MSM.929027

Li, L., Jin, X., Cong, W., Du, T., and Zhang, W. (2022). Acupuncture in the treatment of Parkinson's disease with sleep disorders and dose response. *Biomed. Res. Int.* 2022:7403627. doi: 10.1155/2022/7403627

Li, J., Ran, X., Cui, C., Xiang, C., Zhang, A., and Shen, F. (2018). Instant sedative effect of acupuncture at GV20 on the frequency of electroencephalogram alpha and beta waves in a model of sleep deprivation. *Exp. Ther. Med.* 15, 5353–5358. doi: 10.3892/etm.2018.6123

Li, Y. J., Zhong, F., Yu, P., Han, J. S., Cui, C. L., and Wu, L. Z. (2011). Electroacupuncture treatment normalized sleep disturbance in morphine withdrawal rats. *Evid. Based Complement. Alternat. Med.* 2011:361054. doi: 10.1093/ecam/nep133

Lin, D., De La Pena, I., Lin, L., Zhou, S. F., Borlongan, C. V., and Cao, C. (2014). The neuroprotective role of acupuncture and activation of the BDNF signaling pathway. *Int. J. Mol. Sci.* 15, 3234–3252. doi: 10.3390/ijms15023234

Malhotra, R. K. (2018). Neurodegenerative Disorders and Sleep. *Sleep Med. Clin.* 13, 63–70. doi: 10.1016/j.jsmc.2017.09.006

Miller, M. A. (2015). The role of sleep and sleep disorders in the development, diagnosis, and management of neurocognitive disorders. *Front. Neurol.* 6:224. doi: 10.3389/fneur.2015.00224

Moszczynski, A., and Murray, B. J. (2012). Neurobiological aspects of sleep physiology. *Neurol. Clin.* 30, 963–985. doi: 10.1016/j.ncl.2012.08.001

Murillo-Rodriguez, E., Arias-Carrion, O., Zavala-Garcia, A., Sarro-Ramirez, A., Huitron-Resendiz, S., and Arankowsky-Sandoval, G. (2012). Basic sleep mechanisms: an integrative review. *Cent. Nerv. Syst. Agents Med. Chem.* 12, 38–54. doi: 10.2174/187152412800229107

National Institutes of Health (2005). National Institutes of Health state of the science conference statement on manifestations and Management of Chronic Insomnia in adults, June 13–15, 2005. *Sleep* 28, 1049–1057. doi: 10.1093/sleep/28.9.1049

Park, H., and Poo, M. M. (2013). Neurotrophin regulation of neural circuit development and function. *Nat. Rev. Neurosci.* 14, 7–23. doi: 10.1038/nrn3379

Pei, W., Meng, F., Deng, Q., Zhang, B., Gu, Y., Jiao, B., et al. (2021). Electroacupuncture promotes the survival and synaptic plasticity of hippocampal neurons and improvement of sleep deprivation-induced spatial memory impairment. *CNS Neurosci. Ther.* 27, 1472–1482. doi: 10.1111/cns.13722

Reis, C., Dias, S., Rodrigues, A. M., Sousa, R. D., Gregorio, M. J., Branco, J., et al. (2018). Sleep duration, lifestyles and chronic diseases: a cross-sectional population-based study. *Sleep Sci* 11, 217–230. doi: 10.5935/1984-0063.20180036

Rose, K. M., and Lorenz, R. (2010). Sleep disturbances in dementia. *J. Gerontol. Nurs.* 36, 9–14. doi: 10.3928/00989134-20100330-05

Sateia, M. J. (2014). International classification of sleep disorders-third edition: highlights and modifications. *Chest* 146, 1387–1394. doi: 10.1378/chest.14-0970

Scammell, T. E. (2015). Overview of sleep: the neurologic processes of the sleep-wake cycle. *J. Clin. Psychiatry* 76:e13. doi: 10.4088/JCP.14046tx1c

Seo, S. Y., Moon, J. Y., Kang, S. Y., Kwon, O. S., Bang, S. K., Choi, K. H., et al. (2021). Acupuncture stimulation at HT7 as a non-pharmacological therapy for sleep disorder caused by caffeine administration in rats. *Acupunct. Med.* 39, 691–699. doi: 10.1177/09645284211011489

Seo, S. Y., and Ryu, Y. (2022). Electroacupuncture stimulation of HT7 alleviates sleep disruption following acute caffeine exposure by regulating BDNF-mediated endoplasmic reticulum stress in the rat medial septum. *Biomed. Pharmacother.* 155:113724. doi: 10.1016/j.biopha.2022.113724

Stefani, A., and Hogl, B. (2020). Sleep in Parkinson's disease. *Neuropsychopharmacology* 45, 121–128. doi: 10.1038/s41386-019-0448-y

Su, X. T., Wang, L., Ma, S. M., Cao, Y., Yang, N. N., Lin, L. L., et al. (2020). Mechanisms of acupuncture in the regulation of oxidative stress in treating ischemic stroke. *Oxidative Med. Cell. Longev.* 2020, 7875396–7875315. doi: 10.1155/2020/7875396

Tamtaji, O. R., Naderi Taheri, M., Notghi, F., Alipoor, R., Bouzari, R., and Asemi, Z. (2019). The effects of acupuncture and electroacupuncture on Parkinson's disease: current status and future perspectives for molecular mechanisms. *J. Cell. Biochem.* 120, 12156–12166. doi: 10.1002/jcb.28654

Tao, J., Zheng, Y., Liu, W., Yang, S., Huang, J., Xue, X., et al. (2016). Electro-acupuncture at LI11 and ST36 acupoints exerts neuroprotective effects via reactive astrocyte proliferation after ischemia and reperfusion injury in rats. *Brain Res. Bull.* 120, 14–24. doi: 10.1016/j.brainresbull.2015.10.011

Wang, W. W., Xie, C. L., Lu, L., and Zheng, G. Q. (2014). A systematic review and meta-analysis of Baihui (GV20)-based scalp acupuncture in experimental ischemic stroke. *Sci. Rep.* 4:3981. doi: 10.1038/srep03981

Wattanathorn, J., and Sutalangka, C. (2014). Laser acupuncture at HT7 Acupoint improves cognitive deficit, neuronal loss, oxidative stress, and functions of cholinergic and dopaminergic Systems in Animal Model of Parkinson's disease. *Evid. Based Complement. Alternat. Med.* 2014:937601. doi: 10.1155/2014/937601

Wu, M. F., Zhang, S. Q., Liu, J. B., Li, Y., Zhu, Q. S., and Gu, R. (2015). Neuroprotective effects of electroacupuncture on early- and late-stage spinal cord injury. *Neural Regen. Res.* 10, 1628–1634. doi: 10.4103/1673-5374.167762

Xi, H., Wu, W., Qin, S., Wang, X., and Liu, C. (2023). Effects of electroacupuncture on the ventral tegmental area- nucleus accumbens dopamine pathway in rats with chronic sleep deprivation. *Acupunct. Med.*:9645284221146197. doi: 10.1177/09645284221146197

Xie, C., Wang, J., Zhao, N., Yang, W., Gao, X., Liu, Z., et al. (2021). Effects of Electroacupuncture on sleep via the dopamine system of the HPA axis in rats after cage change. *Evid. Based Complement. Alternat. Med.* 2021, 5527060–5527025. doi: 10.1155/2021/5527060

Xu, Y., Li, X., Man, D., Su, X., and A, G. (2020). iTRAQ-based proteomics analysis on insomnia rats treated with Mongolian medical warm acupuncture. *Biosci. Rep.* 40, 1–13. doi: 10.1042/BSR20191517

Yi, P. L., Lu, C. Y., Jou, S. B., and Chang, F. C. (2015). Low-frequency electroacupuncture suppresses focal epilepsy and improves epilepsy-induced sleep disruptions. *J. Biomed. Sci.* 22:49. doi: 10.1186/s12929-015-0145-z

Yin, X., Gou, M., Xu, J., Dong, B., Yin, P., Masquelin, F., et al. (2017). Efficacy and safety of acupuncture treatment on primary insomnia: a randomized controlled trial. *Sleep Med.* 37, 193–200. doi: 10.1016/j.sleep.2017.02.012

Yu, H., Yu, H., Si, L., Meng, H., Chen, W., Wang, Z., et al. (2022). Influence of warm acupuncture on gut microbiota and metabolites in rats with insomnia induced by PCPA. *PLoS One* 17:e0267843. doi: 10.1371/journal.pone.0267843

Zhao, Y., Zhang, Z., Qin, S., Fan, W., Li, W., Liu, J., et al. (2021). Acupuncture for Parkinson's disease: efficacy evaluation and mechanisms in the dopaminergic neural circuit. *Neural Plast.* 2021:9926445. doi: 10.1155/2021/9926445

Zheng, P., Xu, X., Zhao, H., Lv, T., Song, B., and Wang, F. (2017). Tranquilizing and allaying excitement needling method affects BDNF and SYP expression in Hippocampus. *Evid. Based Complement. Alternat. Med.* 2017:8215949. doi: 10.1155/2017/8215949

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Neural control of cerebral blood flow: scientific basis of scalp acupuncture in treating brain diseases

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Scalp acupuncture (SA), as a modern acupuncture therapy in the treatment of brain diseases, especially for acute ischemic strokes, has accumulated a wealth of experience and tons of success cases, but the current hypothesized mechanisms of SA therapy still seem to lack significant scientific validity, which may not be conducive to its ultimate integration into mainstream medicine. This review explores a novel perspective about the mechanisms of SA in treating brain diseases based on its effects on cerebral blood flow (CBF). To date, abundant evidence has shown that CBF is significantly increased by stimulating specific SA points, areas or nerves innervating the scalp, which parallels the instant or long-term improvement of symptoms of brain diseases. Over time, the neural pathways that improve CBF by stimulating the trigeminal, the facial, and the cervical nerves have also been gradually revealed. In addition, the presence of the core SA points or areas frequently used for brain diseases can be rationally explained by the characteristics of nerve distribution, including nerve overlap or convergence in certain parts of the scalp. But such characteristics also suggest that the role of these SA points or areas is relatively specific and not due to a direct correspondence between the current hypothesized SA points, areas and the functional zones of the cerebral cortex. The above evidence chain indicates that the efficacy of SA in treating brain diseases, especially ischemic strokes, is mostly achieved by stimulating the scalp nerves, especially the trigeminal nerve to improve CBF. Of course, the mechanisms of SA in treating various brain diseases might be multifaceted. However, the authors believe that understanding the neural regulation of SA on CBF not only captures the main aspects of the mechanisms of SA therapy, but also facilitates the elucidation of other mechanisms, which may be of greater significance to further its clinical applications.

KEYWORDS

scalp acupuncture, cerebral blood flow, brain diseases, innervation of the scalp, core SA points or areas, neural stimulation, neural pathway, relative specificity

Introduction

Scalp acupuncture (SA) is a modern acupuncture therapy that first emerged in the early 1970s, and has been widely used globally due to its significant efficacy on various acute or chronic brain diseases, specifically acute ischemic strokes (Lu, 1991; Zheng et al., 2011; Wang et al., 2012; Lee et al., 2013; Zhang et al., 2013; You et al., 2018; Yi et al., 2020; Huang et al., 2021; Xue et al., 2022; Wang J.-H. et al., 2022).

In extensive clinical practice, several different schools of SA have emerged, some revise (mainly by adding) the earliest proposed SA locations (point, area, or zone) corresponding to the functional zones of the cerebral cortex (Jiao, 1997; Wang, 2019); others used classic meridians or acupoints distributed on the scalp as stimulation targets (Zhu, 2007; Xue et al., 2022); some have even integrated the hypothesis of biological holography (Molnar et al., 2018) to create their own unique systems of SA. Therefore, although there are many schools of SA, the primary mechanism explanations generally do not go beyond the above three categories. Of these categories, the meridian theory is considered as a primitive explanation in Traditional Chinese Medicine (TCM), the biological holography theory has not yet shown any biological evidence, while the hypothesis that certain SA locations correspond to the cortical functional zones has been the most attractive and accepted. This is because during body acupuncture, visceral diseases can often manifest as reflex points or zones on the body surface of the corresponding chest, abdomen, or back, based on neural reflexes at the same or nearby spinal segment (Jin et al., 2007). This can easily lead to a common misconception that SA locations have similar correspondence to the cortical functional zones. However, the cranial or occipital nerves innervating the scalp have no such segmental connections with the cerebral cortex. Furthermore, the stimulation of SA is mechanical rather than transcranial electromagnetic, making it impossible to stimulate the cortical functional zones directly below the scalp. Therefore, this hypothesis has been criticized (Ye et al., 2022) or opposed by statements such as "there is currently a lack of complete evidence for the mechanism of SA" (Wang et al., 2020).

This review explores a novel perspective about the mechanisms of SA in treating brain diseases based on its effects on cerebral blood flow (CBF). As supported by the authors' pilot study (Li et al., 1997) and subsequent research applying functional magnetic resonance imaging (fMRI) techniques, CBF has been shown to be improved by acupuncture. Many brain diseases are linked to the reduction of CBF, while SA has been widely reported to treat such brain diseases and/or to improve CBF concurrently. Numerous clinical or laboratory studies have shown stimulation of the trigeminal, the facial, and the cervical nerves innervating the scalp area (including many core SA points or areas) may improve CBF. Accordingly, the authors propose that the efficacy of SA for brain diseases, especially acute ischemic strokes, is likely achieved by stimulating such nerves innervated on the scalp to improve CBF. Moreover, the possible neural pathways that produce these effects and the relative specificity of scalp stimulation at different locations in improving CBF are analyzed. The latter is closely related to the optimal selection of SA points or areas. The elucidation of the scientific basis of SA in treating brain diseases will undoubtedly further its clinical applications and raise its efficacy.

Therapeutic effects of scalp acupuncture for brain diseases

Over the past 50 years, a large body of clinical evidence has been accumulated for the application of SA in the treatment of brain diseases, common cases including ischemic strokes, acute strokes, and post-stroke hemiplegia (Zheng et al., 2011; Wang et al., 2012; You et al., 2018; Huang et al., 2021; Wang Y.-F. et al., 2022). Others include autism (Yi et al., 2020), pediatric cerebral palsy (CP) (Xue et al., 2022), cognitive impairment (Zhang et al., 2013), and Parkinson's disease (PD) (Lee et al., 2013).

Wang et al. (2012) conducted a meta-analysis of SA in the treatment of acute ischemic strokes including eight randomized controlled trials (RCTs) with 538 participants suffering from acute ischemic strokes. The results showed that SA significantly improved neurological deficit score and the clinical effective rate in the patients when compared with the control of conventional medicine (pharmaceuticals). Zheng et al. (2011) conducted a meta-analysis for evaluating the clinical outcome of SA in the treatment of acute intracerebral hemorrhage (ICH). Seven independent trials (230 patients) were included in this study. The results indicate that SA appears to be effective for improving neurological deficit scores in patients with acute hypertensive ICH and SA therapy for acute ICH is generally safe. You et al. (2018) conducted a meta-analysis to assess the clinical effectiveness of SA for stroke. Out of a total of 2,086 papers, 21 RCTs were selected. The results revealed that SA may significantly facilitate the recovery of motor and nervous functions in patients with acute to chronic stroke. A similar conclusion was reached from another meta-analysis by Huang et al. (2021), revealing that SA improves motor function in patients with post-stroke hemiparesis. Therefore, it can be said with confidence that SA has a good therapeutic effect on either acute or chronic stroke, regardless of brain infarction or ICH.

The efficacy of SA has also been well-scrutinized for many types of brain dysfunctions other than strokes. A recent meta-analysis involving 859 cases showed that SA is quite effective in dealing with autism (Yi et al., 2020). A meta-analysis consisting of 731 children of CP showed that SA was more effective than that of conventional rehabilitation in improving the symptoms of CP, and the procedure was deemed safe (Xue et al., 2022). Zhang et al. (2013) evaluated the therapeutic effect of scalp electroacupuncture (SEA) for mild cognitive impairment (MCI) in the early stage. A total of 233 MCI patients were randomly divided into three groups: the medication (nimodipine) group, the SEA group, and the syndrome differentiation group. Each patient was treated for two rounds (courses) of treatment, totaling 8 weeks. The results showed that while all three therapies improved the cognitive function of MCI patients, the efficacy rate of the SEA and syndrome differentiation groups were essentially equal, but both were far more superior to the medication group. In another RCT involving 64 infants with prenatal brain damage syndrome (BDS), Liu et al. (2016) observed that the developmental level of intelligence, motor function, linguistic, and social skills of these infants were enhanced by the intelligence seven-needle therapy that primarily stimulated Shenting (GV24), Benshen (GB13), and Sishencong (Ex.HN1) on the scalp.

Although the above studies all have significant effects of SA for various brain diseases, there are many research limitations, including smaller sample size, unclear stimulation parameters, etc.

Potential mechanisms of scalp acupuncture for strokes

Over the recent years, many animal and clinical studies have been conducted on potential mechanisms of SA in treating brain diseases, especially strokes. According to current hypothesis of SA mechanism, its effect in restoring neurological dysfunction post-stroke is the result of stimulation of specific scalp areas in a reflex somatotopic system, corresponding to the functional zones of the cerebral cortex, with bioelectric effects transmitted to the cerebral cortex through meridians and nerves, thus altering the excitability of cerebral cortical nerve cells and accelerating the establishment of cerebral collateral circulation (Sun et al., 2020).

Certain clinical studies have also observed that applying SA on the region (ISSA_MS6) of the scalp direct above the cortical motor areas could induce vasodilatation of the cerebral blood vessels and better cerebral collateral circulation, raise CBF, lower the risks for infarction, and improve motor function for ischemic stroke cases (Zhang, 2003; Hsing et al., 2012). However, these effects also occur when a number of other scalp regions are stimulated, and it is not necessary to stimulate the specific scalp region corresponding to the cortical motor area. For example, multiple paralleled SA needles inserted at Baihui (GV20) and bilateral ISSA-MS8 could effectively increase the blood flow volume of the common carotid artery, leading to a rise of the energy supply of the cerebral blood circulation (Li et al., 2009). By functional magnetic resonance imaging (fMRI), it was observed that the contralateral somatosensory association cortex, the postcentral gyrus, and the parietal lobe were triggered when SA needles were inserted at the left Sishencong (EX.HN1), Chengling (GB18), Tianchong (GB9), and Jiaosun (TH20) of healthy volunteers (Park et al., 2009). In animal experiments with acute cerebral ischemia-reperfusion injury, it was observed that SA could suppress cytokines-mediated inflammatory reaction, attenuate cerebral ischemia-reperfusion injury, and improve neurofunctional rehabilitation (Zhang et al., 2007; Zhou et al., 2009).

Mechanisms of SA in the treatment of ischemic strokes are somewhat straightforward, especially in the acute stage, as SA stimulations may help reverse brain ischemia and improve neurological function by increasing CBF as it does for the facial nerve (Borsody and Sacristan, 2016). Several studies have shown that SA can prevent persistent thrombosis and increase vasodilation of the neurovasculature in the brain, and maintain blood circulation (Wang Y.-F. et al., 2022). By using fMRI diagnostics, among acute ischemic stroke patients, SA was shown being capable to enhance functional connectivity, particularly between visual, cognitive, motor control, and planning-related brain regions (Liu et al., 2020), or strengthen the functional activities related to sensory integration, language processing, and motor coordination of the dominant cerebral hemisphere and the motor control bilateral frontal lobe (Liu et al., 2021).

Of course, the mechanisms of SA in treating hemorrhagic strokes seems to be vastly different from the treatment of ischemic strokes. The main pathophysiological manifestations after ICH include early hematoma enlargement and perihematomal edema caused by catabolic products released from the hematoma. It was proposed that mechanisms of SA, such as on Baihui (GV20) penetrating Taiyang (EX.HN5) for the brain injury induced by cerebral hemorrhage may include increasing hematoma resorption rate, reducing cerebral edema, decreasing cerebral vascular permeability, and promote repairs for blood–brain barrier damage. It also may inhibit the inflammatory response in brain tissue around hematoma and improve the patient's immune functions. Furthermore, it may modulate vascular function, prevention of additional brain injury, and the improvement of coordination and compensation function between cortical functional zones (Jiang et al., 2001; Bao et al., 2005; Dong et al., 2006; Liu et al., 2012).

However, because hypoperfusion or ischemia of surrounding brain tissue is also common in hemorrhagic strokes or traumatic brain injuries (TBI), resulting in hematoma (Sills et al., 1996; Zazulia et al., 2001; Schubert et al., 2009), the role of SA in the treatment of hemorrhagic brain diseases, especially its sequelae, cannot be separated from the role of improving CBF.

In short, from the above studies, the effect of SA improving CBF is one of most identifiable traits, and therefore has been frequently reported. It not only serves as the basis of various chronic or long-term effects of SA described previously, but is also most closely related to the instant efficacy of SA, to be described below.

Instant effects of scalp acupuncture for stroke

Scalp acupuncture for stroke, whether hemorrhagic or ischemic, often has detectable instant effects, which means that in about 10 min after acupuncture, muscle strength in the paralyzed side could improve by 2 or more grades (Liu et al., 2012). Dong et al. (1994, 1996) found that 60.71% (34/56) ICH patients showed instant effect in the SA group, but no such luck in medication-therapy and surgery hematoma aspiration groups.

In an outpatient stroke rehabilitation unit, Molnar et al. (2018) conducted a prospective, assessor-blinded randomized control trial using Yamamoto's New SA (YNSA), for 520 cases with post-stroke syndrome who had hemorrhagic or ischemic strokes and were admitted within 6 weeks of stroke. The results showed that in the YNSA group, all the sensory, motor, and functional scores improved significantly during the examination period until 3 years after injury. In some instances, effects were instantly observed only after a few minutes of SA, such as improved mobility of the limbs, with such effects lingering even for several weeks.

In a RCT, Wang et al. (2018) assessed the effect of SA on walking pattern of stroke patients, using three-dimensional gait analysis (3D-GA). The RCT was conducted for 30 patients in the subacute stage of ICH (1–3 months), all of whom were able to walk on their own. Participants were divided into two groups: SA (treatment group) or no intervention (control group). The treatment group received SA, a penetrating needle from Baihui (GV20) to Taiyang (EX.HN5), whose manipulation was repeated three times with an interval of 5 min. Shortly after the treatment, a significant difference was observed between the treatment and control groups in terms of spatiotemporal parameters of step length, speed and cadence and bilateral limb support. CBF has been found to be another key factor affecting gait performance (Gatouillat et al., 2015). Moreover, Inoue et al. (2002) also demonstrated that SA has a powerful and instant effect in eliminating limb paralysis caused by cerebral infarction or cerebral hemorrhage in rats.

The instant effect of SA in the treatment of strokes suggests that there must be a rapid response mechanism in the multifaceted mechanisms of SA. Because during the regulation of the physiological functions, only neural control has the characteristic of being rapid and accurate, it can be presumed that the instant effect of SA for strokes must also be achieved through rapid neural regulation. According to Liu et al. (2012), the mechanisms may be that SA improved the disorder of CBF in ischemic region, or that it changed the excitability of cerebral cortex nerve cell and led to the retroconversion of the excitability of cerebral nerve cell that was depressed by the stimulation of hemorrhage or the oppression of hematoma. SA has also shown to be capable of regulating the electrophysiological activity of brain nerve cells and changing excitability of cerebral cortex nerve cells, thus awakening brain nerve cells that were in the state of shock or dormancy after ICH (Wang et al., 2003).

The presence of the instant effect of SA not only illustrates the reliability of the curative effect of SA on acute strokes, but also suggests that its mechanism is definitely a rapid response. The improvement of CBF caused by SA is one of these rapid responses.

Brain diseases related to reduced cerebral blood flow

Reduced CBF has been commonly described in brain aging and related neurodegenerative disorders, including Huntington's disease (HD) (Chen et al., 2012), Alzheimer's disease (AD) (Ruitenberg et al., 2005), PD (Chen et al., 2015; Pelizzari et al., 2019; Taguchi et al., 2019; Yang et al., 2021) and post-stroke sequelae (Hillis and Tippett, 2014), etc., suggesting that it is likely an important and early commonality in their otherwise distinct pathophysiological processes (Chen et al., 2012).

Several reports have suggested reduced CBF in HD, but little is known about the extent. Chen et al. (2012) used pulsed arterial-spin labeling MRI in conjunction with high-resolution anatomical MRI to non-invasively measure regional CBF (rCBF) in 17 early stage HD subjects and 41 healthy controls, and found profound yet heterogeneous CBF reductions in the cortex, extending to the sensorimotor, paracentral, inferior temporal, and lateral occipital regions, with sparing of the neighboring postcentral gyrus, insula and medial occipital areas. CBF in subcortical regions was also profoundly reduced, and to a similar degree.

The relationship between the severity of ischemic strokes and the infarct region is obvious. In fact, even during recovery from stroke, many sequelae symptoms are associated with inadequate CBF in certain brain regions. In a previous study that clinical interventions such as thrombolysis were used to treat acute ischemic strokes, Hillis and Tippett (2014) considered that patients with large areas of hypoperfusion beyond the infarct should be candidates for intervention to restore CBF. In most cases, the salvageable ischemic tissue is mainly confined to the cortex. The degree to which an individual recovers even simple cognitive functions is influenced by changes in CBF in the early period and by their degree of education level, as well as the intensity or level of initial severity of the stroke. Reperfusion of the distinct cortical regions of the left hemisphere, in the absence of infarction in that region, would restore the associated speech function. This suggests that during the rehabilitation, restoring CBF to specific cortical regions may yield improvements in certain symptoms associated with stroke-sequelae patients.

In fact, even in hemorrhagic strokes or TBI, a reduction of CBF also can occur. Schubert et al. (2009) observed that although all 17 patients with subarachnoid hemorrhage had intracranial pressure (ICP) and cerebral perfusion pressure within normal limits, they all had significantly reduced CBF. Those patients in better clinical condition had significantly smaller reductions in CBF than those with more severe hemorrhage, and had a comparably better prognosis. Changes in hypoperfusion were more pronounced in the supratentorial region (including the cerebrum) than in the infratentorial region (including the cerebellum).

Acute ICH also has shown substantial hypoperfusion zones around the hematoma that was interpreted as regional ischemia. Although there is no evidence for ischemia in the periclot zone of hypoperfusion in acute ICH patients studied 5–22 h after hemorrhage onset (Zazulia et al., 2001), substantial regions of reduced perfusion surrounding ICH might contribute to a substandard outcome and be amenable to anti-ischemic therapy (Sills et al., 1996).

Abnormal cerebral perfusion after TBI often leads to vasospasm. Maegawa et al. (2021) studied the relationship between the two in 25 patients, and found that the cerebral hypoperfusion of subarachnoid hemorrhage in the subacute phase could develop into post-traumatic cerebral vasospasm, thus emphasized the importance of aggressive treatment to prevent the development and progression of cerebral perfusion after TBI.

Other studies have also shown CBF is significantly reduced in patients with PD (Pelizzari et al., 2019) that correlates with the severity of the disease (Yang et al., 2021). In addition, previous studies have shown that the reduction in CBF induced by Madopar (levodopa benserazide) had a negative correlation with the Unified Parkinson Disease Rating Scale (UPDRS) scores, suggesting that improvement of symptoms was associated with increased CBF in the relevant areas (Chen et al., 2015).

All in all, there is apparent growing evidence that suggests various aspects of neuro-degenerative diseases are closely associated with the changes in cerebrovascular function (Yang et al., 2021). Changes in CBF could be used as important markers for disease diagnosis, mechanism investigation, and treatment assessment (Taguchi et al., 2019). It is theorized that treatments that improve or restore CBF may attenuate or possibly prevent the onset of these disorders (Sun et al., 2019). Therefore, as long as the SA can effectively enhance CBF, these brain diseases may be indicated for SA therapy. In fact, the clinical application of SA is most effective for patients suffering these types of brain diseases with significantly lower CBF, such as acute ischemic strokes.

Effects of scalp acupuncture on cerebral blood flow

To date, numerous studies have shown that SA can increase rCBF, especially improving CBF disturbances in ischemic regions. This has been observed not only in healthy individuals (Byeon et al., 2011; Im et al., 2014) or animal experiments (Goadsby and Duckworth, 1987; Wang et al., 2017), but also confirmed during the treatment of some patients with brain diseases (Yang et al., 2021). Byeon et al. (2011) discovered that needling Baihui (GV20) increased CBF velocity in the middle cerebral artery (MCA) and anterior cerebral artery (ACA). Im et al. (2014) observed that needling Fengchi (GB20) in selected healthy male subjects increased CO_2 reactivity in the basilar artery, but had no effect on the MCAs. Among other studies on the regulatory effects of acupuncture on CBF in PD, AD, and strokes, etc. (Li et al., 1999; Yong et al., 2009; Chen and Wu, 2011; Ratmansky et al., 2016; Kim et al., 2018; Ding et al., 2019; Yang et al., 2021), the stimulated locations included SA points or areas.

In a study consist of 15 PD patients with moderate symptoms, Yang et al. (2021) observed that needling Dazhui (GV14) and Fengchi (GB20) improved motor functions and subjective perception of the patients, while significantly increasing the total length of the internal carotid artery (ICA), the total length of the MCA, and the distal length of the M3 segment (that supplies blood to important components of the cortex – striatum circuit). From this, it seemed to be the mechanism by which acupuncture benefits PD patients. Besides, since the whole-brain CBF showed no significant difference before and after acupuncture, implying that acupuncture modulated the rCBF rather than increasing the whole-brain CBF.

Yong et al. (2009) observed in 30 patients of PD that SA combined with Madopar could improve the rigidity, tremor, dyskinesia and rCBF, indicating that the improvement of PD symptoms had a close relationship with the effect of SA on rCBF. Another study in the treatment of acute cerebral hemorrhage observed that the more significant the original CBF abnormality, the more significant the improvement after SA (Li et al., 1999).

Different SA points or areas from various parts of the scalp may produce different effects on rCBF, as such, the duration or intensity of acupuncture may also affect these effects. In a meta-analysis study, the effect of acupuncture was measured on the posterior circulation infarction vertigo (PCIV) that included 20 RCTs (1,541 participants) (Li et al., 2022). This study showed that acupuncture improved the vertebrobasilar blood flow velocity and achieved good efficacy for patients with PCIV. Moreover, longer duration of acupuncture interventions (more courses or sessions) and stronger stimulation (intensity) are generally more effective in improving vertebrobasilar blood flow velocity. For the acupoint selection, 33 main acupoints including SA points were used in the 20 studies, and Fengchi (GB20) was the most frequently used. Other researchers also indicated that needling Fengchi (GB20) may improve posterior cerebral circulation (Wang X.-X. et al., 2021; Wang Z.-Z. et al., 2021). Therefore, the use of Fengchi (GB20) for PCIV in clinical practice is highly recommended. Besides, Wu et al. (2017) observed the effect of long-term SA (treating for 5 months) on CBF in children with CP that SA increased the CBF, decreased the vascular resistance of ACA, MCA, and posterior cerebral artery (PCA), and improved the overall motor functions of the patients.

As mentioned above, the quantitative whole-brain CBF showed no significant difference before and after SA, implying that SA was modulating the distribution of cerebral blood supply. Therefore, in SA research or clinical practice, it may be better to observe and analyze the effect of SA on rCBF rather than the whole-brain CBF to show its impact on brain functions (Yong et al., 2009).

Innervation of the scalp and its overlap and communication

The above-mentioned effect of SA on significantly improving CBF is clearly inseparable from stimulating the sensory nerves that innervate the scalp. Regarding the innervation of the scalp region, in brief, the anterior half of the scalp is dominated by the trigeminal nerve, while the posterior half of the scalp mainly receives occipital nerves from the cervical roots (C1∼C3) (Kemp et al., 2011).

The supratrochlear nerve (STN) and supraorbital nerves (SON) originate from the ophthalmic branch (V1) of the trigeminal nerve. The STN innervates the lower part of the forehead, while the SON innervates the skin from the forehead to the lambdoidal suture. The zygomaticotemporal nerve (ZTN), arising from the maxillary division (V2) of the trigeminal nerve, innervates the anterior portion of the skin in the temporal region. The auriculotemporal nerve (ATN), derived from the mandibular division (V3) of the trigeminal nerve, innervates the posterior portion of the skin in the temporal region.

The cervical nerves innervating the scalp include the greater occipital nerve (GON), the lesser occipital nerve (LON), the greater auricular nerve (GAN), and the third occipital nerve (TON). The grouping of these three nerves is also called the occipital nerve. The GON originates from the medial branch of the dorsal ramus of the C2 spinal nerve and provides cutaneous innervation to most of the posterior scalp regions as it travels up to the vertex. The LON originates from the ventral rami of spinal nerves C2 and C3, and innervates skin behind the ear. The GAN originates from the anterior branches of C2 and C3, and supplies the skin of the posterior ear and mandibular angle. The TON, the superficial medial branch of the C3 dorsal ramus innervates an area of skin just below the superior nuchal line.

It is of the utmost importance to note the possible overlap and communication between these sensory nerves. The overlap (also known as convergence) of nerve fibers refers to two or more nerves innervating the same scalp area (Tubbs et al., 2011; Capek et al., 2015), which mainly occurs at the junction of adjacent or bilateral innervation areas (such as at the midline of the scalp). It was reported that small branches were found to cross the midline and communicate with the contralateral TON inferior to the inion in 66.7% of patients (Tubbs et al., 2011). In contrast, communications between neurons refers to the synaptic connection between two or more neurons (Lovinger, 2008). It is known that all three occipital nerves (GON, LON, and TON) located in the posterior neck and scalp regions are interconnected through their communicating branches regularly (Kemp et al., 2011).

Therefore, by needling an acupoint or area with overlaps of innervations, afferent stimulation information can be transmitted in parallel through different nerves. When needling an acupoint or area filled with communication, afferent stimulation information is transmitted, often through synaptic connections. Presumably, the stimulation amount of acupuncture or the degree of induced effects would be different from that of the stimulation of a single innervation area.

Many areas on the scalp have overlap or communication of different innervating nerves, such as between the trigeminal nerve or the GON distributed on either side of the midline; between two or more adjacent nerves distributed on the front and back of the same side of the scalp [such as between the ATN and the temporozygomatic nerve (TZN), the SON, or the GON or the LON, etc.]. In fact, in some scalp areas, due to the close proximity of two different nerves, even if they do not overlap or communicate, they may be successively stimulated with a single needle (such as 1-inch horizontal needling) (Li et al., 1999) inserted into the subcutaneous area at a certain distance. If SEA is applied, the propagation of the current is impossible to be limited to the stimulation of a single nerve.

Herewith, it is also important to note another type of overlap, or strictly speaking, a convergence mechanism that could occur after two sensory afferents enter the brain (Piovesan et al., 2003), as in the secondary sensory neurons of the trigeminal nerve. It also affects the specificity of the effects of SA when stimulating different nerves. In addition, the frontal and occipital muscles on the scalp are innervated by the superior zygomatic nerve (SZN) of the facial nerve and the posterior auricular nerve (PAN) (Yu and Wang, 2022), respectively, so that when stimulating the frontal or occipital areas with SA, it will also stimulate these two branches of the facial nerve besides stimulating the trigeminal nerve or the occipital nerve, respectively. It is currently known that in the face, there are dense and rich communication connections between the trigeminal nerve and facial nerve (Hwang et al., 2015).

The core scalp acupuncture points or areas for brain diseases and their innervation

Recently, a systematic review and meta-analysis was conducted by Wang Y.-F. et al. (2022) where the researchers extracted 33 SA points or areas from 35 RCT studies of SA for post-stroke hemiparesis. The study analyzed the SA point-selections data by applying an association rule based on the *Apriori* algorithm. The results showed that Baihui (GV20), Shenting (GV24), Yintang (Ex.HN3), and ISSA_MS6_i (ISSA Anterior Oblique Line of Vertex-Temporal, Lesion-Ipsilateral), ISSA_MS7_i (ISSA Posterior Oblique Line of Vertex-Temporal, Lesion-Ipsilateral), ISSA_PR (ISSA Parietal Region, comprised of ISSA_MS5, ISSA_MS6, ISSA_MS7, ISSA_MS8, and ISSA_MS9) (WHO Scientific Group, 1991; Liu et al., 2012) could be considered as the core SA location-combos in the treatment of post-stroke hemiparesis.

In daily SA clinical practice, the modern stimulation locations of SA are often labeled with meridian-based acupoints well-known to most acupuncturists (Wang et al., 2018). For examples, ISSA_MS5 is the connecting line from Baihui (GV20) to Qianding (GV21), along the vertex midline; ISSA_MS6 is the anterior oblique line of vertex-temporal from the anterior one of Shishencong (Ex.HN1) obliquely to Xuanli (GB6); ISSA_MS7 is the posterior oblique line of vertex-temporal from Baihui (GV20) obliquely to Qubing (GB7).

In a database of acupoints for PD established by Li et al. (2020), 184 acupoints, across 168 eligible papers, were selected. These points were mainly distributed in the head and neck areas and at the extremities. Among them, Taichong (LR3), Baihui (GV20), Fengchi (GB20), Hegu (LI4) and Chorea-Tremor Controlled Area were the most frequently used acupoints/areas. In a separate study to evaluate the effects of acupuncture on vascular dementia (VD) by Feng et al. (2015), 238 acupuncture prescriptions were included for analysis. Baihui (GV20), Sishencong (EX.HN1), Fengchi (GB20), Shenting (GV24), and Shuigou (GV26) of the scalp were the most frequently used acupoints for treatment of VD.

Yu et al. (2018) conducted a correlation analysis on the data of acupuncture prescriptions in the clinical literature regarding acupuncture treatment for AD, and observed the high-usage of 15 acupoints. Among them, those located on the scalp are Baihui (GV20), Sishencong (EX.HN1), Fengchi (GB20), and Shenting (GV24). Out of these, Baihui (GV20) seems to be the most frequently selected acupoint for AD. The intelligence seven needle therapy used by Liu et al. (2016) for infants with prenatal BDS primarily stimulates Shenting (GV24), Benshen (GB13), and Sishencong (Ex.HN1).

Since modern anatomical studies have been completed on almost all the acupoints of the body, including the scalp (Yan, 1983), one can easily label the core SA points or areas using the scalp nerves as follows: the frontal nerve originating from the V1 can be divided into the STN and the SON. The STN innervates Shenting (GV24), which is at 0.5 inches directly above the midpoint of the anterior hairline. SON innervates Benshen (GB13), which is on the lateral part of the forehead, 0.5 inches within the anterior hairline, 3 inches lateral to Shenting (GV24), in the frontalis muscle; Baihui (GV20) is primarily governed by the GON and the frontal nerve. Sishencong (Ex.HN1) has branches of the GON, ATN, and SON; Fengchi (GB20) is distributed with branches of the LON; Fengfu (GV16) is distributed with branches of the TON and the GON; there are branches of the GON in the ISSA_MS13 (Optic Area) and ISSA_MS14 (Balance Area) located on both sides of the midline of scalp. The ATN and the SON innervate the Chorea-Tremor Controlled Area, located on a parallel line, 1.5 cm in front of ISSA-MS6.

Among the points used from the YNSA method, the Parietal Acupoints (PTAT) are located on the midline from 5 cm anterior of the coronal suture to over the lambdoid suture, with a 2-cm width on the scalp (Aoyama et al., 2017). This area almost completely overlaps with the segment of GV on the vertex, including the ISSA_MS5 and Sishencong (EX.HN1). Their anterior and posterior parts are innervated by the trigeminal nerve and GON, respectively. Moreover, the Ypsilon points are distributed on the anterior and posterior sides of the auricle, in both sides of the scalp, innervated by the ATN and the LON.

Figure 1 shows the core SA points or areas frequently used for brain diseases, with the distribution of scalp nerves referenced in Khansa et al. (2016). Because acupoint is an area instead of a spot (Jin et al., 2007), in this figure, solid blue dots represent acupoints, while red bands represent the connecting areas between them or those of the international standard scalp acupuncture

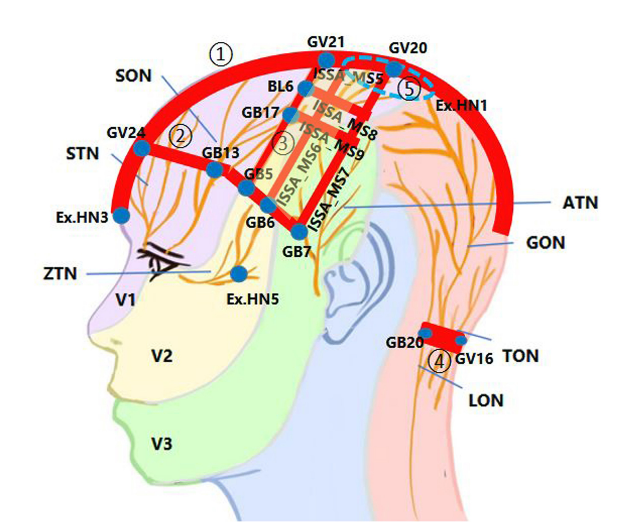

FIGURE 1
The innervation in the core scalp acupuncture (SA) points or areas used for brain diseases. The scalp nerve distribution patterns were redrawn based on the inspiration of a figure in Khansa et al. (2016). ATN, auriculotemporal nerve; GON, greater occipital nerve; LON, lesser occipital nerve; SON, supraorbital nerve; STN, supratrochlear nerve; TON, third occipital nerve; V1, ophthalmic branch of the trigeminal nerve; V2, maxillary branch of the trigeminal nerve; V3, mandibular branch of the trigeminal nerve; ZTN, zygomaticotemporal nerve. ① The midline area from Yintang (Ex.HN3) to above the lambdoid suture, with a 2-cm width on the scalp (red band). ② The region between Shenting (GV24) and Benshen (GB13) along the anterior hairline (red band), that includes ISSA_MS1, ISSA_MS2, ISSA_MS3, etc. ③ The region is surrounded by Baihui (GV20), Qianding (GV21), Xuanlu (GB5), Xuanli (GB6), and Qubin (GB7), which is a horizontal trapezoid composed of ISSA_MS5, the Chorea-Tremor Controlled Area, the lateral hairline, and ISSA_MS7 (light yellow box). ④ The region between Fengchi (GB20) and Fengfu (GV16) along the posterior hairline (red band). ⑤ The EX.HN1 region is surrounded by the four points of EX.HN1 (blue dash circle).

(ISSA) system (WHO Scientific Group, 1991); five particular SA regions are remarked by circled Arabic numbers.

From this figure, the distribution of core SA points or areas have at least three key features: 1. Centered around Baihui (GV20) with the scalp segment of the GV as the main axis. 2. Centered around the boundary between the front and back halves of the scalp along the oblique line from the vertex to the front of the auricle, especially the posterior edge of the front half. 3. Centered along the anterior, lateral and posterior hairline.

From the perspective of scalp innervation, most of these locations, such as ISSA_MS5, ISSA_MS6, ISSA_MS7, ISSA_MS8, ISSA_MS9, the Chorea-Tremor Controlled Area, and those SA points or areas along the frontier hairline, are located in the region innervated by the trigeminal nerve, or at the overlap of the bilateral same nerves or several different nerves on the same side of the scalp. This may also be the main neural basis for stimulating these areas with strong needle sensation or significant therapeutic effects. Of course, certain branches of the facial nerve innervating these areas may be stimulated simultaneously. Actually, 11 of the 14 ISSA lines (WHO Scientific Group, 1991) are distributed on the front half of the scalp innervated by the trigeminal nerve. Although Yintang (Ex.HN3) is not located on the scalp, it is still categorized as part of the SA points because it extends from Shenting (GV24) and also is innervated by the V1 of the trigeminal nerve. The significance of stimulating the trigeminal nerve with SA can be attributed to the direct axonal reflex pathway between the sensory afferent of trigeminal nerve and certain intracranial arteries (White et al., 2021).

However, it should be noted, however, that these so-called "core SA points or areas" were selected mainly on the basis of their high frequency of application in the related literature to date, without comparison to other SA points or areas in terms of efficacy. Their high frequency of application is also due to the current hypothesis of the SA mechanisms, which speculates the role of these points or areas corresponding to the cortical functional zones. Therefore, further research is warranted to determine whether they are the most effective stimulation locations for treated brain diseases. Moreover, the frequently used SA points or areas are not limited to locations shown in **Figure 1**.

Stimulating the trigeminal, facial, and cervical nerves to improve cerebral blood flow

Cerebral blood flow is usually determined by cerebral perfusion pressure and cerebrovascular resistance, while the latter itself depends on the degree of vasodilation and blood viscosity (Fantini et al., 2016). The brain relies mainly on changes in vascular caliber and systemic arterial pressures to maintain CBF (ter Laan, 2014). In the recent decades, many experiments or clinical trials are beginning to investigate the impact of CBF under direct stimulation of the trigeminal nerve or facial nerve innervating the scalp (or head and face).

It is known that the trigeminal nerve innervates the majority of the cerebral arteries and significantly contributes to the control of cerebrovascular tone in both healthy and diseased states (White et al., 2021). Upon stimulation of the trigeminal nerve, it permits direct modulation of CBF (Li et al., 2021). Research has shown that stimulation of the trigeminal nerve may be topographically oriented, with specific branches (the V1, V2, and V3) leading to an increased CBF in specific regions of the intracranial arterial tree (White et al., 2021).

Most intracranial vessels and dura mater, as well as the prefrontal skin are innervated by the ophthalmic branch of the trigeminal nerve. In some healthy subjects, both studies by Suzuki et al. (2020) and Waki et al. (2017) found that electroacupuncture at the V1 of the trigeminal nerve enhanced CBF in the prefrontal cortex. The nasociliary nerve, which originates from the V1, contains major vasodilator innervation for the MCA, and its stimulation results in the release of vasoactive neuropeptides in the MCA region (Atalay et al., 2002). Li et al. (2019, 2021) in recent studies observed that stimulation of the infraorbital nerve of the V2 dilates the ICA and that such vasodilatory response of cerebrovasculares is retained after SAH-induced cerebral vasospasm. The lingual nerve (LN) from the V3 of the trigeminal nerve is known to innervate the ICA. Ishii et al. (2014) demonstrated that stimulation of the LN leads to an enhanced CBF within the ICA. When considering the geographic distribution of the trigeminal nerve in the face, a possible distinction exists between different branches of the trigeminal system and the areas that they innervate, and this geographic specificity has the potential to guide a more specific treatment of trigeminal nerve stimulation (Li et al., 2021).

The facial nerve is a mixed nerve composed of motor, sensory, and parasympathetic fibers. To date, numerous studies have shown that stimulation of the parasympathetic fibers of the facial nerve rapidly dilates the cerebral arteries and increases CBF, no matter if that stimulation is delivered at the facial nerve trunk or at distal points such as the sphenopalatine ganglion (SPG, or called pterygopalatine ganglion, PTG). Generally, facial nerve stimulation increases CBF in a manner that it is deemed sufficient in reversing brain ischemia and improving neurological function (Borsody and Sacristan, 2016).

The facial nerve is the efferent limb in a cerebrovascular reflex with one or more sensory afferent limbs (e.g., trigeminal nerve). In the brainstem reflex evoked by stimulation of the face, the sensory endings of the trigeminal nerve are excited first in response to tissue damage, while the facial nerve might serve as the effector (Borsody and Sacristan, 2016). Therefore, the significance of stimulating the facial nerve in a clinical setting is to provide means to counteract brain injuries. For example, it could be used as an emergency treatment for conditions of brain ischemia such as ischemic strokes. Presently, several methods have been developed to activate the facial nerve, including non-invasive medical devices to give the facial nerve or the geniculate ganglion region magnetic stimulation through coil devices, placed on either side of the head or the ears (Borsody and Sacristan, 2016; Baker et al., 2021).

The effect of stimulating the occipital nerve on CBF was observed mainly by stimulating the cervical medulla of experimental animals. For example, stimulation of the cervical medulla in rats leads to cerebral vasodilation and a significant increase in cortical CBF (Sagher and Huang, 2000). This similar result was also observed in humans, and the ability to stimulate the spine to increase CBF is peculiar to higher cervical medullary stimulation with moderately low frequencies (Hosobuchi, 1985; Isono et al., 1995). Since the nerves innervating the posterior scalp mainly originate from the C1-C2 of the higher cervical medulla, there are many studies in recent years on the effect of SA on CBF, in which Fengchi (GB20) innervated by the LON was stimulated; most of these studies observed the improvement of the cerebral posterior circulation (Dong et al., 2020; Wang X.-X. et al., 2021; Wang Z.-Z. et al., 2021; Li et al., 2022).

The above-mentioned changes in CBF observed by direct stimulation of the trigeminal, facial nerves, etc. can satisfactorily explain why similar results of SA improving CBF are commonly observed in clinical practice. In other words, the improvement in CBF resulting from different SA points or areas is achieved by stimulating the corresponding nerves of the scalp.

Mechanisms of stimulating scalp nerves to improve cerebral blood flow

Mechanisms of stimulating scalp nerves to improve CBF can be explored by reviewing the structural characteristics of cerebral circulation with its neuromodulation. The blood supply to the brain tissue is accomplished by the ICA system and the vertebrobasilar artery system, the latter also known as the posterior circulation system, composed of the vertebral artery, basilar artery, and their branches. The brain is one of the most metabolically demanding tissues in the body, thus the cerebral arteries have several specificities that help differentiate themselves from other arteries in the body in order to meet the high demand for blood flow.

First, the “Circle of Willis” is formed from several interconnecting arteries, including the PCA, the posterior communicating artery, the ACA and the anterior communicating artery, In humans, CBF is mainly provided by the ICAs whereas the vertebral and spinal arteries supply the brainstem. The unique blood vessel organization of the “Circle of Willis” acts as a distribution center permitting blood to flow in any direction to meet increased demand and overcome stenosis (Roloff et al., 2016). When one of the arteries is blocked or narrowed, blood from the health side flows compensatory to the ischemic area, alleviating or eliminating the symptoms caused by the vascular blockage or stenosis.

Second, a unique characteristic of cerebral arteries is that they are both conduits and resistance vessels that are unlike any other arteries that constrict and dilate. In other words, the contraction and dilation of cerebral blood vessels have an autoregulatory capability (Faraci and Heistad, 1990), which protects the brain from sudden rises in blood pressure to prevent stroke, whereas vasodilatation can prevent ischemia (Roloff et al., 2016).

Third, cerebral arteries differ from peripheral arteries in another major way: they are richly innervated with neuronal fibers of parasympathetic origin, providing the most powerful vasodilatory mechanism (Goadsby, 2013). Parasympathetic fibers follow the sympathetic nerves along cerebral vasculatures (Duckles, 1981). The parasympathetic fibers innervating the anterior circulation of the cerebral artery are mainly derived from four parasympathetic ganglia: they are SPG, cavernous ganglion (CG), otic ganglion (OG), and carotid mini ganglion (CmG). Although there is some evidence for parasympathetic innervation of the vertebrobasilar arteries, their origin remains unknown (Roloff et al., 2016).

Fourth, cross talk between parasympathetic and sympathetic nerves exists in the cerebral arterial wall. This is because parasympathetic and sympathetic nerves usually are intertwined on cerebral arteries or run parallel within the same perineural sheath. The axo-axonal contact distance between them can be as low as 25 nm, compared to the 100 nm commonly seen for neuromuscular contacts in the same vessel. This close apposition suggests cross-talk between parasympathetic and sympathetic nerve fibers (Roloff et al., 2016). It has been observed that during a “fight or flight” situation where there is an increased sympathetic drive, the vertebrobasilar arteries may vasodilate (rather than constrict) via the mechanism to ensure the brainstem and/or cortex remain well perfused at times when peripheral organs experience vasoconstriction and reduced vascular conductance (Lee et al., 2011).

Fifth, the cerebrovasculares are innervated by sympathetic, parasympathetic, and sensory nerve fibers, all of which play an important role in cerebrovascular regulation. The trigeminal nerve is the largest cranial sensory nerve, with three bilaterally paired branches that spread across the face. In the brain, the trigeminal nerve converges at the trigeminal nucleus and has known connections to various regions of vasoregulatory control. Outside the brain substance, the trigemino-cerebrovascular network, which originates from the V2 and V1, innervates most of the cerebral vasculature system, including the large arteries, pial vessels, and venous sinuses. Therefore, stimulation of the trigeminal nerve can directly modulate CBF via these networks (Li et al., 2021). Those sensory nerve fibers that can affect cerebrovascular tone are called "sensory-motor nerves" (Rubino and Burnstock, 1996) and contain substance P and calcitonin gene–related peptide (cGRP) (Roloff et al., 2016).

Based on the above characteristics of the cerebral vascular structure, stimulation of the parasympathetic nerve fibers innervating the cerebral vasculature results in an increase in CBF accompanied by cerebral vasodilation; while stimulation of the sympathetic nerves innervating the cerebral vasculature may experience brief vasoconstriction, the subsequent effects are mostly increases in CBF.

It is known that there are two sources of innervation controlling blood flow to cerebrovasculares: extrinsic and intrinsic. The intrinsic innervation originates from local neurons [γ-aminobutyric acid (GABA)–ergic interneurons in the cerebral cortex (Suzuki et al., 2020)] within the central nervous system targeting arterioles to control blood flow in the brain parenchyma. The extrinsic innervation from peripheral ganglia innervate all major cerebral arteries before they enter the brain parenchyma (Roloff et al., 2016).

Extrinsic neuromodulation of CBF is mainly involved in the primary sensory neurons of the trigeminal nerve and postganglionic fibers of the facial nerve. They cause cerebral vasodilation by releasing different neurotransmitters: primary sensory neurons of the trigeminal nerve induce vasodilation by releasing cGRP, substance P, and pituitary adenylate cyclase-activated peptide. Facial nerve postganglionic fibers originate from the SPG and OG induce vasodilation by releasing acetylcholine (ACh) and vasoactive intestinal peptide (VIP). Originating in the superior salivatory nucleus, the greater petrosal nerve triggers vasodilation by synapsing with nitric oxide (NO)–ergic neurons in the SPG, which causes them to release NO from their terminals. In addition, the dural arteries are innervated by postganglionic fibers from the facial nerve, which originates from trigeminal primary sensory neurons and the SPG; trigeminal stimulation may increase blood flow in the dural arteries via an axonal reflex mechanism (Suzuki et al., 2020).

Stimulation of the trigeminal nerve at least has a threefold effect on cerebral vasculature, with each individual action leading to increased CBF (Suzuki et al., 2020): (1) The antidromic pathway: stimulation of sensory branches of the trigeminal nerve activates a pathway originating at the trigeminal ganglion that leads to antidromic release of neurotransmitters, vasodilation, and increases in CBF. (2) The trigeminal parasympathetic pathway: the sensory afferent from the trigeminal nerve results in parasympathetic vasodilation of the cerebral vasculature via interactions with the facial nerve and the SPG. (3) The central pathway: activation of the rostral ventral lateral medulla (RVLM) induces cerebral vasodilation and increase in mean arterial pressure (MAP), leading to an increased CBF (White et al., 2021).

As for mechanisms by which stimulation of the parasympathetic fibers of the facial nerve increases CBF, the most important is the anatomical connection about the greater superficial petrosal branch of the facial nerve to the terminal SPG, which then projects to the cerebral arteries. Next, connections for the parasympathetic facial nerve to the cerebral arteries other than the SPG likely exist, because the proximal stimulation of the facial nerve trunk produces larger CBF responses than does distal stimulation of the SPG (Borsody and Sacristan, 2016). In addition, stimulation of the facial nerve can also affect the blood supply to the ICA system through the connection with the trigeminal nerve, or affect the blood supply of the vertebral artery system through the connection with the branches of the cervical plexus such as the great auricular, greater, and lesser occipital, and transverse cervical nerves (Diamond et al., 2011). However, its effect is most significant through its own parasympathetic fibers via the SPG.

How does cervical nerve stimulation affect CBF, especially in the posterior circulation system? Although there is evidence indicating parasympathetic innervation of the vertebrobasilar arteries, their origin is still unclear (Roloff et al., 2016). There are hypotheses that innervation of the posterior circulation could originate from the CG (Hardebo et al., 1991; Suzuki and Hardebo, 1991), or the OG. Here the authors postulate the following two potential pathways:

The first is through the connection between the cervical nerve and the trigeminal nerve or facial nerve. There is plenty of evidence to date that shows the convergence of the trigeminal and occipital nerves. For example, the trigeminal cervical complex is such a convergence location (Bartsch and Goadsby, 2003). The overlap between the trigeminal nerve and cervical nerve is known as a convergence mechanism (Piovesan et al., 2003). Because the connection between the V1 of the trigeminal nerve and the cervical nerve through this complex is bidirectional, it is highly likely that stimulation of the scalp in the occipital nerve innervated area may affect CBF through the role of the V1. The connections between the cervical and facial nerves include the connections between the facial nerve and the GON or the LON from C2, and the communication between the facial nerve and the transverse cervical nerve from C2 to C3 (Diamond et al., 2011).

Another possibility is through the connection between the superior cervical ganglion (SCG) and the CmG of the ICA. Stimulation of the GON, LON or the TON from C2 to C3 can communicate with the SCG through the gray ramus communicans. The CmG is a small gangliform swelling, located on the under surface of the ICA, some fibers of which communicate with the trigeminal ganglion, the abducens nerve, and the SPG are distributed to the wall of the ICA (Gray and Lewis, 1918). The SCG is part of the sympathetic nervous system, a division of the autonomic nervous system most commonly associated with the "fight or flight" response. Sympathetic cerebral vasodilation has also been reported to be a compensatory response to vasoconstriction by adrenergic neurons originating in the SCG (Suzuki et al., 2020). Due to the cross talk of parasympathetic and sympathetic fibers on the aforementioned cerebral vascular walls, when stimulating the occipital nerve, the relevant cerebral blood vessels dilate rather than constrict.

Understanding the above mechanisms by which stimulation of different scalp nerves improves CBF not only opens the door to unveil the mechanisms of SA but also helps to elucidate why the effects of stimulating SA points or areas are relatively specific.

The relative specificity of scalp acupuncture in regulating cerebral blood flow

As of now, many clinical and experimental studies have clearly observed the relative specificity of SA in regulating CBF, which is generally manifested in the following aspects:

(1) The stimulation of the scalp area (SA) has shown significant improvement in CBF compared to stimulation of other parts of the body (body acupuncture) (Hyun et al., 2014; Ratmansky et al., 2016). A study compared the CBF impact of various GV acupoints, and found that effects of Fengfu (GV16), Baihui (GV20), and Dazhui (GV14) on the head and the neck had relatively better effects than other GV points on the trunk. In a similar study, results showed the largest impact or the most significant changes occurred when Fengfu (GV16) was needled (Kim et al., 2018).

(2) Stimulation at any parts of the scalp may improve CBF, but there must exist regional specificity. This is mainly because of the different innervation to the anterior and posterior halves of the scalp. It has been reported that SA with Baihui (GV20) can more significantly affect the ACA and the MCA, while needling Fengfu (GV16) can more significantly affect the PCA (Kim et al., 2018). In other studies, needling Fengchi (GB20) has been shown to improve CBF in posterior (vertebral and basilar) arteries but not the MCAs (Yuan et al., 1998; Im et al., 2014). whereas 20-min of needling at Baihui (GV20) increased CBF and CO2 reactivity in both MCA and ACA (basilar not measured; Byeon et al., 2011). In addition, stimulation of the V1, V2, or V3 of the trigeminal nerve also had different effects on CBF (White et al., 2021). Because the majority of the intracranial blood vessels and dura are innervated by the V1 of the trigeminal nerve, stimulation of SA areas or acupoints innervated by the V1 may have a more broader effect on rCBF. For the characteristics that acupuncture can improve CBF and stimulate different acupoints in different parts of the body to induce different cerebrovascular reactions, some researchers call it "acupoint-cerebrovascular specificity" (Sun et al., 2019).

On the other hand, due to the overlap or convergence of different innervation in many parts of the scalp, stimulation at these overlapping areas may also induce a broader or more significant improvement in CBF compared to stimulation of a single innervated area. This may be the neural characteristic of SA core points or areas frequently used for brain disease. However, it may also be a reason resulting in weakening the regional specificity of SA on CBF. For example, due to the functional connectivity or convergence between the trigeminal and occipital nerves innervating the anterior and the posterior parts of the scalp, respectively (Busch et al., 2006), the effect of stimulating the scalp areas innervated by the trigeminal nerve or GON, ION on CBF may extend to the cerebral arteries innervated by other connecting nerves. It has been reported that needling Baihui (GV20) affected the CBF of the posterior circulation system (Dong et al., 2020), and that needling Fengchi (GB20) and Dazhui (GV14) could improve the CBF of the ICA and MCA in PD patients (Yang et al., 2021).

(3) Stimulating only on a single side of the scalp nerve has an instant effect on CBF on both sides. In a study, Chen and Wu (2011) applied SA in the anterior oblique parietotemporal line on either the affected or the unaffected side for 30 stroke patients, there was an increased CBF bilaterally, but later it was found there was no statistically difference in CBF between SA on the affected and the unaffected side.

(4) The degree of improvement in CBF is related to stimulation parameters (intensity, frequency, duration, etc.) of SA. Although it has been reported that needling the scalp could improve CBF even without repeatedly manipulation of the needles, for example, when the needles were inserted at Fengchi (GB20) and Dazhui (GV14) clockwise to a depth of 15–20 mm, twirled at a angle, and retained for 30 min, an improvement in CBF in ICA and MCA could be observed (Yang et al., 2021), traditionally, SA usually emphasizes the need for repeated rapid rotation or lifting-thrusting of needles during treatment (Wang, 2019). It was reported that when SA was used to treat patients with PCIV, a longer treatment course (sessions) and stronger stimulation (intensity) are more effective in improving vertebrobasilar blood flow velocity and clinical efficacy (Li et al., 2022).

It was observed that SEA of the V1 of the trigeminal nerve to a level of 100 Hz, using 0.2-mA, stimulation enhances CBF of the prefrontal cortex more strongly than 0.1 mA. The intensity of the SEA was set so that no pain was felt but the stimulus was perceptible (Suzuki et al., 2020). In the electromagnetic stimulation experiment of the facial nerve, it was observed that the frequency of 10 Hz could most effectively improve the CBF (Borsody and Sacristan, 2016).

In summary, the effect of SA therapy on improving CBF has both specificity and relativity, the extent of which is determined by the innervated nerves and their density or overlap at the stimulated site, as well as the parameters of the stimulation. The relative specificity of SA points or areas affecting the cerebral vascular system or improving CBF may explain why there is the similar effectiveness of multiple SA schools using different models of SA areas in treating brain diseases. Of course, the presence of some frequently applied core SA points or areas, such as those primary lines described in the international standard of SA are still rational. However, selecting optimal stimulation locations of SA should be based on the relative specificity in future clinical trials.

Clinical significance of elucidating mechanisms of scalp acupuncture

In clinical practice, elucidating the mechanisms of SA improving CBF would be significant, at least in terms of the following aspects: First, indications of SA therapy shall be clarified. All brain disease patients with reduced CBF would be candidates of SA therapy, no matter if they are acute or chronic, ischemic or hemorrhagic. Second, selection and determination of core SA points or areas for various brain diseases shall be based on a solid scientific foundation. The optimal SA points or areas for brain disease shall be on the locations innervated by overlapping nerves, which would improve rCBF. Third, changes to CBF, especially rCBF can be a quick objective marker used to assess instant or long-term therapeutic effects, or to determine the proper stimulation parameters of SA.

In short, such clinical strategies based on the effect of improving CBF will further enhance the efficacy of SA for brain diseases, as well as to avoid SA research pitfalls based on the misconceptions of SA points or areas corresponding to the cortical functional zones.

Conclusion

It has been known that reduced CBF is often associated with the occurrence of many brain diseases, not only acute ischemic strokes, but also serious neurodegenerative diseases including PD, AD, and other forms of cognitive decline. Thus, improving or restoring CBF can prevent and treat these types of diseases theoretically. In fact, presently, there is an adequate amount of evidence-based clinical research (RCTs) that shows SA does have significant therapeutic effects on these brain diseases, especially ischemic strokes, though certain research limitations remained in such RCTs.

Furthermore, stimulating the SA points, areas or nerves innervating the scalp, especially the trigeminal nerve, can significantly improve CBF, which parallels the instant or long-term improvement of clinical symptoms of brain diseases. Neural pathways to improve CBF by stimulation of the trigeminal, facial, or cervical nerves, have also been gradually revealed. The presence of the core SA points or areas frequently used can also be rationally explained from the characteristics of nerve distribution, including nerve overlap or convergence in different parts of the scalp. However, these characteristics that would determine the role of these SA points or areas are relatively specific, and not due to a direct correspondence between the current hypothesized SA points, areas and the functional zones of the cerebral cortex.

Based on the above evidence chain, the efficacy of SA in treating brain diseases, especially ischemic, is mostly achieved by stimulating the nerves innervating the scalp, specifically the trigeminal nerve, to improve CBF. However, the therapeutic role of improving CBF is not always easily comprehended for brain diseases caused by other etiologies. It has been known that mechanisms of SA in treating different brain diseases might also be multifaceted, such as anti-inflammatory, reducing central sensitization, promoting angiogenesis and tissue repair, etc. Among these mechanisms, the anti-inflammatory response by SA may be secondary to the improvement of CBF in the treatment of brain diseases.

There have been a number of studies in which SA has an impact on the local or systemic immune function and inflammatory response (Liu et al., 2012; Wang J.-H. et al., 2022). The cholinergic anti-inflammatory mechanism might be activated by trigeminal parasympathetic pathways (such as trigeminal-vagal reflex) during SA. It is entirely possible that SA, while improving CBF, could also arouse the anti-inflammatory reflex, which is critical to those brain diseases with significant inflammation due to brain tissue injury (hemorrhagic stroke, traumatic brain injury, etc.). However, since the anti-inflammatory effect induced by acupuncture occurs slower than the improvement in CBF, the latter seems more suitable to explain the instant effect of SA in the treatment of brain diseases. Moreover, the improvement of rCBF in brain injury also facilitates the recruitment of leukocytes and pro-inflammatory cytokines in the early stages of injury while increasing the concentration of anti-inflammatory cytokines in later stages. Thus, there is an obvious need to further investigate which of these mechanisms are independent of improving CBF or whether they act synergistically, or have different levels of significance in the treatment of various types of brain diseases with SA.

The authors believe that understanding the neural control of SA on CBF is not only key in revealing the underlying mechanism in treating brain diseases, but also helps clarify above-mentioned roles evoked by SA. Moreover, the improvement of CBF by SA can be used as an objective indicator for effective SA stimulation in future studies.

The effort of elucidating this scientific foundation of SA therapy will undoubtedly further advance the clinical applications of SA in treating brain diseases, such as broadening the scope of indications based on its effect on improving CBF; clarifying or expanding the applications of SA; selecting the optimal stimulation points or areas of SA and stimulation parameters based on different conditions and scalp innervation characteristics and establishing a rapid objective indicator based on changes in rCBF to evaluate instant or long-term therapeutic effects. Such clinical strategies will all further improve the efficacy of SA in treating brain diseases and increase its repeatability, as well as avoid the pitfalls of SA research or practice due to misconceptions of SA points or areas corresponding to the cortical functional zones.

Author contributions

GJ conceptualized the theme of the review and drafted the manuscript. LJ critically reviewed and revised the manuscript. BJ performed the literature search and drew the figure. JZ and BH reviewed and edited the manuscript. SL helped with the final revision of the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

GJ was employed by the International Institute of Systems Medicine, Inc. and Ace Acupuncture Clinic of Milwaukee, LLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aoyama, N., Fujii, O., and Yamamoto, T. (2017). Efficacy of parietal acupoint therapy: scalp acupuncture for neck/shoulder stiffness with related mood disturbance. *Med. Acup.* 29, 383–389. doi: 10.1089/acu.2017.1250

Atalay, B., Bolay, H., Dalkara, T., Soylemezoglu, F., Oge, K., and Ozcan, O. E. (2002). Transcorneal stimulation of trigeminal nerve afferents to increase cerebral blood flow in rats with cerebral vasospasm: a noninvasive method to activate the trigeminovascular reflex. *J. Neurosurg.* 97, 1179–1183. doi: 10.3171/jns.2002.97.5.1179

Baker, T. S., Robeny, J., Cruz, D., Bruhat, A., Iloreta, A. M., Costa, A., et al. (2021). Stimulating the facial nerve to treat ischemic stroke: a systematic review. *Front. Neurol.* 12:753182. doi: 10.3389/fneur.2021.753182

Bao, C.-L., Dong, H.-S., Dong, G.-R., and Luo, E.-L. (2005). Effects of scalp penetration acupuncture on plasma endothelin (ET) and calcitonin related peptide (CGRP) contents in the patient of acute intracerebral hemorrhage. *Chin. Acup. Moxi.* 25, 717–719.

Bartsch, T., and Goadsby, P. J. (2003). The trigeminocervical complex and migraine: current concepts and synthesis. *Curr. Pain Headache Rep.* 7, 371–376. doi: 10.1007/s11916-003-0036-y

Borsody, M. K., and Sacristan, E. (2016). Facial nerve stimulation as a future treatment for ischemic stroke. *Brain Circ.* 2, 164–177. doi: 10.4103/2394-8108.195281

Busch, V., Jakob, W., Juergens, T., Schulte-Mattler, W., Kaube, H., and May, A. (2006). Functional connectivity between trigeminal and occipital nerves revealed by occipital nerve blockade and nociceptive blink reflexes. *Cephalalgia* 26, 50–55. doi: 10.1111/j.1468-2982.2005.00992.x

Byeon, H. S., Moon, S. K., Park, S. U., Jung, W. S., Park, J. M., Ko, C., et al. (2011). Effects of GV20 acupuncture on cerebral blood flow velocity of middle cerebral artery and anterior cerebral artery territories, and CO2 reactivity during hypocapnia in normal subjects. *J Altern. Compl. Med.* 17, 219–224. doi: 10.1089/acm.2010.0232

Capek, S., Tubbs, R. S., and Spinner, R. J. (2015). Do cutaneous nerves cross the midline? *Clin. Anat.* 1, 96–100. doi: 10.1002/ca.22427

Chen, J.-J., Salat, D. H., and Rosas, H. D. (2012). Complex relationships between cerebral blood flow and brain atrophy in early Huntington's disease. *Neuroimage* 59, 1043–1051. doi: 10.1016/j.neuroimage.2011.08.112

Chen, Y., Pressman, P., Simuni, T., Parrish, T. B., and Gitelman, D. R. (2015). Effects of acute levodopa challenge on resting cerebral blood flow in Parkinson's Disease patients assessed using pseudo-continuous arterial spin labeling. *PeerJ* 3:e1381. doi: 10.7717/peerj.1381

Diamond, M., Wartmann, C. T., Tubbs, R. S., Shoja, M. M., Cohen-Gadol, A. A., and Loukas, M. (2011). Peripheral facial nerve communications and their clinical implications. *Clin. Anat.* 24, 10–18. doi: 10.1002/ca.21072

Ding, N., Jiang, J., Xu, A., Tang, Y., and Li, Z. (2019). Manual acupuncture regulates behavior and cerebral blood flow in the SAMP8 mouse model of Alzheimer's disease. *Front. Neurosci.* 13:37. doi: 10.3389/fnins.2019.00037

Dong, G.-R., Wang, Z., and Wu, B.-Z. (1996). Discussion on the mechanism of immediate effect of head needling on acute encephalorrhagia. *Chin. Acup. Moxi.* 14, 26–28.

Dong, G.-R., Zhang, X., Li, D., and Han, R.-H. (1994). Research on scalp acupuncture for acute hypertensive cerebral hemorrhage. *Chin. Acup. Moxi.* 14, 13–15.

Dong, J., Bao, C.-L., and Gong, W.-Z. (2006). Clinical observation on effect of scalp penetration acupuncture in treating acute intracerebral hemorrhage. *Clin. J. Tradit. Chin. Med.* 18, 341–342.

Dong, Y.-S., Xing, S.-L., Zhou, H.-Y., Zhang, W., Sun, W., and Fan, J.-M. (2020). Effects of fast-twisting long-retaining acupuncture therapy on apoptosis and expression of related proteins in vestibular nucleus in rats with vertigo induced by posterior circulation ischemia. *Zhongguo Zhen Jiu* 40, 179–184. doi: 10.13703/j.0255-2930.20190305-k00034

Duckles, S. P. (1981). Evidence for a functional cholinergic innervation of cerebral arteries. *J. Pharmacol. Exp. Ther.* 217, 544–548.

Fantini, S., Sassaroli, A., Tgavalekos, K., and Kornbluth, J. (2016). Cerebral blood flow and autoregulation: current measurement techniques and prospects for noninvasive optical methods. *Neurophotonics* 3:031411. doi: 10.1117/1.NPh.3.3.031411

Faraci, F. M., and Heistad, D. D. (1990). Regulation of large cerebral arteries and cerebral microvascular pressure. *Circ. Res.* 66, 8–17. doi: 10.1161/01.res.66.1.8

Feng, S., Ren, Y., Fan, S., Wang, M., Sun, T., Zeng, F., et al. (2015). Discovery of acupoints and combinations with potential to treat vascular dementia: a data mining analysis. *Evid. Based Compl. Altern. Med.* 2015:310591. doi: 10.1155/2015/310591

Gatouillat, A., Bleton, H., VanSwearingen, J., Perera, S., Thompson, S., Smith, T., et al. (2015). Cognitive tasks during walking affect cerebral blood flow signal features in middle cerebral arteries and their correlation to gait characteristics. *Behav. Brain Funct.* 11:29. doi: 10.1186/s12993-015-0073-9

Goadsby, P. J. (2013). "Autonomic nervous system control of the cerebral circulation," in *Handbook of clinical neurology*, Chap. 16, eds M. Ruud and F. Dick (Amsterdam: Elsevier), 193–201.

Goadsby, P. J., and Duckworth, J. W. (1987). Effect of stimulation of trigeminal ganglion on regional cerebral blood flow in cats. *Am. J. Physiol.* 253(2 Pt. 2), R270–R274. doi: 10.1152/ajpregu.1987.253.2.R270

Gray, H., and Lewis, W. (1918). *Anatomy of the human body*, 20th Edn. Philadelphia: Lea and Febiger, 977–978.

Hardebo, J. E., Arbab, M., Suzuki, N., and Svendgaard, N. A. (1991). Pathways of parasympathetic and sensory cerebrovascular nerves in monkeys. *Stroke* 22, 331–342. doi: 10.1161/01.str.22.3.331

Hillis, A. E., and Tippett, D. C. (2014). Stroke recovery: surprising influences and residual consequences. *Adv. Med.* 2014:378263. doi: 10.1155/2014/378263

Hosobuchi, Y. (1985). Electrical stimulation of the cervical spinal cord increases cerebral blood flow in humans. *Appl. Neurophysiol.* 48, 372–376. doi: 10.1159/000101161

Hsing, W. T., Imamura, M., Weaver, K., Fregni, F., and Azevedo Neto, R. S. (2012). Clinical effects of scalp electrical acupuncture in stroke: a sham-controlled randomized clinical trial. *J. Altern. Compl. Med.* 18, 341–346. doi: 10.1089/acm.2011.0131

Huang, Y.-J., Huang, C.-S., Leng, K.-F., Sung, J.-Y., and Cheng, S.-W. (2021). Efficacy of scalp acupuncture in patients with post-stroke hemiparesis: meta-analysis of randomized controlled trials. *Front. Neurol.* 12:746567. doi: 10.3389/fneur.2021.746567

Hwang, K., Yang, S. C., and Song, J. S. (2015). Communications between the trigeminal nerve and the facial nerve in the face: a systematic review. *J. Craniofac. Surg.* 26, 1643–1646. doi: 10.1097/SCS.0000000000001810

Hyun, S., Im, J. W., Jung, W. S., Cho, K. H., Kim, Y. S., Ko, C. N., et al. (2014). Effect of ST36 Acupuncture on hyperventilation-induced CO2 reactivity of the basilar and middle cerebral arteries and heart rate variability in normal subjects. *Evid. Based Compl. Altern. Med.* 2014:574986. doi: 10.1155/2014/574986

Im, J. W., Moon, S. K., Jung, W. S., Cho, K. H., Kim, Y. S., Park, T. H., et al. (2014). Effects of acupuncture at GB20 on CO2 reactivity in the basilar and middle cerebral arteries during hypocapnia in healthy participants. *J. Altern. Compl. Med.* 20, 764–770. doi: 10.1089/acm.2013.0240

Inoue, I., Chen, L., Zhou, L., Zeng, X., and Wang, H. (2002). Reproduction of scalp acupuncture therapy on strokes in the model rats, spontaneous hypertensive rats-stroke prone (SHR-SP). *Neurosci. Lett.* 333, 191–194. doi: 10.1016/s0304-3940(02)01032-7

Ishii, H., Sato, T., and Izumi, H. (2014). Parasympathetic reflex vasodilation in the cerebral hemodynamics of rats. *J Comp. Physiol. B.* 184, 385–399. doi: 10.1007/s00360-014-0807-2

Isono, M., Kagam, A., Fujiki, M., Mori, T., and Hori, S. (1995). Effect of spinal cord stimulation on cerebral blood flow in cats. *Stereotact Funct. Neurosurg.* 64, 40–46. doi: 10.1159/000098732

Jiang, S.-Y., Du, J., and Dong, G.-R. (2001). Clinical immunological study on scalp acupuncture therapy for acute cerebral apoplexy. *Heilongjiang J. Tradit. Chin. Med.* 30, 53–54.

Jiao, S.-F. (1997). *Scalp acupuncture and clinical cases*. Beijing: Foreign Languages Press.

Jin, G.-Y., Xiang, J.-J., and Jin, L. L. (2007). *Contemporary medical acupuncture - a systems approach*. Berlin: Springer-Verlag and Higher Education Press, PRC.

Kemp, W. J. III, Tubbs, R. S., and Cohen-Gado, A. A. (2011). The innervation of the scalp: a comprehensive review including anatomy, pathology, and neurosurgical correlates. *Surg. Neurol. Int.* 2:178. doi: 10.4103/2152-7806.90699

Khansa, I., Barker, J. C., and Janis, J. E. (2016). "10 Sensory nerves of the head and neck," in *Anatomy for plastic surgery of the face, head, and neck*, eds K. Watanabe, M. M. Shoja, M. Loukas, and R. S. Tubbs (New York, NY: Thieme), 87. doi: 10.1055/b-0036-141935

Kim, Y. I., Kim, S. S., Sin, R. S., Pu, Y. J., Ri, G., and Rim, K. S. (2018). Study on the cerebral blood flow regulatory features of acupuncture at acupoints of the governor vessel. *Med. Acup.* 30, 192–197. doi: 10.1089/acu.2018.1285

Lee, H. S., Park, H. L., Lee, S. J., Shin, B. C., Choi, J. Y., and Lee, M. S. (2013). Scalp acupuncture for Parkinson's disease: a systematic review of randomized controlled trials. *Chin. J. Integr. Med.* 19, 297–306. doi: 10.1007/s11655-013-1431-9

Lee, T. J. F., Chang, H. H., Lee, H. C., Chen, P., Lee, Y. C., Kuo, J. S., et al. (2011). Axo-axonal interaction in autonomic regulation of the cerebral circulation. *Acta. Physiol.* 203, 25–35. doi: 10.1111/j.1748-1716.2010.02231.x

Li, B., Zhao, Q., Du, Y., Li, X., Li, Z., Meng, X., et al. (2022). Cerebral blood flow velocity modulation and clinical efficacy of acupuncture for posterior circulation infarction vertigo: a systematic review and meta-analysis. *Evid. Based Compl. Altern. Med.* 2022:3740856. doi: 10.1155/2022/3740856

Li, C., Chiluwal, A., Afridi, A., Chaung, W., Powell, K., Yang, W.-L., et al. (2019). Trigeminal nerve stimulation: a novel method of resuscitation for hemorrhagic shock. *Crit. Care Med.* 47, e478–e484. doi: 10.1097/CCM.0000000000003735

Li, C., White, T. G., Powell, K., Chaung, W., Shah, K., Wang, P., et al. (2021). Percutaneous trigeminal nerve stimulation induces cerebral vasodilation in a dose-dependent manner. *Neurosurgery* 88:nyab053. doi: 10.1093/neuros/nyab053

Li, J., Xiao, J.-H., and Dong, G.-R. (1999). Clinical study on the effect of scalp-acupuncture in treating acute cerebral hemorrhage. *Chin. J Integr. Tradit. West. Med.* 5, 265–268. doi: 10.1007/BF02935400

Li, L., Gong, J.-Q., Ding, G.-H., Cai, D.-H., and Cai, Y. (2009). Effect of multiple paralleled acupuncture needles stimulation of scalp points on hemodynamics and blood flow energy of the common carotid artery in stroke patients. *Zhen Ci Yan Jiu* 34, 334–338.

Li, Z., Hu, Y.-Y., Zheng, C. Y., Su, Q. Z., An, C., Luo, X.-D., et al. (2020). Rules of meridians and acupoints selection in treatment of Parkinson's disease based on data mining techniques. *Chin. J. Integr. Med.* 26, 624–628. doi: 10.1007/s11655-017-2428-6

Li, S.-J., Wang, Y., Xiang, J.-J., and Jin, G.-Y. (1997). FMRI studies with acupunctural stimulation. *Poster presented at the Fifth Scientific Meeting Intl. Sci. Megn. Reson. Med. April 12–18*, Vancouver, BC.

Liu, H., Chen, L., Zhang, G., Jiang, Y., Qu, S., Liu, S., et al. (2020). Scalp acupuncture enhances the functional connectivity of visual and cognitive-motor function network of patients with acute ischemic stroke. *Evid. Based Compl. Alt. Med.* 2020:8836794. doi: 10.1155/2020/8836794

Liu, H., Jiang, Y., Wang, N., Yan, H., Chen, L., Gao, J., et al. (2021). Scalp acupuncture enhances local brain regions functional activities and functional connections between cerebral hemispheres in acute ischemic stroke patients. *Anat. Rec.* 304, 2538–2551. doi: 10.1002/ar.247461

Liu, Z. H., Li, Y. R., Lu, Y.-L., and Chen, J.-K. (2016). Clinical research on intelligence seven needle therapy treated infants with brain damage syndrome. *Chin. J. Integr. Med.* 22, 451–456. doi: 10.1007/s11655-015-1977-9

Liu, Z., Guan, L., Wang, Y., Xie, C.-L., Lin, X.-M., and Zheng, G.-Q. (2012). History and mechanism for treatment of intracerebral hemorrhage with scalp acupuncture. *Evid. Based Compl. Altern. Med.* 2012:895032. doi: 10.1155/2012/895032

Lovinger, D. M. (2008). Communication networks in the brain: neurons, receptors, neurotransmitters, and alcohol. *Alcohol Res. Health* 31, 196–214.

Lu, S.-K. (1991). Scalp acupuncture therapy and its clinical application. *J. Tradit. Chin. Med.* 11, 272–280.

Maegawa, T., Sasahara, A., Ohbuchi, H., Chernov, M., and Kasuya, H. (2021). Cerebral vasospasm and hypoperfusion after traumatic brain injury: combined CT angiography and CT perfusion imaging study. *Surgic. Neurol. Intl.* 12:361. doi: 10.25259/SNI_859_2020

Molnar, I., Mate, A., Szöke, H., and Hegyi, G. (2018). YNSA permanent acupuncture application for post-stroke syndrome. *Med. Clin. Arch.* 2, 7–8. doi: 10.15761/MCA.1000132

Park, S. U., Shin, A. S., Jahng, G. H., Moon, S. K., and Park, J. M. (2009). Effects of scalp acupuncture versus upper and lower limb acupuncture on signal activation of blood oxygen level dependent fMRI of the brain and somatosensory cortex. *J. Altern. Compl. Med.* 5, 1193–1200. doi: 10.1089/acm.2008.0602

Pelizzari, L., Laganà, M. M., Rossetto, F., Bergsland, N., Galli, M., Baselli, G., et al. (2019). Cerebral blood flow and cerebrovascular reactivity correlate with severity of motor symptoms in Parkinson's disease. *Ther. Adv. Neurol. Disord.* 12:1756286419838354. doi: 10.1177/1756286419838354

Piovesan, E. J., Kowacs, P. A., and Oshinsky, M. L. (2003). Convergence of cervical and trigeminal sensory afferents. *Curr. Pain Headache Rep.* 7, 377–383. doi: 10.1007/s11916-003-0037-x

Ratmansky, M., Levy, A., Messinger, A., Birg, A., Front, L., and Treger, I. (2016). The effects of acupuncture on cerebral blood flow in post-stroke patients: a randomized controlled trial. *J. Altern. Compl. Med.* 22, 33–37. doi: 10.1089/acm.2015.0066

Roloff, E. V., Tomiak-Baquero, A. M., Kasparov, S., and Paton, J. F. (2016). Parasympathetic innervation of vertebrobasilar arteries: is this a potential clinical target? *J. Physiol.* 594, 6463–6485. doi: 10.1113/JP272450

Rubino, A., and Burnstock, G. (1996). Capsaicin-sensitive sensory-motor neurotransmission in the peripheral control of cardiovascular function. *Cardiovasc. Res.* 31, 467–479.

Ruitenberg, A., den Heijer, T., Bakker, S. L., van Swieten, J. C., Koudstaal, P. J., Hofman, A., et al. (2005). Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam Study. *Ann. Neurol.* 57, 789–794. doi: 10.1002/ana.20493

Sagher, O., and Huang, D. L. (2000). Effects of cervical spinal cord stimulation on cerebral blood flow in the rat. *J. Neurosurg.* 1 Suppl, 71–76. doi: 10.3171/spi.2000.93.1.0071

Schubert, G. A., Seiz, M., Hegewald, A. A., Manville, J., and Thomé, C. (2009). Acute hypoperfusion immediately after subarachnoid hemorrhage: a xenon contrast-enhanced CT study. *J. Neurotrauma* 26, 2225–2231. doi: 10.1089/neu.2009.0924

Sills, C., Villar-Cordova, C., Pasteur, W., Ramirez, A., Lamki, L., Barron, B., et al. (1996). Demonstration of hypoperfusion surrounding intracerebral hematoma in humans. *J. Stroke Cerebrovasc. Dis.* 6, 17–24. doi: 10.1016/S1052-3057(96)80021-8

Sun, J., Ashley, J., and Kellawan, J. M. (2019). Can acupuncture treatment of hypertension improve brain health? A mini review. *Front. Aging Neurosci.* 11:240. doi: 10.3389/fnagi.2019.00240

Sun, L., Fan, Y., Fan, W., Sun, J., Ai, X., and Qiao, H. (2020). Efficacy and safety of scalp acupuncture in improving neurological dysfunction after ischemic stroke: a protocol for systematic review and meta-analysis. *Medicine* 99:e21783. doi: 10.1097/MD.000000000002178

Suzuki, N., and Hardebo, J. E. (1991). Anatomical basis for a parasympathetic and sensory innervation of the intracranial segment of the internal carotid artery in man. Possible implication for vascular headache. *J. Neurol. Sci.* 104, 19–31. doi: 10.1016/0022-510x(91)90211-o

Suzuki, T., Waki, H., Imai, K., and Hisajima, T. (2020). Electroacupuncture of the ophthalmic branch of the trigeminal nerve: effects on prefrontal cortex blood flow. *Med. Acup.* 32, 143–149. doi: 10.1089/acu.2019.1406

Taguchi, S., Tanabe, N., Niwa, J. I., and Doyu, M. (2019). Motor improvement-related regional cerebral blood flow changes in Parkinson's disease in response to antiparkinsonian drugs. *Parkinsons Dis.* 2019:7503230. doi: 10.1155/2019/7503230

ter Laan, M. (2014). *Neuromodulation of cerebral blood flow. Ph. D Thesis*. Groningen: University Medical Center Groningen.

Tubbs, R. S., Mortazavi, M. M., Loukas, M., D'Antoni, A. V., Shoja, M. M., Chern, J. J., et al. (2011). Anatomical study of the third occipital nerve and its potential role in occipital headache/neck pain following midline dissections of the craniocervical junction. *J. Neurosurg. Spine* 15, 71–75.

Waki, H., Suzuki, T., Tanaka, Y., Tamai, H., Minakawa, Y., Miyazaki, S., et al. (2017). Effects of electroacupuncture to the trigeminal nerve area on the autonomic nervous system and cerebral blood flow in the prefrontal cortex. *Acup. Med.* 35, 339–344. doi: 10.1136/acupmed-2016-011247

Wang, H.-Q., Dong, G.-R., Bao, C.-L., and Jiao, Z.-H. (2018). Immediate effect of scalp acupuncture on the gait of patients with subacute intracerebral hemorrhage analyzed by three-dimensional motion: secondary analysis of a randomized controlled trial. *Acup. Med.* 36, 71–79. doi: 10.1136/acupmed-2016-011272

Wang, J.-H., Wang, N.-N., Yuan, B., He, W.-J., Du, X.-Z., Jiang, H., et al. (2022). Anti-inflammation mechanism of electro-scalp acupuncture in treatment of ischemic stroke based on IL-12 mediated JAK/STAT signaling pathway. *Zhongguo Zhen Jiu* 42, 1137–1144. doi: 10.13703/j.0255-2930.20210821-0006

Wang, M. (2019). "Acupuncture styles in current practice," in *Translational acupuncture research*, ed. Y. Xia (Cham: Springer), 155–163. doi: 10.1007/978-3-030-16089-0_2

Wang, S., Liu, K., Wang, Y., Wang, S., He, X., Cui, X., et al. (2017). A proposed neurologic pathway for scalp acupuncture: trigeminal nerve-meninges-cerebrospinal fluid - contacting neurons-brain. *Med. Acup.* 29, 322–326. doi: 10.1089/acu.2017.1231

Wang, S., Wang, J., Liu, K., Bai, W., Cui, X., Han, S., et al. (2020). The shortcut pathway between scalp acupuncture and brain. *Zhen Ci Yan Jiu* 45, 947–953. doi: 10.13702/j.1000-0607.200730

Wang, X.-X., He, A.-L., Liu, G.-Q., Yang, T.-J., and Gao, L. (2021). Clinical study on lingguizhugan decoction combined with electroacupuncture at fengchi blood supply point for vertebrobasilar insufficiency vertigo. *Progr. Mod. Biomed.* 21, 515–519. doi: 10.13241/j.cnki.pmb.2021.03.024

Wang, Y., Shen, J., Wang, X.-M., Fu, D.-L., Chen, C.-Y., Lu, L.-Y., et al. (2012). Scalp acupuncture for acute ischemic stroke: a meta-analysis of randomized controlled trials. *Evid. Based Compl. Altern. Med.* 2012:480950. doi: 10.1155/2012/480950

Wang, Y.-F., Chen, W.-Y., Lee, C.-T., Shen, Y.-Y., Lan, C.-C., Liu, G.-T., et al. (2022). Combinations of scalp acupuncture location for the treatment of post-stroke hemiparesis: a systematic review and *Apriori* algorithm-based association rule analysis. *Front. Neurosci.* 16:956854. doi: 10.3389/fnins.2022.956854

Wang, Y.-Q., Luo, C.-F., and Teng, X.-Y. (2003). Effect of somatosensory evoked potential of rats with acute cerebral hemorrhage treated with scalp acupuncture. *Inform. Tradit. Chin. Med.* 20, 47–48.

Wang, Z.-Z., Zhou, D., and Gao, Z.-Y. (2021). Clinical observation of the treatment of 40 cases of posterior circulation ischemic vertigo with western medicine combined with "yang hidden in yin" manipulation acupuncture at fengchi (GB20) point. *Chin. J. Ethnomed. Ethnopharm.* 30, 113–117.

White, T. G., Powell, K., Shah, K. A., Woo, H. H., Narayan, R. K., and Li, C. (2021). Trigeminal nerve control of cerebral blood flow: a brief review. *Front. Neurosci.* 15:649910. doi: 10.3389/fnins.2021.649910

WHO Scientific Group (1991). *WHO Scientific Group on International Acupuncture Nomenclature and World Health Organization. A proposed standard international acupuncture nomenclature : report of a WHO scientific group*. Available online at: https://apps.who.int/iris/handle/10665/40001 (accessed February 15, 2023).

Chen, X.-Y., and Wu, F.-D. (2011). A comparative study of the effect on cerebral blood flow of scalp acupuncture on the affected vs unaffected side in stroke patients. *Shanghai J. Acup. Moxi.* 30, 586–588.

Wu, M.-H., Liang, L.-P., Zeng, J., Li, R.-H., Luo, X.-G., Liang, W., et al. (2017). Effects of scalp acupuncture on cerebral blood flow and gross motor function in children with spastic cerebral palsy. *Chin. J. Rehab. Theory Pract.* 12, 942–945.

Xue, Y., Shi, S., Zheng, S., Yang, Z., Xu, J., and Gong, F. (2022). Therapeutic effect of scalp-based acupuncture and moxibustion as an adjunctive treatment on children with cerebral palsy comparing to conventional rehabilitation therapy: a systematic review and meta-analysis of randomized controlled trials. *Transl. Pediatr.* 11, 631–641. doi: 10.21037/tp-22-85

Yan, Z.-G. (1983). *Anatomical acupuncture point diagrams*. China: Chinese Medicine Health Press.

Yang, Y., He, L., Miao, S., Zhou, R., Zhang, Y., and Ma, Y. (2021). Quantitative analysis of cerebrovascular characteristics of Parkinson's disease treated with acupuncture based on magnetic resonance angiography. *Brain Sci. Adv*. 7, 141–154. doi: 10.26599/BSA.2021.9050015

Ye, M., Fan, A. Y., and Faggert Alemi, S. (2022). Questioning the fundamentals of Jiao's scalp acupuncture: point-positioning based on the hypothesis of the cerebral Cortex's functional zone. *Altern. Ther. Health Med*. [Epub ahead of print].

Yi, H. M., Han, Y. J., Li, M. X., Wang, J., and Yang, L. P. (2020). Scalp acupuncture for Autism spectrum disorder: a systematic review. *IOP Conf. Ser. Earth Environ. Sci.* 440:042094. doi: 10.1088/1755-1315/440/4/042094

Yong, H., Ying, Z., Jia, X.-M., Tang, A.-W., Li, D., Shao, M., et al. (2009). Effect of scalp acupuncture on regional cerebral blood flow in Parkinson's disease patients. *Chin. J. Tradit. Chin. Med. Pharm.* 24, 305–308.

You, Y. N., Song, M. Y., Park, G. C., Na, C. S., Han, J., Cho, M. R., et al. (2018). Meta-analysis on randomized controlled trials for scalp acupuncture treatment of stroke: a systematic review. *J. Tradit. Chin. Med.* 38, 465–479. doi: 10.1016/S0254-6272(18)30879-3

Yu, C., Wang, L., Kong, L., Shen, F., Du, Y., Kong, L., et al. (2018). Acupoint combinations used for treatment of Alzheimer's disease: a data mining analysis. *J. Tradit. Chin. Med.* 38, 943–952.

Yu, M., and Wang, S.-M. (2022). *Anatomy, head and neck, occipital nerves*. Treasure Island, FL: StatPearls Publishing.

Yuan, X., Hao, X., Lai, Z., Zhao, H., and Liu, W. (1998). Effects of acupuncture at fengchi point (GB 20) on cerebral blood flow. *J Tradit. Chin. Med.* 18, 102–105.

Zazulia, A. R., Diringer, M. N., Videen, T. O., Adams, R. E., Yundt, K., Aiyagari, V., et al. (2001). Hypoperfusion without ischemia surrounding acute intracerebral hemorrhage. *J. Cereb. Blood Flow Metab.* 21, 804–810. doi: 10.1097/00004647-200107000-00005

Zhang, H. (2003). Effect of scalp acupuncture on blood rheology, blood lipid, apoprotein of patients with apoplexy. *J. Emerg. Tradit. Chin. Med.* 12, 409–410. doi: 10.1097/MD.0000000000021783

Zhang, H., Zhao, L., Yang, S., Chen, Z., Li, Y., Peng, X., et al. (2013). Clinical observation on effect of scalp electroacupuncture for mild cognitive impairment. *J. Tradit. Chin. Med.* 33, 46–50. doi: 10.1016/s0254-6272(13)60099-0

Zhang, H.-X., Liu, L.-G., Zhou, L., Huang, H., Li, X., and Yang, M. (2007). Effect of scalp acupuncture on inflammatory response in rats with acute cerebral ischemia-reperfusion injury. *Zhong Xi Yi Jie He Xue Bao* 5, 686–691. doi: 10.3736/jcim20070617

Zheng, G.-Q., Zhao, Z.-M., Wang, Y., Gu, Y., Li, Y., Chen, X., et al. (2011). Meta-analysis of scalp acupuncture for acute hypertensive intracerebral hemorrhage. *J. Altern. Compl. Med.* 17, 293–299. doi: 10.1089/acm.2010.0156

Zhou, L., Zhang, H.-X., Wang, Q., Liu, L.-G., Yang, X., Yang, M., et al. (2009). Effect of scalp acupuncture on the expression of NF-kappaB mRNA, COX-2 mRNA and their proteins in rats with acute cerebral ischemia-reperfusion injury. *Zhen Ci Yan Jiu* 34, 304–308.

Zhu, M.-Q. (2007). *Color atlas of Zhu's scalp acupuncture*. San Jose, CA: Zhu's Neuro-Acupuncture Center, Inc.

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The durable effect of acupuncture for episodic migraine: a systematic review and meta-analysis

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Background: Migraine is a common and recurrent type of headache. Avoiding trigger factors is not often successful in reducing headache frequency, duration, and severity. Prophylactic medications may be effective but are limited by strict indications and daily medication intake. This review aimed to investigate the durable effect of acupuncture on episodic migraine.

Methods: Seven databases including Medline, Embase, PubMed, etc., were searched for English and Chinese literature from their inception to 23 November 2022. Two independent reviewers screened the retrieved studies and extracted the data. Primary outcomes were monthly migraine days, monthly migraine attacks, and VAS score at 3 months post-treatment. The risk of bias in included studies was assessed using the Cochrane Risk of Bias 2.0 tool. Meta-analysis was conducted where applicable.

Results: Fifteen studies were included in this review. Acupuncture reduced the number of migraine attacks (MD -0.68; 95% CI −0.93, −0.43; $p < 0.001$), the number of days with migraine (MD −0.86; 95% CI −1.18, −0.55; $p < 0.001$), and VAS score (MD −1.01; 95% CI −1.30, −0.72; $p < 0.001$) to a greater degree than sham acupuncture at 3 months after treatment. Significant differences in reducing pain intensity of migraine in favor of acupuncture compared with waitlist (MD −1.84; 95% CI −2.31, −1.37; $p < 0.001$) or flunarizine (MD −2.00; 95% CI −2.35, −1.65; $p < 0.001$) at 3 months after treatment were found, and the differences reached the minimal clinically important difference (MCID).

Conclusion: This review found that the durable effect of acupuncture for episodic migraine lasted at least 3 months after treatment. More high-quality studies with longer follow-up periods in the future are needed to confirm the findings.

KEYWORDS

acupuncture, migraine, durable effect, systematic review, RCTs

1. Introduction

Migraine is a disabling disorder that is typically characterized by recurrent moderate to severe attacks of headache, often lasting hours to days (Steiner et al., 2019), which is very common among all age groups and more prevalent in women than men (Stovner et al., 2022). It was ranked as the second most disabling disease worldwide with a global prevalence of 15%, associated with an annual financial burden estimated at $23 billion in the United States (Steiner et al., 2020; Ashina et al., 2021). Among migraine patients, young people reported the highest incidence rate and older adults reported the highest 1-year prevalence which increased with age. According to the International Headache Society (IHS) classification, the most frequent type of migraine is episodic migraine, with attacks occurring randomly with or without aura (Headache Classification Committee of the International Headache Society (IHS), 2018). Although there are verified migraine triggers, including stress, premenstrual periods, alcohol, and bad weather, most patients experience unpredictable attacks from month to month and fail to prevent migraine attacks by simply avoiding triggers (Marmura, 2018). The uncertainty of episodic migraine and the accompanying symptoms impair the quality of daily life and possibly lead to anxiety and depression (May and Schulte, 2016). With unsatisfied management and other risk factors, 2.5% of the patients with episodic migraine ultimately turn to chronic migraine (Andreou and Edvinsson, 2019).

The clinical recommendations for migraine consist of two situations: analgesics for acute attacks including nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin, triptan, and paracetamol; and prophylactic medication including metoprolol, propranolol, flunarizine, valproic acid, and topiramate (Evers et al., 2009; Kennis et al., 2013; Tfelt-Hansen, 2013). Using analgesics does not prevent future attacks but increases the risk of chronic migraine (Su and Yu, 2018). Prophylactic drugs are carefully prescribed only for patients with frequent attacks or severe auras that significantly impair quality of life and with no contraindication. Daily intake of preventive drugs usually lasts 3–6 months protecting patients from frequent attacks (Evers et al., 2009). However, the long-term effect after drug withdrawal has rarely been studied. Both flunarizine and beta-blockers failed to maintain the success of prophylaxis with a marked decrease after treatment discontinuation (Wöber et al., 1991). Considering the huge burden of migraine, the Global Campaign Against Headache has recently emphasized that more effort is needed to reduce migraine attack frequency and duration, reduce disability, and reduce health-related distress (The American Headache Society Position Statement On Integrating New Migraine Treatments Into Clinical Practice, 2019; Olesen and Jensen, 2023).

Acupuncture has been recommended as an optional treatment for episodic migraine by the National Institute for Health and Care Excellence (NICE) (Kennis et al., 2013). Besides, recent reviews consistently suggested that acupuncture's benefit for migraine is similar to preventive drugs and superior to sham acupuncture (Linde et al., 2016; Wells et al., 2019; Zhang et al., 2020). Another network meta-analysis reported that acupuncture showed a better effect than propranolol in reducing the number of migrainous attacks (Chen et al., 2020). Another notable feature of acupuncture is the durable effect after treatment, which can achieve longer-term therapeutic effects with fewer sessions, greatly reducing the burden of patients with episodic migraine. Latest clinical trials have revealed the long-lasting effect of acupuncture for episodic migraine in the post-treatment periods (Zhao et al., 2017; Xu et al., 2020) although the previous study did not find any significant difference between acupuncture and sham acupuncture in reducing migraine attacks at post-treatment follow-ups (Linde et al., 2005). However, the durable effect of acupuncture for episodic migraine has not been systematically reviewed to date. Therefore, we conducted this focused, systematic review of high-quality, randomized controlled trials (RCTs) to investigate the durable effect of acupuncture for episodic migraine after discontinuation of treatment. Figure 1 shows the flow chart of our research.

2. Materials and methods

We performed this systematic review in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement (Page et al., 2021). The protocol has been previously registered on PROSEPERO (ID: CRD42023394096).

2.1. Inclusion criteria

We identified relevant original studies following the PICOS principle (Table 1): Steiner et al. (2019) study type: RCTs; (Stovner et al., 2022) object of study: adult (age ≥ 18) patients with episodic migraine (with or without aura); (Ashina et al., 2021) intervention: acupuncture (including manual acupuncture, electroacupuncture, fire needling acupuncture, auricular acupuncture, scalp acupuncture, and warm needle moxibustion) compared with sham acupuncture, waitlist, or any pharmacological therapy (acute or prophylactic treatment); (Steiner et al., 2020) primary outcomes: the article must report at least one of the following primary outcomes and follow-up the patients for at least 3 months after treatment: ① total migraine days per month, ② attacks per month, ③ visual analog scale (VAS) score or numerical rating scale (NRS); [Headache Classification Committee of the International Headache Society (IHS), 2018] peer-reviewed articles that have been published in a journal; (Marmura, 2018) published in English or Chinese.

2.2. Exclusion criteria

Literature with the following characteristics was excluded: Steiner et al. (2019) studies investigating the effect of acupressure, moxibustion, laser acupuncture, or acupoint injection; Stovner et al. (2022) studies including patients with chronic migraine, cluster headache, tension-type headache, or menstrual migraine; Ashina et al. (2021) studies involving Chinese herbal medicine in any group, or comparing different needle insertion sites (different acupoints) or different forms of acupuncture; Steiner et al. (2020) studies with invalid sham control (inserting needles as deeply as verum acupuncture at acupoints or non-acupoints); Headache Classification Committee of the International Headache Society (IHS) (2018) quasi-RCTs; Marmura (2018) duplicate publications; May and Schulte (2016) articles without available data or research without full text; Andreou and Edvinsson (2019) articles with unclear follow-up timepoints.

2.3. Literature retrieval, screening, and data extraction

The RCT search strategy published by the Cochrane Collaboration was used to perform the literature search. We searched Seven databases including Medline, Embase, PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), China National Knowledge

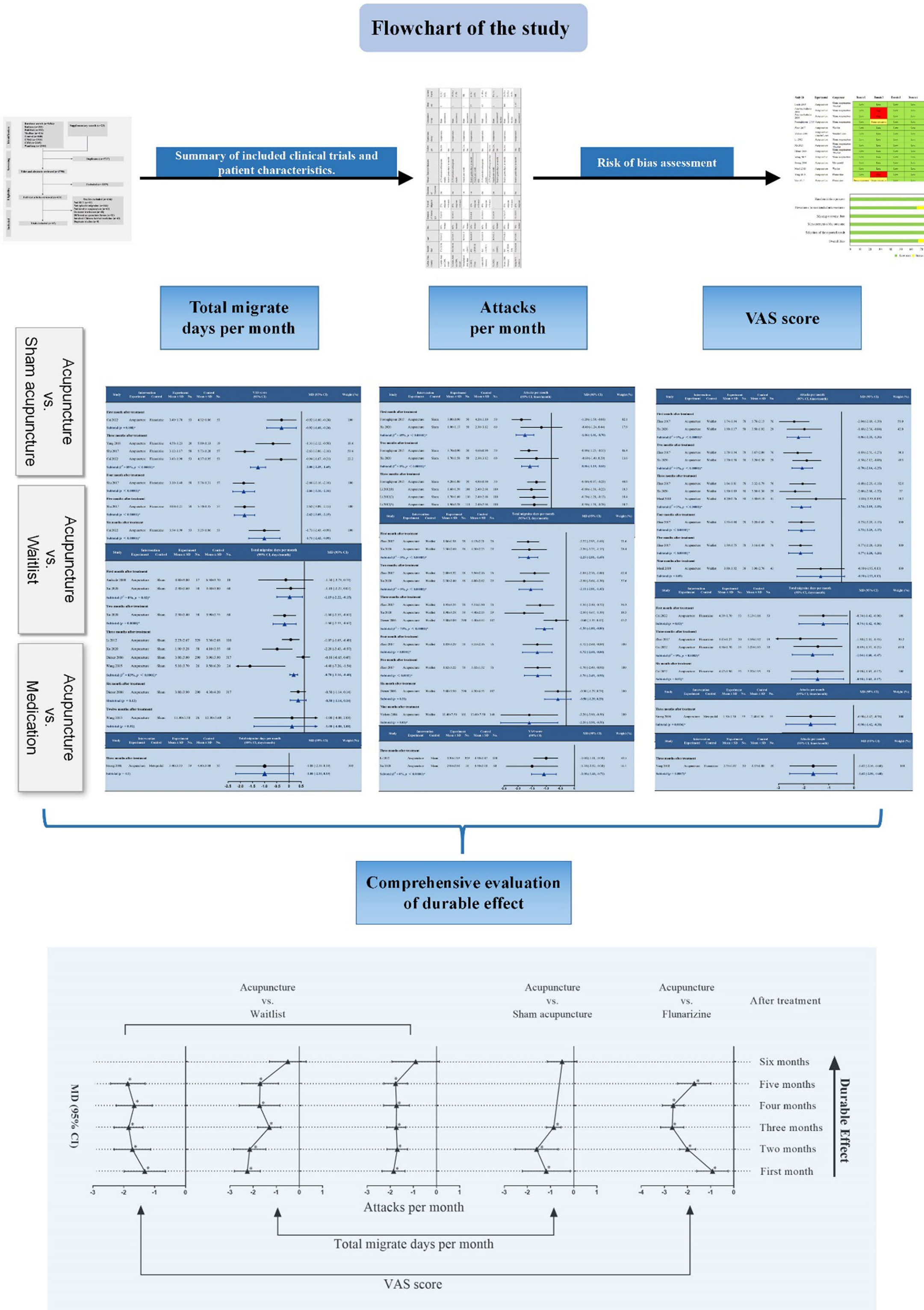

FIGURE 1
Flow chart of the study. RCT, randomized controlled trial.

Infrastructure (CNKI), WanFang Database, and Chinese Biomedical Literature Database (CBM) for English and Chinese literature from their inception to 23 November 2022. Please refer to Supplementary Data Sheet S1 for more information about the search strategy. We also searched references of current reviews, ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP), and the Chinese Clinical Trial Registry (ChiCTR) for potentially eligible studies.

The retrieved literature was included in the Endnote X9 software to remove duplicates. Two researchers were assigned to double-check the retrieved literature, read the title and abstract for preliminary screening, and read the full text for further screening. Data extraction also adopted double entry and cross-checking. In case of disagreement, a third senior researcher was consulted to reach a consensus. We contacted the authors to obtain complete data for literature with incomplete data. Data extraction included title, author, number of patients, average age, gender, study type, intervention and comparison, duration of treatment, outcome measures, follow-ups, and adverse events. Both primary outcomes and secondary outcomes were extracted. Secondary outcomes included response rate, safety, disability, quality of life, and anxiety and depression.

2.4. Risk of bias assessment

Based on the Cochrane Risk of Bias 2.0 tool for RCTs (Sterne et al., 2019), a revised domain-based evaluation introduced by Cochrane Collaboration, two researchers independently assessed the risk of bias of included studies. This tool considered five domains of bias: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. The overall assessment was classified into three categories "low," "high," and "some concerns," corresponding to the worst risk of bias identified across all domains.

2.5. Statistical analyses

RevMan (version 5.3) and Prism (version 7.0) were used to analyze the data. The effect value of categorical variables was expressed by risk ratio (RR), the effect value of continuous variables was expressed by mean difference (MD), and the 95% confidence interval (CI) was used to express the statistical analysis results. I^2 statistics were used to measure heterogeneity. A fixed-effect model was used if $I^2 < 50\%$; otherwise, the random-effect model was used. Publication bias was explored through a funnel-plot analysis.

3. Results

3.1. Study characteristics

We found 853 articles from PubMed, 314 from Embase, 416 from Medline, 840 from Central, 1968 from CNKI, 2149 from CBM, and 2,944 from WanFang. After results from these searches were combined and duplicates removed, the total number of articles was 3,790. Of these, 3,359 were excluded based on their titles and abstracts. Of the 416 that underwent full-text evaluation, 15 met the inclusion criteria and were included for analysis (Figure 2).

A total number of 3,035 patients with episodic migraine were enrolled in the 15 included studies. There was no statistical difference between the baselines of all study descriptions. Eight studies compared acupuncture with sham acupuncture (Linde et al., 2005; Alecrim-Andrade et al., 2006; Diener et al., 2006; Alecrim-Andrade et al., 2008; Li et al., 2012; Foroughipour et al., 2014; Wang et al., 2015; Xu et al., 2020), five studies compared acupuncture with waitlist (Vickers et al., 2004; Diener et al., 2006; Zhao et al., 2017; Musil et al., 2018; Xu et al., 2020), and four studies compared acupuncture with prophylactic drugs (Streng et al., 2006; Shu et al., 2017; Yang et al., 2018; Cai et al., 2022). Patients received 8–16 treatments over 4–12 weeks in most studies; the details of interventions are shown in Supplementary Data Sheet S2. Only Wang's study (Wang et al., 2015) had a longer treatment duration, 20 weeks. We also summarized each trial's clinical and methodological characteristics and primary outcomes (Table 2).

TABLE 1 Population, intervention, comparison, outcomes, and study design (PICOS) criteria for study selection.

Parameters	Descriptions
Object of study	Adult with episodic migraine.
Intervention	Acupuncture, electroacupuncture, fire needling acupuncture, auricular acupuncture, scalp acupuncture, and warm needle moxibustion.
Comparison	Sham acupuncture, waitlist, or any pharmacological therapy.
Outcome	(1) Total migraine days per month, (2) attacks per month, and (3) VAS score.
Setting	Peer-reviewed articles (RCTs) in English or Chinese.

3.2. Risk of bias assessment

Ten studies (Vickers et al., 2004; Linde et al., 2005; Diener et al., 2006; Streng et al., 2006; Li et al., 2012; Wang et al., 2015; Zhao et al., 2017; Musil et al., 2018; Xu et al., 2020; Cai et al., 2022) had a low RoB. One study (Shu et al., 2017) did not report the concealment of the allocation sequence. Five studies (Alecrim-Andrade et al., 2006, 2008; Foroughipour et al., 2014; Shu et al., 2017; Yang et al., 2018) did not sufficiently describe the process of intervention deviation. However, three studies (Alecrim-Andrade et al., 2006, 2008; Yang et al., 2018) with high dropout rates neither described the intervention deviation nor used intention-to-treat analysis to avoid the potential of a substantial impact on the result. Thus, one study (Shu et al., 2017) had a considerable RoB in the randomization process, three studies (Alecrim-Andrade et al., 2006, 2008; Yang et al., 2018) had high RoBs, and two studies (Foroughipour et al., 2014; Shu et al., 2017) had considerable RoBs in the domain of deviations from intended interventions (Figure 3).

3.3. Acupuncture versus sham acupuncture

Eight studies (Linde et al., 2005; Alecrim-Andrade et al., 2006; Diener et al., 2006; Alecrim-Andrade et al., 2008; Li et al., 2012; Foroughipour et al., 2014; Wang et al., 2015; Xu et al., 2020) were found comparing acupuncture with sham acupuncture with follow-ups ranging from 1 to 12 months after treatment (Figure 4). Acupuncture reduced significantly more migraine attacks than sham acupuncture at 3 months after treatment (MD -0.66; 95% CI −0.96, −0.37; $p < 0.001$; and $I^2 = 0\%$). Patients in the acupuncture group had significantly fewer days with migraine per month than in the sham acupuncture group at 3 months after treatment (MD -0.78; 95% CI −1.16, −0.40; $p < 0.001$). The random effect model was

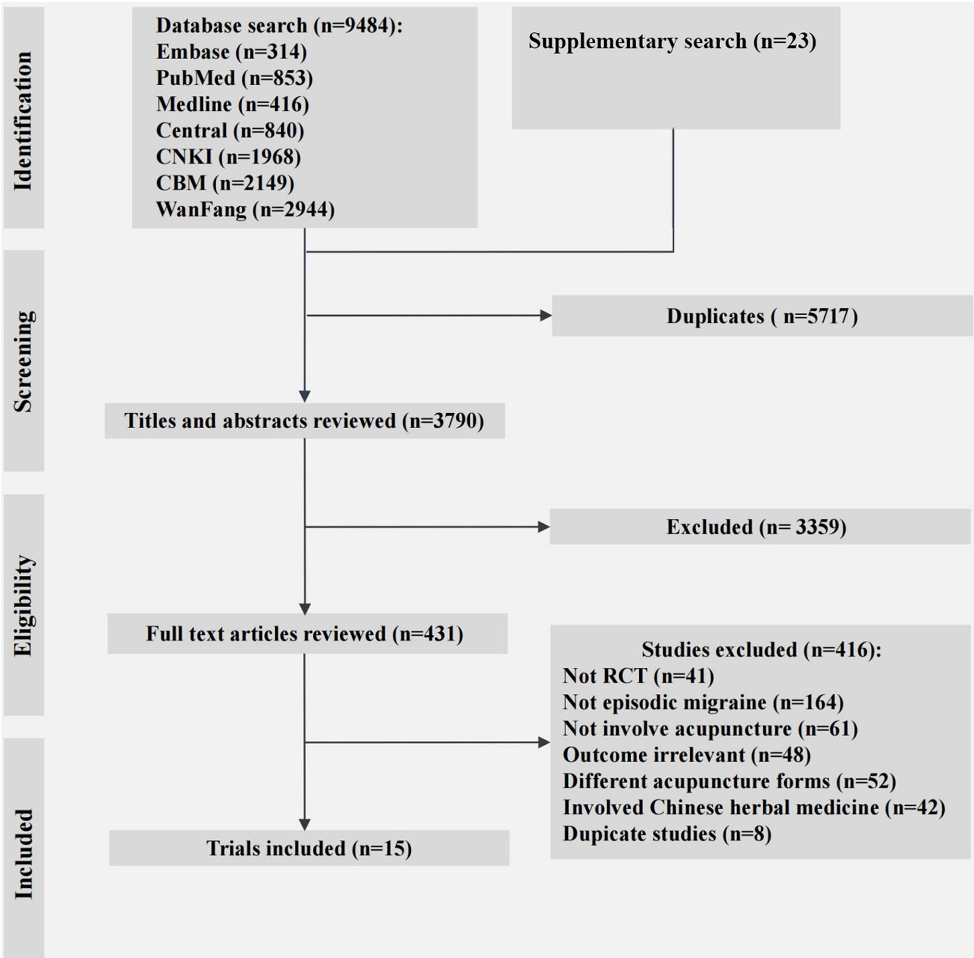

FIGURE 2
PRISMA flow diagram. RCT, randomized controlled trial.

employed due to the heterogeneity among the four trials ($I^2 = 83\%$). Two studies (Diener et al., 2006; Wang et al., 2015) reported that acupuncture was probably better than sham acupuncture in reducing the number of days with migraine per month at 6 and 12 months after treatment, separately, but the differences were not significant. No more evidence on the durable effect of acupuncture beyond 3 months was found (Supplementary Data Sheets S3, S4).

Three studies reported a reduction of pain intensity at 3 months after treatment. In Li et al. (2012) and Xu et al. (2020) studies, pain intensity was assessed by the VAS score. The minimal clinically important difference (MCID) in VAS score was defined as a 1.3 difference (Gallagher et al., 2001). The pooled estimate of VAS score at 3 months between acupuncture and sham acupuncture was significant (MD −1.08; 95% CI −1.46, −0.71; $p < 0.001$) in both trials but did not reach MCID (Figure 4). A study by Wang et al. (2015) employed a six-point Likert scale to assess pain intensity. Acupuncture reported a lower score than sham acupuncture at 3 months after treatment, but no significant difference was found (MD 0.3; 95% CI −0.5, 0.0; $p = 0.087$).

Two studies (Wang et al., 2015; Xu et al., 2020) reported the response rate, defined as the proportion of patients with a reduction in the number of migraine days by 50% or more, at 3 months after treatment (Figure 5). Acupuncture reached a significantly higher response rate than sham acupuncture (RR 1.93; 95% CI 1.46, 2.53; $p < 0.001$; and $I^2 = 74\%$). Furthermore, in two trials by Alecrim-Andrade et al. (2006, 2008), patients in the acupuncture group experienced fewer migraine days with nausea as well as fewer migraine days with vomiting than in the sham acupuncture group at 6 months after treatment; however, no significant difference has been found.

3.4. Acupuncture versus waitlist

Five studies (Vickers et al., 2004; Diener et al., 2006; Zhao et al., 2017; Musil et al., 2018; Xu et al., 2020) were found comparing acupuncture with waitlist with follow-ups ranging from 1 to 9 months after treatment. The durable effect of acupuncture sustained over 3 months after treatment is shown in Figure 6. The pooled estimated effect of three studies (Zhao et al., 2017; Musil et al., 2018; Xu et al., 2020) showed that acupuncture reduced significantly more migraine attacks than waitlist at 3 months (MD −1.74; 95% CI −2.15, −1.33; $p < 0.001$; $I^2 = 0\%$). For the number of days with migraine (Diener et al., 2006; Zhao et al., 2017; Xu et al., 2020), acupuncture also provided significantly more benefit than waitlist at 3 months after treatment (MD −1.30; 95% CI −1.80,

TABLE 2 Characteristics of included studies.

Author, Year, Country	Sample size	Age	Sex	Duration of disease (y)	Type of migraine	Duration of treatment	Primary outcome measures	Follow-ups	Experiment group	Comparison group	Drop out	Adverse events
Alecrim-Andrade 2008, Brazil	37 (19/18)	35.0 ± 9.2	89% women	17.6 ± 9.8	27% migraine with aura	3 m	≥50% reduction of migraine attacks	1 m, 6 m	Acupuncture	Sham acupuncture	1	8.2%/8.8%
							Total migraine days per month	1 m				
Alecrim-Andrade 2006, Brazil	28 (14/14)	24.7 ± 11.7	89% women	18.5 ± 8.3	21% migraine with aura	3 m	≥50% reduction of migraine attacks	6 m	Acupuncture	Sham acupuncture	0	19.6%/11.2%
Foroughipour 2014, Iran	100 (50/50)	36.5 ± 11.0	79% women	–	–	1 m	Attacks per month	1 m, 2 m, 3 m	Acupuncture	Sham acupuncture	0	NR
Li 2012, China	476 (358/118)	36.8 ± 12.2	59% women	8.2 ± 0.4	12% migraine with aura	1 m	Total migraine days per month	3 m	Acupuncture	Sham acupuncture	37	8.1%/6.8%
							Attacks per month					
							VAS					
Linde 2005, Germany	226 (145/81)	43.3 ± 11.8	82% women	20.9 ± 12.1	28% migraine with aura	2 m	≥50% reduction of migraine attacks	1 m	Acupuncture	Sham acupuncture	23	41.4%/17.3%
							≥50% reduction of migraine days	1 m, 4 m				
Xu 2020, China	150 (60/60/30)	36.3 ± 11.4	80% women	10.0 ± 5.0	–	2 m	Attacks per month	1 m, 2 m, 3 m	Acupuncture	Sham acupuncture/ Waitlist	2	8%/0/0
							Total migraine days per month					
Diener 2006, Germany	960 (313/339/308)	37.7 ± 10.5	83% women	16.7 ± 11.7	50% migraine with aura	6w	Total migraine days per month	3 m, 6 m	Acupuncture	Sham acupuncture / Waitlist	166	24.0%/23.6% /19.5%
Wang 2015, Australia	50 (26/24)	42.7 ± 14.1	74% women	19.7 ± 12.9	42% migraine with aura	5 m	Total migraine days per month	3 m, 12 m	Acupuncture	Sham acupuncture	3, 25*	NR
Zhao 2017, China	165 (83/82)	36.4 ± 14.2	76% women	9.2 ± 7.6	–	1 m	Total migraine days per month	1 m,2 m, 3 m, 4 m, 5 m	Acupuncture	Waitlist	0	6.0%/2.4%
							Attacks per month					
							VAS					
Vickers 2004, the United Kingdom	401 (205/196)	46.3 ± 10.4	85% women	21.6 ± 13.9	–	3 m	Total migraine days per month	9 m	Acupuncture	Waitlist	100	2.4%/0

(Continued)

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TABLE 2 (Continued)

Author, Year, Country	Sample size	Age	Sex	Duration of disease (y)	Type of migraine	Duration of treatment	Primary outcome measures	Follow-ups	Experiment group	Comparison group	Drop out	Adverse events
Musil 2018, Czech Republic	86 (42/44)	46.1 ± 11.5	89% women	24.9 ± 13.6	–	3 m	Attacks per month VAS	3 m, 9 m 3 m	Acupuncture	Waitlist	2	2.4%/0
Yang et al. (2018), China	42 (21/21)	33.0 ± 3.4	89% women	–	–	1 m	Total migraine days per month VAS	3 m	Acupuncture	Flunarizine	3	33.3%/42.9%
Shu et al. (2017), China	120 (60/60)	47 ± 9	76% women	0.9 ± 0.5	–	1 m	VAS	3 m, 4 m, 5 m	Acupuncture	Flunarizine	3	1.7%/0
Cai et al. (2022), China	110 (55/55)	41.5 ± 9	60% women	11.1 ± 4.8	–	1 m	Total migraine days per month VAS	1 m, 3 m, 6 m	Acupuncture	Flunarizine	0	1.7%/5.5%
Streng 2006, Germany	114 (59/55)	40.2 ± 11.0	84% women	–	90% migraine with aura	3 m	Total migraine days per month Attacks per month	3 m	Acupuncture	Metoprolol	26	15.3%/78.2%

*Three patients dropped out at 3 months and 25 dropped out at 12 months.

−0.80; $p < 0.001$; and $I^2 = 74\%$). Four studies followed up with the patients for more than 3 months after treatment and found inconsistent results (Supplementary Data Sheets S5, S6). Two studies (Vickers et al., 2004; Zhao et al., 2017) reported significant between-group differences (at 4 and 5 months after treatment in Zhao's study, and at 9 months after treatment in Vickers' study), while the differences were not significant in another two studies (Diener et al., 2006; Musil et al., 2018) (at 6 months after treatment in Diener's study, and at 9 months after treatment in Musil's study).

Two trials (Zhao et al., 2017; Musil et al., 2018) comparing acupuncture with waitlist measured the VAS score at 3 months after treatment. We pooled the data from both studies and found a statistically significant difference between groups on VAS score (MD −1.84; 95% CI −2.31, −1.37; $p < 0.001$; and $I^2 = 58\%$), and the difference reached MCID. Only one study by Zhao et al. (2017) reported longer follow-ups. It seemed that the effect of acupuncture on alleviating pain still existed at 4 months after treatment (MD −1.66; 95% CI −2.24, −1.08; $p < 0.001$) and 5 months after treatment (MD −1.87; 95% CI −2.43, −1.31; $p < 0.001$), showing both statistically and clinically important differences (Supplementary Data Sheet S7).

Four studies reported the response rate for 3 months or more after treatment. Three out of the four trials (Vickers et al., 2004; Musil et al., 2018; Xu et al., 2020) suggested that acupuncture reached significantly higher rates of responders than waitlist at 3 months after treatment 64.7 (MD 64.7%; 95% CI 44.2, 85.1%; $p < 0.001$), 6 months after treatment (RR 2.26; 95% CI 1.44, 3.53; $p = 0.0004$), and 9 months after treatment (MD 15%; 95% CI 6, 25%; $p = 0.02$). However, one study (Diener et al., 2006) reported no significant difference between acupuncture and waitlist in response rate at 5 months after treatment (RR 1.14; 95% CI 0.92, 1.42; $p = 0.22$).

3.5. Acupuncture versus medication

3.5.1. Acupuncture versus flunarizine

Three trials (Shu et al., 2017; Yang et al., 2018; Cai et al., 2022) comparing acupuncture with flunarizine for episodic migraine were analyzed (Supplementary Data Sheets S8–S10). Yang's study (Yang et al., 2018) reported that acupuncture led to fewer migraine attacks per month than flunarizine with a statistically significant difference between groups (MD −1.62; 95% CI −2.56, −0.68; $p < 0.001$). For the number of days with migraine, we pooled the data from studies by Yang and Cai (Yang et al., 2018; Cai et al., 2022) and found a statistically significant difference between groups (MD −1.04; 95% CI −1.60, −0.47; $p < 0.001$), as shown in Figure 7. There was non-significant heterogeneity between the studies ($I^2 = 0\%$), hence fixed effect model was adopted. Three studies (Shu et al., 2017; Yang et al., 2018; Cai et al., 2022) were found assessing pain intensity at 3 months after treatment. The pooled estimate of VAS score at 3 months between acupuncture and sham acupuncture was significant (MD −2.00; 95% CI −2.35, −1.65; $p < 0.001$) in both trials but did not reach MCID (Figure 7). The random effect model was employed due to the significant heterogeneity amongst the three trials ($I^2 = 89\%$).

3.5.2. Acupuncture versus metoprolol

A study by Streng et al. (2006) compared acupuncture with metoprolol in patients with episodic migraine

Study ID	Experimental	Comparator	Domain 1	Domain 2	Domain 3	Domain 4	Domain 5	Overall
Linde 2005	Acupuncture	Sham acupuncture /Waitlist	Low	Low	Low	Low	Low	Low
Alecrim-Andrade 2008	Acupuncture	Sham acupuncture	Low	High	Low	Low	Low	High
Alecrim-Andrade 2006	Acupuncture	Sham acupuncture	Low	High	Low	Low	Low	High
Foroughipour 2015	Acupuncture	Sham acupuncture	Low	Some concerns	Low	Low	Low	Some concerns
Zhao 2017	Acupuncture	Waitlist	Low	Low	Low	Low	Low	Low
Vickers 2004	Acupuncture+ standard care	Standard care	Low	Low	Low	Low	Low	Low
Li 2012	Acupuncture	Sham acupuncture	Low	Low	Low	Low	Low	Low
Xu 2020	Acupuncture	Sham acupuncture /Waitlist	Low	Low	Low	Low	Low	Low
Diener 2006	Acupuncture	Sham acupuncture /Waitlist	Low	Low	Low	Low	Low	Low
Wang 2015	Acupuncture	Sham acupuncture	Low	Low	Low	Low	Low	Low
Streng 2006	Acupuncture	Metoprolol	Low	Low	Low	Low	Low	Low
Musil 2018	Acupuncture	Waitlist	Low	Low	Low	Low	Low	Low
Yang 2018	Acupuncture	Flunarizine	Low	High	Low	Low	Low	High
Shu 2017	Acupuncture	Flunarizine	Some concerns	Some concerns	Low	Low	Low	Some concerns
Cai 2022	Acupuncture	Flunarizine	Low	Low	Low	Low	Low	Low

FIGURE 3
(A) Risk of bias graph presented as percentages. **(B)** Cochrane Risk of Bias 2.0 Tool of included studies.

(Supplementary Data Sheets S11, S12). The number of migraine attacks per month was significantly lower in the acupuncture group than in the metoprolol group at 3 months after acupuncture treatment (MD −0.90; 95% CI −1.42, −0.38; $p < 0.001$). However, the between-group difference in total migraine days per month was not significant (MD −1.00; 95% CI −2.19, 0.19; $p = 0.1$).

3.6. Adverse event

Overall, 13 studies reported information on adverse events. The rates of AEs in the acupuncture group were 1.7–41.4%, and those in the sham acupuncture group were reported by six of the 13 studies as 0–23.6% (Table 2). The main pattern of AEs was similar in both acupuncture and sham acupuncture groups, all reported as mild to moderate and not requiring special intervention. The most frequent type of AE was subcutaneous hemorrhage at needling sites. Also, some patients complained about pain in the puncture area (19 received acupuncture vs. 6 received sham acupuncture) and other AEs, including fatigue (6 vs. 1), palpitation (2 vs. 0), and ankle swelling (1 vs. 0). In addition, two studies (Vickers et al., 2004; Linde et al., 2005) reported a total of 23 cases of headache after treatment (17/383 in the acupuncture group vs. 6/112 in the sham acupuncture group).

4. Discussion

In this meta-analysis, 15 studies were analyzed to evaluate the durable effect of acupuncture for patients with episodic migraine. Acupuncture was significantly better than sham acupuncture, waitlist, and flunarizine in reducing the number of days with migraine per month and migraine attacks per month at 3 months after treatment. According to pooled estimates, acupuncture achieved a significant reduction in clinical importance in pain intensity measured by VAS score than waitlist and prophylactic drugs. Evidence on the pain-alleviating effect of acupuncture compared with sham acupuncture was inadequate.

As a worldwide prevalent life-span disorder, migraine negatively impacts patients' quality of life and causes disability and comorbidity. We conducted this systematic review and meta-analysis to evaluate the durable effect of acupuncture for episodic migraine in response to the need to reduce migraine frequency, severity, and headache-related depression. Overall, the therapeutic successes of acupuncture for episodic migraine lasted for at least 3 months after discontinuation of treatment. Compared with waitlist or sham control, the response rate (≥50% reduction of migraine days) of acupuncture was reported to be significantly higher in most of the included studies. Previous literature also focused on the efficacy of acupuncture in comparison

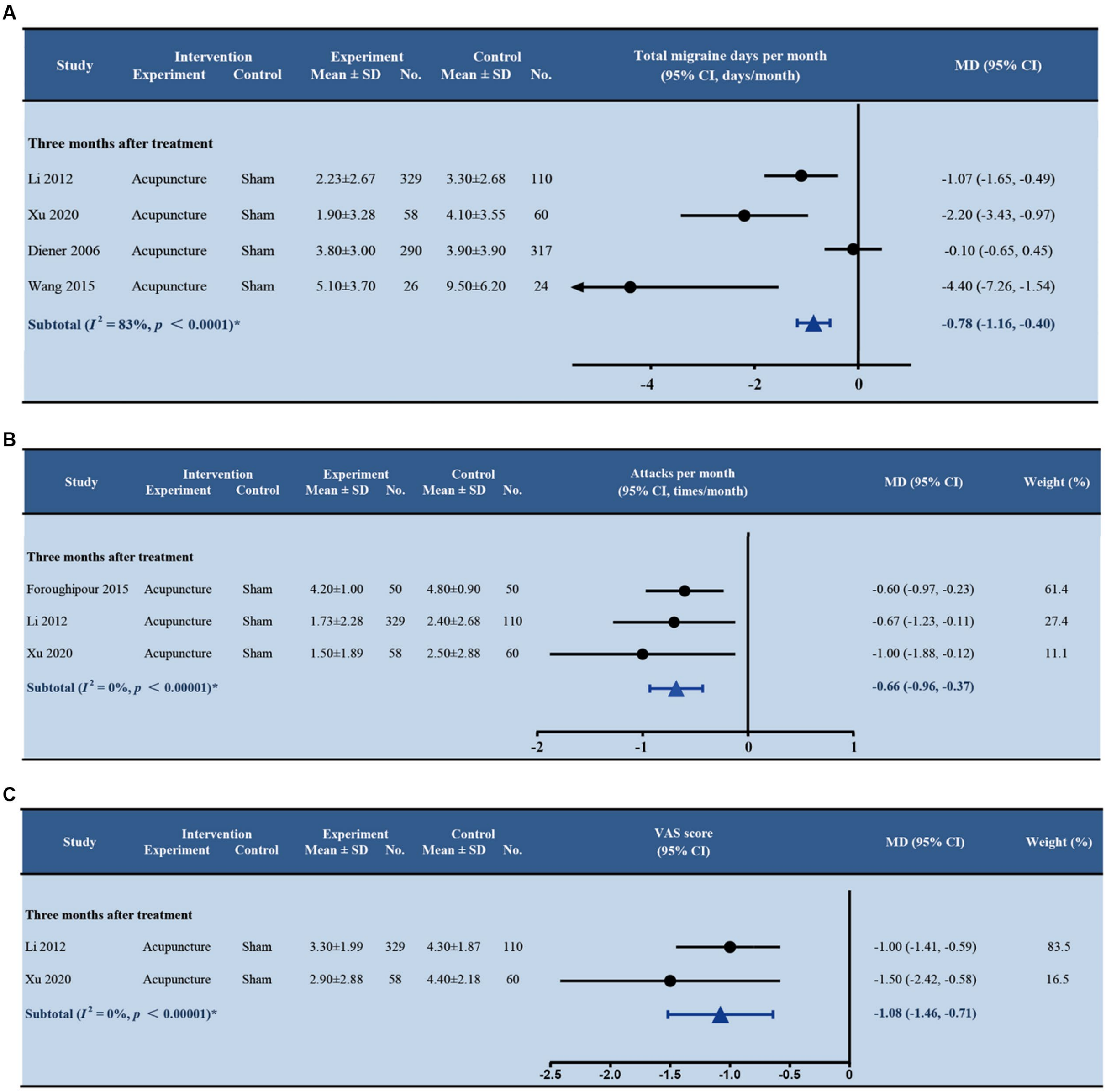

FIGURE 4
Forest plot of primary outcomes (acupuncture vs. sham acupuncture). **(A)** Total migraine days per month; **(B)** Attacks per month; **(C)** VAS score. SD, standard deviation; No., number of subjects; MD, mean difference; CI, confidence interval.

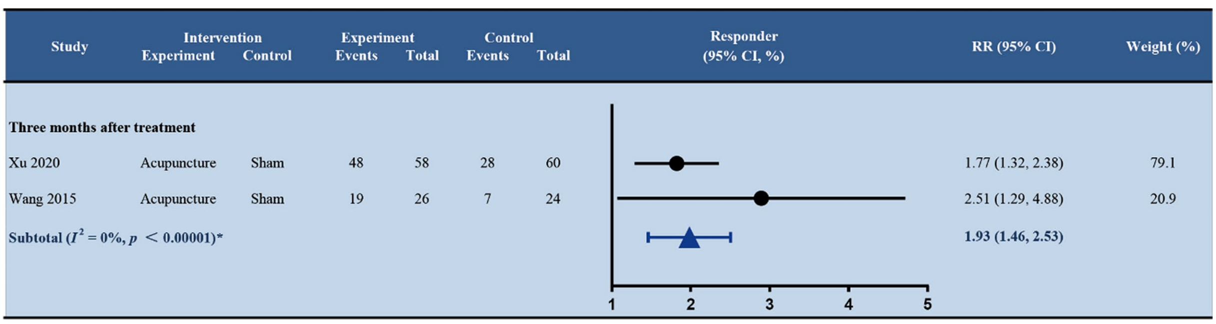

FIGURE 5
Forest plot of response rate (acupuncture vs. sham acupuncture). RR, relative risk.

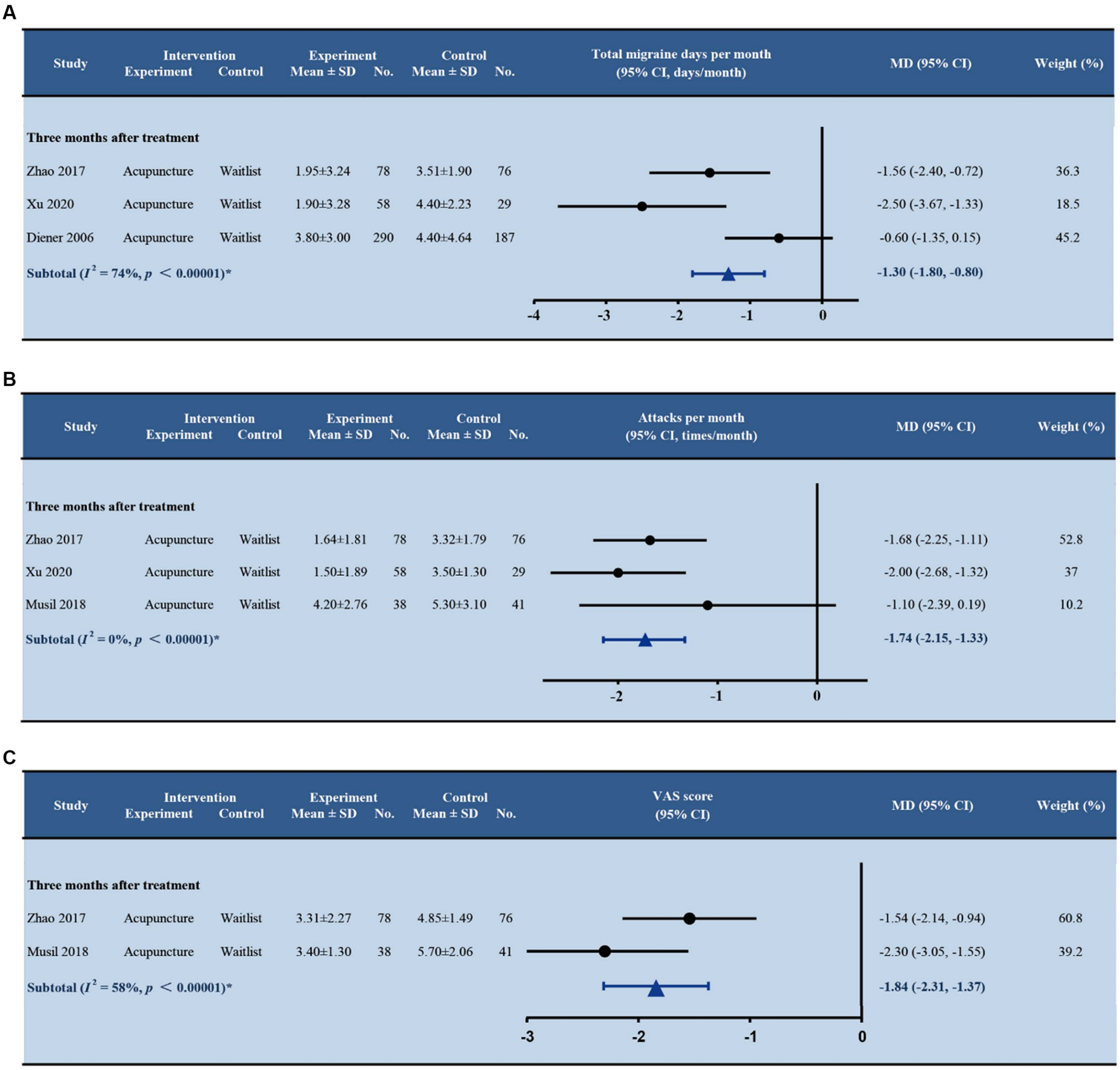

FIGURE 6
Forest plot of primary outcomes (acupuncture vs. waitlist). **(A)** Total migraine days per month; **(B)** Attacks per month; **(C)** VAS score. SD, standard deviation; No., number of subjects; MD, mean difference; CI, confidence interval.

with pharmacological treatment in episodic migraine (Hesse et al., 1994; Allais et al., 2002; Diener et al., 2006; Streng et al., 2006; Wang et al., 2011; Facco et al., 2013; Zhang et al., 2020). The majority of these studies compared acupuncture with monotherapy as a prophylactic treatment. Recently, one prospective, randomized controlled trial compared acupuncture with the best prophylactic drugs for patients taking into consideration comorbidities (i.e., depression, insomnia, hypertension, etc.) and previous preventive treatment (Giannini et al., 2020). This trial showed that acupuncture was as effective as the most appropriate pharmacological treatment for migraine prophylaxis. On the total sample completing the treatment, 33.0 and 25.4% required prophylaxis therapy after 3 and 6 months, respectively, with a higher proportion in patients randomized to the pharmacological group ($n = 19/46$, 41.3% after T2; $n = 8/46$, 17.4% after T3) than those randomized to the acupuncture group ($n = 15/57$, 26.3% after T2; $n = 7/57$, 12.3% after T3). The improvements observed at the end of treatment persisted after therapy in 57.3% (59/103) after 3 months (T3) and in 38.8% (40/103) after 6 months (T4), especially in patients randomized to acupuncture treatment.

However, current evidence was insufficient to reach a conclusive recommendation. According to results from current RCTs evaluating the durable effect of acupuncture for episodic migraine, no conclusion could be drawn about how the durable effect of acupuncture changes with time. Figure 8 shows a rough schematic of results from included studies. Besides, AEs of acupuncture rarely persist or appear newly after treatment discontinuation because the reported AEs were all mild to moderate and soon disappeared without particular intervention.

In the past decades, acupuncture has been pointed out as a valuable non-pharmacological tool in patients with migraine. In acupuncture research, true acupuncture is often compared with sham acupuncture. There are many different types of sham acupuncture intervention; these include lack of skin penetration by the needle, shallow penetration of the needle, insertion at points that are not traditional acupuncture points, or not achieving "deqi" which is an expected needling response (the subjective sensation of local warmth and paresthesia tenderness) that is considered an integral element of the healing process. Any intervention involving skin penetration cannot be considered an inert placebo. Sham acupuncture may still induce a wide range of peripheral, segmental, and central physiological responses to an unpredictable degree.

In this review, 11 out of 15 studies followed up patients for 3 months after treatment (73%), 4 studies followed up for 6 months after treatment (27%), and only one study followed up for 12 months after treatment (7%). Of the eight studies that evaluated the durable effects of acupuncture for episodic migraine compared with sham acupuncture, the results of the five studies with 3-month post-treatment follow-ups showed a significant reduction in the number of migraine attacks, the number of migraine days, and VAS score, in favor of acupuncture. However, the results of the only study that reported the primary outcome 6 months after treatment found no significant difference between acupuncture and sham acupuncture in reducing the number of migraine days (MD −0.50; 95% CI −1.14, 0.14; $p = 0.13$). Similarly, at 12 months after treatment, only one study with 50 patients (26/24) reported no significant difference in reducing the number of migraine days between acupuncture and sham acupuncture (MD −1.00; 95% CI −4.08 2.08; $p = 0.52$). Future studies with longer follow-up periods are required to evaluate the duration of acupuncture's treatment effect after the completion of therapy.

According to the included studies above, patients received 8–15 treatments over 4–12 weeks and obtained durable therapeutic effects after treatment for at least 3 more months. Since the current therapies could barely provide sustained effects after drug withdrawal (Wöber et al., 1991), the durable effect of acupuncture showed potential advantages in decreasing the burden of migraine,

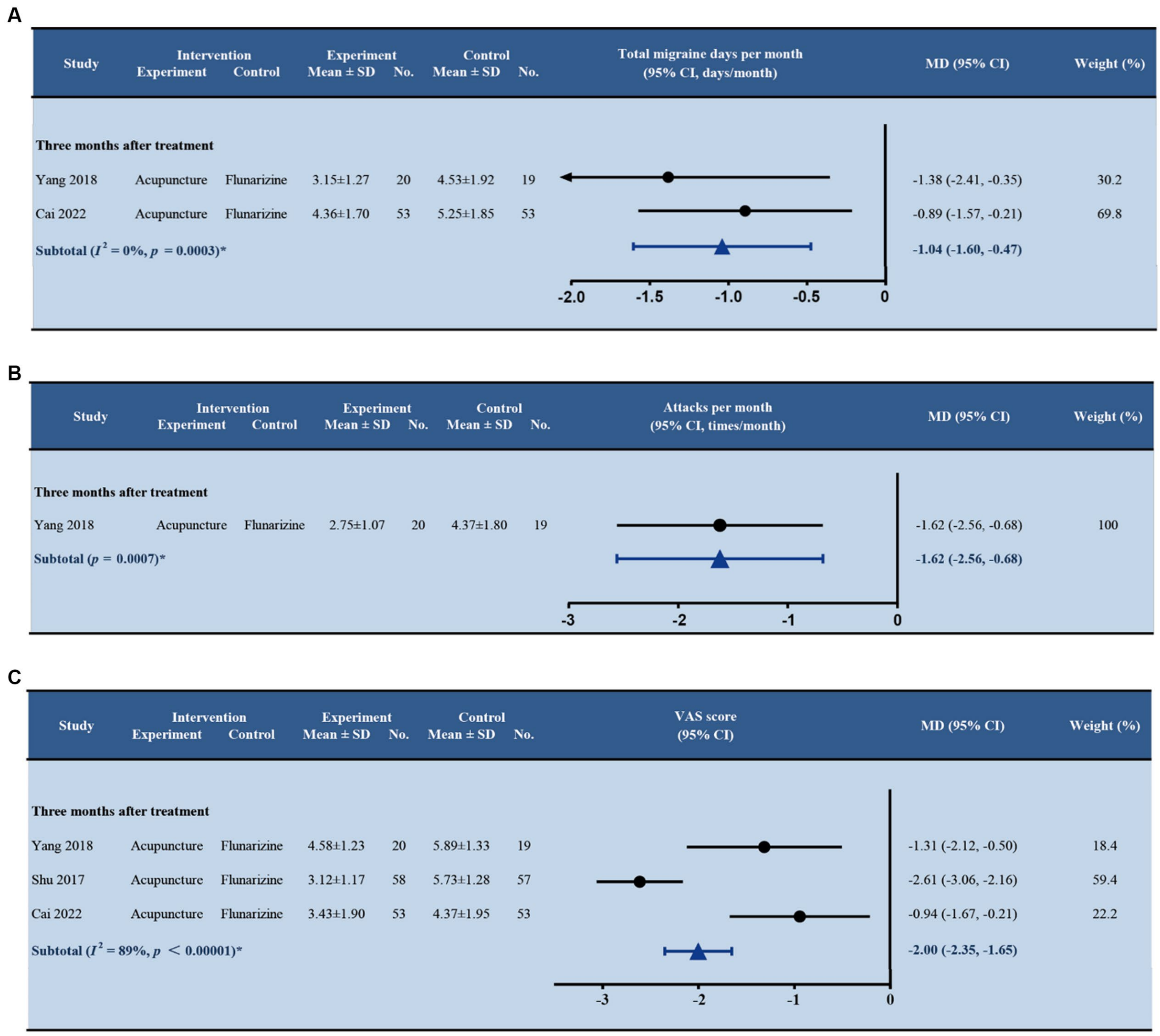

FIGURE 7
Forest plot of primary outcomes (acupuncture vs. flunarizine). **(A)** Total migraine days per month; **(B)** Attacks per month; **(C)** VAS score. SD, standard deviation; No., number of subjects; MD, mean difference; CI, confidence interval.

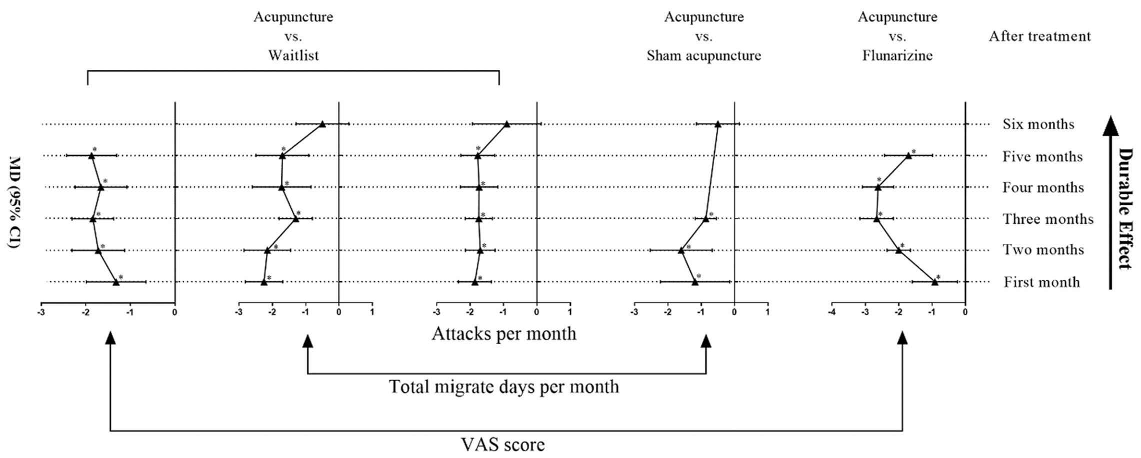

FIGURE 8
Schematic of the durable effect of acupuncture for episodic migraine.

improving the patient's mood and quality of life (Dodick, 2018), and slowing the chronic evolutive process of migraine (May and Schulte, 2016; Andreou and Edvinsson, 2019). More high-quality clinical trials on the durable effect of acupuncture for migraine with longer follow-up periods are needed to investigate the benefits of acupuncture. Meanwhile, cost-effectiveness studies of acupuncture for migraine should consider the durable effect when measuring the time horizon.

Several previous reviews in the last decade evaluated the treatment effect of acupuncture for migraine, while only one systematic review collected follow-up outcomes after treatment. In the 2016 review by Linde et al. (2016), the authors pooled the follow-up outcomes at different time points together and reported significant post-treatment benefits on headache frequency and response rate (at least 50% frequency reduction) in favor of acupuncture compared with sham acupuncture or no acupuncture. Our results were consistent with the results of the previous review by Linde et al. (2016). Furthermore, our research clarified the durable effect of acupuncture 3 months after treatment and identified the pain-alleviating effect of acupuncture using the VAS score. Recently, a systematic review assessed the efficacy and safety of acupuncture for the prophylaxis of episodic or chronic migraine in adult patients compared to pharmacological treatment (Giovanardi et al., 2020), including nine randomized trials (1,484 patients). At the end of the intervention, the authors found a small reduction in favor of acupuncture for the number of days with migraine per month: (SMD: −0.37; 95% CI −1.64 to −0.11), and for response rate (RR: 1.46; 95% CI 1.16–1.84), a moderate effect in the reduction of pain intensity in favor of acupuncture (SMD: −0.36; 95% CI −0.60 to −0.13), and a large reduction in favor of acupuncture in both the dropout rate due to any reason (RR 0.39; 95% CI 0.18–0.84) and the dropout rate due to adverse event (RR 0.26; 95% CI 0.09–0.74). The quality of evidence was moderate for all these primary outcomes. These results seem partially in contrast with the results of the present review.

There are some limitations to this review. First, although most of the included studies had a low risk of bias, five studies still had a high or considerable risk of bias, causing potential heterogeneity. Second, the review was not able to assess the durable effect of acupuncture at follow-ups longer than three months after treatment due to a lack of data. Third, studies with different types of sham acupuncture (with or without penetration) and different drug dosages were all included, which may impact the pooled estimate effect. Therefore, the interpretation of the results of this review should be cautious.

5. Conclusion

Current studies suggested that acupuncture had a durable effect on episodic migraine for at least 3 months after treatment discontinuation. Acupuncture should be recommended to the migraine population, considering the rising global problem with medication-overuse headache (MOH) and the 15% non-responders to pharmacological management. Future clinical trials with robust methodological quality and longer follow-ups are needed to further investigate the durable effect of acupuncture.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HS and RM contributed equally to this manuscript. HS and ZL designed and conceptualized the study. HS, SG, and JF searched the databases, extracted data from included studies, and assessed risk of bias. HS, RM, and LZ contributed to the data analysis. HS and ZL contributed to the revising of the manuscript. All authors contributed to drafting the initial manuscript and approved the final version of this manuscript to be published.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1211438/full#supplementary-material

References

Alecrim-Andrade, J., Maciel-Júnior, J. A., Carnè, X., Severino Vasconcelos, G. M., and Correa-Filho, H. R. (2008). Acupuncture in migraine prevention: a randomized sham controlled study with 6-months posttreatment follow-up. *Clin. J. Pain* 24, 98–105. doi: 10.1097/AJP.0b013e3181590d66

Alecrim-Andrade, J., Maciel-Júnior, J. A., Cladellas, X. C., Correa-Filho, H. R., and Machado, H. C. (2006). Acupuncture in migraine prophylaxis: a randomized sham-controlled trial. *Cephalalgia* 26, 520–529. doi: 10.1111/j.1468-2982.2006.01062.x

Allais, G., De, L. C., Quirico, P. E., Airola, G., Tolardo, G., Mana, O., et al. (2002). Acupuncture in the prophylactic treatment of migraine without aura: a comparison with flunarizine. *Headache* 42, 855–861. doi: 10.1046/j.1526-4610.2002.02203.x

American Headache Society (2019). The American Headache Society position statement on integrating new migraine treatments into clinical practice. *Headache* 59, 1–18. doi: 10.1111/head.13456

Andreou, A. P., and Edvinsson, L. (2019). Mechanisms of migraine as a chronic evolutive condition. *J. Headache Pain* 20:117. doi: 10.1186/s10194-019-1066-0

Ashina, M., Katsarava, Z., Do, T. P., Buse, D. C., Pozo-Rosich, P., Özge, A., et al. (2021). Migraine: epidemiology and systems of care. *Lancet* 397, 1485–1495. doi: 10.1016/S0140-6736(20)32160-7

Cai, Y., Pei, J., Fu, Q., Xu, J., Shen, F., Zhan, Y., et al. (2022). Electroacupuncture at Siguan points for migraine of liver yang hyperactivity: a randomized controlled trial. *Zhongguo zhen jiu = Chinese acupuncture & moxibustion* 42. doi: 10.13703/J.0255-2930.20210403-0001

Chen, Y.-Y., Li, J., Chen, M., Yue, L., She, T.-W., and Zheng, H. (2020). Acupuncture versus propranolol in migraine prophylaxis: an indirect treatment comparison meta-analysis. *J. Neurol.* 267, 14–25. doi: 10.1007/s00415-019-09510-x

Diener, H.-C., Kronfeld, K., Boewing, G., Lungenhausen, M., Maier, C., Molsberger, A., et al. (2006). Efficacy of acupuncture for the prophylaxis of migraine: a multicentre randomised controlled clinical trial. *Lancet Neurol.* 5, 310–316. doi: 10.1016/S1474-4422(06)70382-9

Dodick, D. W. (2018). Migraine. *Lancet* 391, 1315–1330. doi: 10.1016/S0140-6736(18)30478-1

Evers, S., Afra, J., Frese, A., Goadsby, P. J., Linde, M., May, A., et al. (2009). EFNS guideline on the drug treatment of migraine--revised report of an EFNS task force. *Eur. J. Neurol.* 16, 968–981. doi: 10.1111/j.1468-1331.2009.02748.x

Facco, E., Liguori, A., Petti, F., Fauci, A. J., Cavallin, F., and Zanette, G. (2013). Acupuncture versus valproic acid in the prophylaxis of migraine without aura: a prospective controlled study. *Minerva Anestesiol.* 79, 634–642.

Foroughipour, M., Golchian, A. R., Kalhor, M., Akhlaghi, S., Farzadfard, M. T., and Azizi, H. (2014). A sham-controlled trial of acupuncture as an adjunct in migraine prophylaxis. *Acupunct. Med.* 32, 12–16. doi: 10.1136/acupmed-2013-010362

Gallagher, E. J., Liebman, M., and Bijur, P. E. (2001). Prospective validation of clinically important changes in pain severity measured on a visual analog scale. *Ann. Emerg. Med.* 38, 633–638. doi: 10.1067/mem.2001.118863

Giannini, G., Favoni, V., Merli, E., Nicodemo, M., Torelli, P., Matrà, A., et al. (2020). A randomized clinical trial on acupuncture versus best medical therapy in episodic migraine prophylaxis: the ACUMIGRAN study. *Front. Neurol.* 11:570335. doi: 10.3389/fneur.2020.570335

Giovanardi, C. M., Cinquini, M., Aguggia, M., Allais, G., Campesato, M., Cevoli, S., et al. (2020). Acupuncture vs. pharmacological prophylaxis of migraine: a systematic review of randomized controlled trials. *Front. Neurol.* 11:5762. doi: 10.3389/fneur.2020.576272

Headache Classification Committee of the International Headache Society (IHS) (2018). The international classification of headache disorders, 3rd edition. *Cephalalgia* 38, 1–211. doi: 10.1177/0333102417738202

Hesse, J., Møgelvang, B., and Simonsen, H. (1994). Acupuncture versus metoprolol in migraine prophylaxis: a randomized trial of trigger point inactivation. *J. Intern. Med.* 235, 451–456. doi: 10.1111/j.1365-2796.1994.tb01102.x

Kennis, K., Kernick, D., and O'Flynn, N. (2013). Diagnosis and management of headaches in young people and adults: NICE guideline. *Br. J. Gen. Pract.* 63, 443–445. doi: 10.3399/bjgp13X670895

Li, Y., Zheng, H., Witt, C. M., Roll, S., Yu, S., Yan, J., et al. (2012). Acupuncture for migraine prophylaxis: a randomized controlled trial. *CMAJ* 184, 401–410. doi: 10.1503/cmaj.110551

Linde, K., Allais, G., Brinkhaus, B., Fei, Y., Mehring, M., Vertosick, E. A., et al. (2016). Acupuncture for the prevention of episodic migraine. *Cochrane Database Syst. Rev.* 28:CD001218. doi: 10.1002/14651858.CD001218.pub3

Linde, K., Streng, A., Jürgens, S., Hoppe, A., Brinkhaus, B., Witt, C., et al. (2005). Acupuncture for patients with migraine: a randomized controlled trial. *JAMA* 293, 2118–2125. doi: 10.1001/jama.293.17.2118

Marmura, M. J. (2018). Triggers, protectors, and predictors in episodic migraine. *Curr. Pain Headache Rep.* 22:81. doi: 10.1007/s11916-018-0734-0

May, A., and Schulte, L. H. (2016). Chronic migraine: risk factors, mechanisms and treatment. *Nat. Rev. Neurol.* 12, 455–464. doi: 10.1038/nrneurol.2016.93

Musil, F., Pokladnikova, J., Pavelek, Z., Wang, B., Guan, X., and Valis, M. (2018). Acupuncture in migraine prophylaxis in Czech patients: an open-label randomized controlled trial. *Neuropsychiatr. Dis. Treat.* 14, 1221–1228. doi: 10.2147/NDT.S155119. eCollection 2018.

Olesen, J., and Jensen, R. H. (2023). The global campaign against headache and its future relation to IHS and WHO. *Cephalalgia* 43:3331024231159625. doi: 10.1177/03331024231159625

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372:n71. doi: 10.1136/bmj.n71

Shu, W., Peng, T., Huang, X., Hu, S., Zhou, C., Xie, G., et al. (2017). Observations on the Efficacy of Intermittent Liver-nourishing and Mind-regulating Acupuncture in Preventive Treatment of Migraine. *Shanghai Journal of Acupuncture and Moxibustion* 36, 727–730. doi: 10.13460/j.issn.1005-0957.2017.06.0727

Steiner, T. J., Jensen, R., Katsarava, Z., Linde, M., MacGregor, E. A., Osipova, V., et al. (2019). Aids to management of headache disorders in primary care (2nd edition): on behalf of the European headache federation and lifting the burden: the global campaign against headache. *J. Headache Pain* 20:57. doi: 10.1186/s10194-018-0899-2

Steiner, T. J., Stovner, L. J., Jensen, R., Uluduz, D., and Katsarava, Z. (2020). Migraine remains second among the world's causes of disability, and first among young women: findings from GBD2019. *J. Headache Pain* 21:137. doi: 10.1186/s10194-020-01208-0

Sterne, J. A. C., Savović, J., Page, M. J., Elbers, R. G., Blencowe, N. S., Boutron, I., et al. (2019). RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 366:l4898. doi: 10.1136/bmj.l4898

Stovner, L. J., Hagen, K., Linde, M., and Steiner, T. J. (2022). The global prevalence of headache: an update, with analysis of the influences of methodological factors on prevalence estimates. *J. Headache Pain* 23:34. doi: 10.1186/s10194-022-01402-2

Streng, A., Linde, K., Hoppe, A., Pfaffenrath, V., Hammes, M., Wagenpfeil, S., et al. (2006). Effectiveness and tolerability of acupuncture compared with metoprolol in migraine prophylaxis. *Headache* 46, 1492–1502. doi: 10.1111/j.1526-4610.2006.00598.x

Su, M., and Yu, S. (2018). Chronic migraine: a process of dysmodulation and sensitization. *Mol. Pain* 14:1744806918767697. doi: 10.1177/1744806918767697

Tfelt-Hansen, P. C. (2013). Evidence-based guideline update: pharmacologic treatment for episodic migraine prevention in adults: report of the quality standards subcommittee of the American Academy of Neurology and the American headache society. *Neurology* 80, 869–870. doi: 10.1212/01.wnl.0000427909.23467.39

Vickers, A. J., Rees, R. W., Zollman, C. E., McCarney, R., Smith, C. M., Ellis, N., et al. (2004). Acupuncture for chronic headache in primary care: large, pragmatic, randomised trial. *BMJ* 328:744. doi: 10.1136/bmj.38029.421863.EB

Wang, Y., Xue, C. C., Helme, R., Da Costa, C., and Zheng, Z. (2015). Acupuncture for frequent migraine: a randomized, patient/Assessor blinded, controlled trial with one-year follow-up. *Evid. Based Complement. Alternat. Med.* 2015:920353. doi: 10.1155/2015/137321

Wang, L.-P., Zhang, X.-Z., Guo, J., Liu, H.-L., Zhang, Y., Liu, C.-Z., et al. (2011). Efficacy of acupuncture for migraine prophylaxis: a single-blinded, double-dummy, randomized controlled trial. *Pain* 152, 1864–1871. doi: 10.1016/j.pain.2011.04.006

Wells, R. E., Beuthin, J., and Granetzke, L. (2019). Complementary and integrative medicine for episodic migraine: an update of evidence from the last 3 years. *Curr. Pain Headache Rep.* 23:10. doi: 10.1007/s11916-019-0750-8

Wöber, C., Wöber-Bingöl, C., Koch, G., and Wessely, P. (1991). Long-term results of migraine prophylaxis with flunarizine and beta-blockers. *Cephalalgia* 11, 251–256. doi: 10.1046/j.1468-2982.1991.1106251.x

Xu, S., Yu, L., Luo, X., Wang, M., Chen, G., Zhang, Q., et al. (2020). Manual acupuncture versus sham acupuncture and usual care for prophylaxis of episodic migraine without aura: multicentre, randomised clinical trial. *BMJ* 368:m697. doi: 10.1136/bmj.m697

Yang, J., Shen, Y., and Wang, S. (2018). Acupuncture versus flunarizine hydrochloride in the prophylaxis of migraine: a prospective controlled study. *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica* 20, 750–755. doi: 10.11842/wst.2018.05.021

Zhang, N., Houle, T., Hindiyeh, N., and Aurora, S. K. (2020). Systematic review: acupuncture vs standard pharmacological therapy for migraine prevention. *Headache* 60, 309–317. doi: 10.1111/head.13723

Zhao, L., Chen, J., Li, Y., Sun, X., Chang, X., Zheng, H., et al. (2017). The long-term effect of acupuncture for migraine prophylaxis: a randomized clinical trial. *JAMA Intern. Med.* 177, 508–515. doi: 10.1001/jamainternmed.2016.9378

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Mobilizing endogenous neuroprotection: the mechanism of the protective effect of acupuncture on the brain after stroke

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Given its high morbidity, disability, and mortality rates, ischemic stroke (IS) is a severe disease posing a substantial public health threat. Although early thrombolytic therapy is effective in IS treatment, the limited time frame for its administration presents a formidable challenge. Upon occurrence, IS triggers an ischemic cascade response, inducing the brain to generate endogenous protective mechanisms against excitotoxicity and inflammation, among other pathological processes. Stroke patients often experience limited recovery stages. As a result, activating their innate self-protective capacity [endogenous brain protection (EBP)] is essential for neurological function recovery. Acupuncture has exhibited clinical efficacy in cerebral ischemic stroke (CIS) treatment by promoting the human body's self-preservation and "Zheng Qi" (a term in traditional Chinese medicine (TCM) describing positive capabilities such as self-immunity, self-recovery, and disease prevention). According to research, acupuncture can modulate astrocyte activity, decrease oxidative stress (OS), and protect neurons by inhibiting excitotoxicity, inflammation, and apoptosis via activating endogenous protective mechanisms within the brain. Furthermore, acupuncture was found to modulate microglia transformation, thereby reducing inflammation and autoimmune responses, as well as promoting blood flow restoration by regulating the vasculature or the blood–brain barrier (BBB). However, the precise mechanism underlying these processes remains unclear. Consequently, this review aims to shed light on the potential acupuncture-induced endogenous neuroprotective mechanisms by critically examining experimental evidence on the preventive and therapeutic effects exerted by acupuncture on CIS. This review offers a theoretical foundation for acupuncture-based stroke treatment.

KEYWORDS

ischemic stroke, endogenous brain neuroprotection, acupuncture, astrocytes, microglia, brain blood vessels

1 Background

Ischemic stroke (IS) remains the second leading cause of death and disability in adults worldwide. According to research (Wang, 2005; Neuhaus et al., 2017; Ornello et al., 2018), the combination of thrombolytic and neuroprotective agents is the first-line treatment for acute ischemic stroke (AIS). However, commonly used thrombolytic drugs, such as the recombinant tissue plasminogen activator (rtPA), have several drawbacks, including a narrow effective time window (<4.5 h) and bleeding risk, among other disadvantages. Furthermore, only ∼5% of patients finally receive rtPA, with the vast majority only receiving supportive care in the acute AIS phase (Montaño et al., 2013; Chang et al., 2015; Auboire et al., 2021). Although more than 1,000 neuroprotective agents could ameliorate nerve damage post-AIS over the past 50 years, most of them failed in large-scale clinical trials (Park et al., 2012; Kim et al., 2017). Despite the current experimental studies on stroke treatment primarily focus on protecting neurons from ischemic pathogenic factors, such as excitatory neurotransmitter toxicity, oxidative stress (OS), inflammation, and apoptosis, applying these findings to clinical practice poses a significant challenge. In this context, a recent study has added a vital perspective, reporting that the brain possesses inherent self-protection mechanisms, which can fully mobilize endogenous protective measures, and harnessing this natural defense mechanism is identified as crucial for stroke prevention and treatment (Iadecola and Anrather, 2011). Endogenous brain protection (EBP) encompasses a collaborative process of multicellular programs; if one cell type is threatened, a coordinated multicellular response emerges to maintain the tissue's dynamic equilibrium, resulting in a coherent defense system program that can prioritize the survival of critical cells. By acting on the coordinated neuro–glial–vascular protection process, EBP induces multicellular cooperation between neurons, astrocytes, microglia, T cells, and cerebral vessels. In order to maintain internal tissue stability when a specific cell type is threatened, this process might be initiated to confront OS, excitatory toxicity, inflammation, apoptosis, and other ischemic cascade reactions, thereby reducing ischemic injury and prioritizing the survival of critical cells (Datta et al., 2020). Therefore, the prospective promotion of stroke research is primarily based on comprehensively strengthening endogenous protective measures.

In the history of Chinese medicine, acupuncture has been used to treat diseases for thousands of years. Its functions include dredging meridians, harmonizing yin and yang, and boosting positive qi. In *Ling Shu, Nine Needles and Twelve Originals (灵枢·九针十二原),* acupuncture is described as "Using microneedles to unblock the meridians, harmonize qi and blood, and improve the in and out flow of qi and blood." Homeostasis regulation, which enhances the body's endogenous protection to attain disease prevention and treatment goals, is the fundamental mechanism of acupuncture (Zhang et al., 2020; Jia et al., 2022).

High-quality clinical evidence-based theory has confirmed the medical efficacy and safety of acupuncture in treating acute ischemia (MacPherson et al., 2013; Qiu, 2013; Liu et al., 2015, 2016a; Xin et al., 2015; Zhao et al., 2017; Zhan et al., 2018). Specifically, recent studies have shown that acupuncture exerts a synergistic effect on EBP cells (astrocytes, microglia, and vascular cells, among others) (Cao et al., 2021). Therefore, to elucidate the theoretical evidence for ischemic stroke prevention and treatment, this review summarizes the mechanism of acupuncture in mobilizing endogenous neuroprotection.

2 Stroke and its endogenous protection mechanism

After stroke, the supply of oxygen and glucose to the brain is impeded, resulting in an inadequate production of ATP to meet the brain's energy demands. Consequently, brain cells die quickly due to energy loss, which is particularly severe in areas with the lowest blood flow. Furthermore, in the relatively mild ischemic area (ischemic penumbra), a continuous depolarization wave induces the release of neurotransmitters, accompanied by a glial cell reuptake disorder, causing extracellular glutamate and other excitatory neurotransmitters to aggregate and produce excitatory toxicity. Continuous glutamate receptor activation leads to the accumulation of extracellular calcium ions, eventually resulting in a chain of ischemic cascade reactions, including antioxidant stress, excitotoxicity, and inflammatory apoptosis. Meanwhile, ischemia also triggers a cascade of inflammatory signals, causing white blood cell aggregation in blood vessels and between cells. By producing cytotoxic mediators, these inflammatory cells further damage the brain tissue, eventually causing brain damage.

To alleviate brain injury and restore internal environment homeostasis, the brain's local and remote protection mechanisms can resist harmful events as follows.

Early IS will result in reactive astrocyte production, cell proliferation and rapid aggregation, and glial scar formation around the lesions (Sun et al., 2019; Tanabe et al., 2019; Kim et al., 2021), thereby inhibiting or aggravating neuronal injury. On the one hand, activated astrocytes can protect neurons through the inhibition of glutamate excitatory toxicity by antioxidant stress and anti-inflammatory responses, among other neuroprotective effects (de Pablo et al., 2013; Jeong et al., 2014; Roy Choudhury et al., 2014); on the other hand, the glial scar could increase inflammation and intracerebral pressure and decrease vascular perfusion and the accelerated brain edema process (Kim et al., 2015).

Microglia, a permanent immune cell in the brain, is involved in the first-line central nervous system (CNS) innate immunity defending the brain against injury and diseases (Franco-Bocanegra et al., 2019). Following ischemic brain injury, microglia rapidly migrate to the lesion site, eliminate cell debris, and generate anti-inflammatory/pro-inflammatory cytokines (Ma et al., 2017). Microglia activation post-IS was previously assumed to aggravate brain injury. However, at present, growing evidence indicates that microglia activation may reduce neuronal apoptosis and exert beneficial effects on later neural repair stages (Sherafat et al., 2021).

Increasing arterial pressure by activating the sympathetic nerve-releasing hormone during cerebral ischemia enhances blood flow through communicating branch vessels (collateral circulation) adjacent to the normal perfusion area, aiding blood supply to the ischemic area. Within 24 h post-stroke, T cells appear in the brain

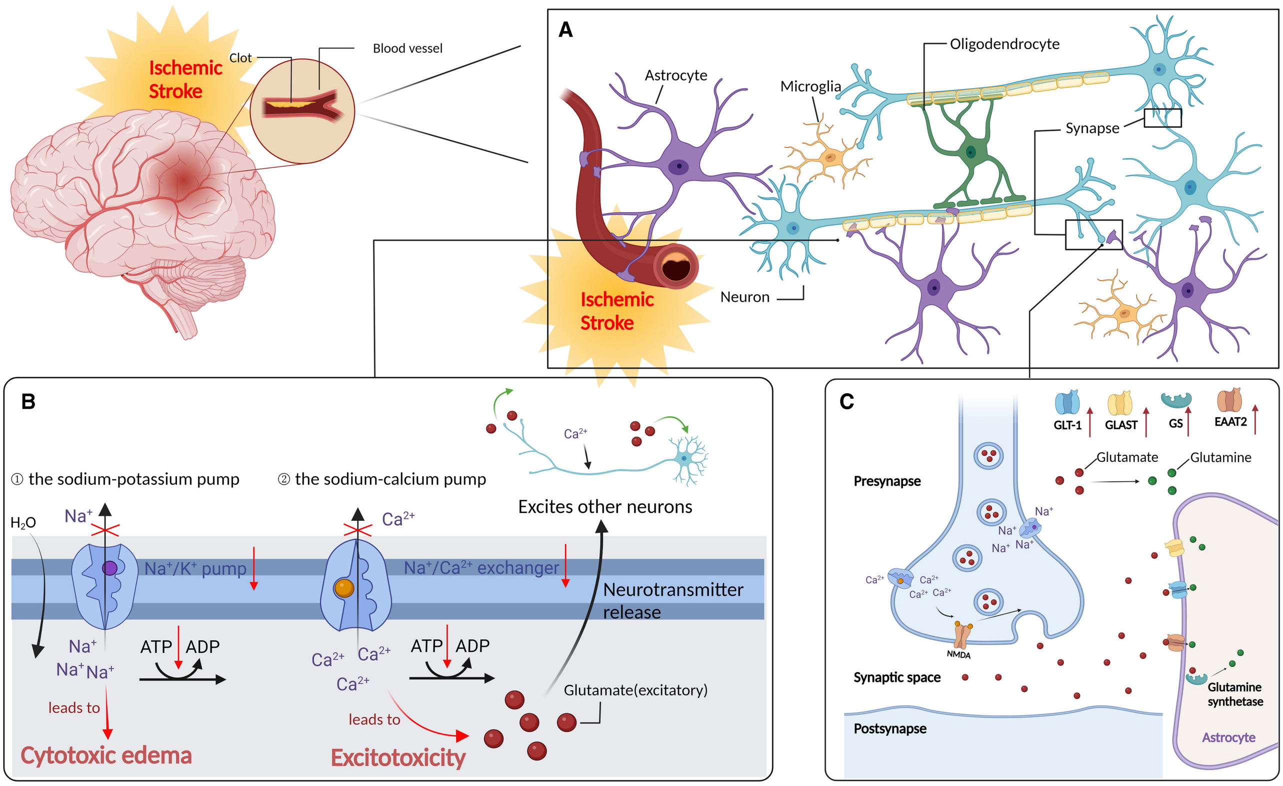

FIGURE 1
Regulate astrocytes to reduce oxidative stress and after stroke. **(A)** Ischemic stroke: distribution of neurone in the brain. **(B)** Ischemic cascade: after an ischemic stroke, neurons in the ischemic core are rapidly deprived of oxygen due to lack of blood supply, resulting in reduced ATP production, which in turn lead to the failure of Na^+/k^+ pumps (1) and plasma membrane Ca2+/ATP pumps (2). **(C)** Acupuncture can enhance the ability of astrocytes to clear glutamate, inhibit the expression of glutamate NMDA receptor NR1, NR2B and other proteins, up-regulate the expression of glutamate transporter-1 (GLT-1), NR2A, cannabinoid receptor type 1 and type 2 proteins; expression of glutamate asparate transporter (GLAST) and glutamine synthetase (GS), and reduce the MCAO rat brain tissue glutamate content and attenuate neurotoxicity, thus exerting a protective effecr in ischemic brain tissues. In addition, electropuncture pretreatment could reduce glutamate toxicity by upregulating EAAT2 and activating CB1R in the ischemic penumbra of AS, thus protecting neurons from cerebral ischemia (the red upward arrow indicates that acupuncture promotes its upregulation).

tissue and could infiltrate the brain for a long time (Gelderblom et al., 2009), exerting an essential impact on stroke outcomes.

The clearing function of the "brain lymphatic system" has neuroprotective effects (Toro et al., 2019; Cheng and Wang, 2020; Segawa et al., 2021). Some anti-inflammatory and neuroprotective cytokines produced by regulatory lymphocytes, including interleukin 10 (IL-10) and transforming growth factor β (TGF- β), can limit white blood cell infiltration and suppress natural and acquired immune responses, thus promoting the survival of ischemic neurons. The protective signaling pathway activated in the late stages of ischemic cascade reactions can promote the repair of injured brain tissues. Furthermore, growth factors secreted by microglia, macrophages, neurons, astrocytes, and intravascular cells, such as erythropoietin (EPO) and insulin-like growth factor 1 (IGF-1), can be produced by peripheral organs and infiltrate the brain through cerebral vessels (Jelkmann, 2005). Meanwhile, following ischemia, neural precursor cells and bone marrow-derived endothelial progenitor cells infiltrate the ischemic injury site, restoring tissue homeostasis and reconstructing neural networks by recombining the extracellular matrix (ECM) and replacing damaged cells, thus playing a crucial role in brain microvascular network reconstruction.

3 Acupuncture plays a neuroprotective role by regulating astrocytes

Astrocytes are positioned between the cell body and nerve cell processes. They function by supporting and guiding neurons and enhancing their survival. Notably, astrocytes are the brain's most widely distributed cell type. The location, size, and time of the physical barrier formed by reactive astrocytes influence the acceleration of nerve cell death. Furthermore, research shows that acupuncture can play the role of an EBP through astrocyte regulation.

3.1 Acupuncture can reduce oxidative stress and inhibit excitatory toxicity by regulating astrocytes after stroke

Acupuncture could impede the sudden depletion of oxygen after excitotoxicity in IS, and when the endogenous redox balance cannot be maintained, OS will occur, yielding reactive oxygen species (ROS) and activating the release of various inflammatory factors (Sun et al., 2008; Shen et al., 2012; Jiang et al., 2013; Lin et al., 2022), as well as destroying the integrity of the blood–brain barrier (BBB) and increasing the infarction volume. The nuclear factor E2-related factor 2 (Nrf2), which plays a vital role in cell redox homeostasis, is expressed in astrocytes (Yang et al., 2016; Hoxhaj and Manning, 2020; Terada et al., 2020). According to research (Jin, 2017), electroacupuncture (EA) can regulate downstream targets along the Nrf2 signaling pathway γ- (Choudhury and Ding, 2015). Additionally, GCS expression can enhance the body's antioxidant capacity and influence ischemic cerebrovascular diseases. Zhao showed that acupuncture at "Baihui" and "Sishencong" can significantly upregulate antioxidant enzymes SOD and GSH-px in the ischemic penumbra of middle cerebral ischemia reperfusion (MCAO/R) rats, with its mechanism of action speculated to be associated with the activation of the Nrf2 signaling pathway (Zhao and Ma, 2018).

Upon neuronal anoxic depolarization, Ca2+ flows into the terminals of presynaptic neurons, resulting in an abundance of excitatory neurotransmitter glutamate released into the synaptic space, thereby causing excitotoxicity. Depending on their specific Na+-dependent glutamate transporter, astrocytes absorb glutamate [glutamate aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1)] from the extracellular space, regulate the extracellular Ca2+ levels, and critically influence injured neuron repair (Kobayashi et al., 2019; Pajarillo et al., 2019). Acupuncture can enhance the astrocyte glutamate clearance ability; inhibit the expression of glutamate NMDA receptors (NR1 and NR2B) and other proteins; and upregulate GLT-1, NR2A, and cannabinoid receptor type 1 (CB1R) and type 2 (CB2R) proteins, by reducing neurotoxicity, thus protecting the ischemic brain tissue (Dai, 2016). Furthermore, acupuncture at "Neiguan" can upregulate the GLAST and glutamine synthetase (GS) and downregulate glutamate in the brain tissue of MCAO rats. Furthermore, acupuncture at "Neiguan" will increase the astrocyte GLT1 and GS protein expression, reduce excessive glutamate, and upregulate glutamine synthetase in the brain tissue of MCAO rats. As the main transporter for clearing excitatory neurotransmitter glutamate from the CNS, the excitatory amino-acid transporter 2 (EAAT2) is responsible for the reuptake of more than 90% of glutamate in the brain and is abundant in astrocytes (Danbolt, 2001; Wei et al., 2019). Electroacupuncture pretreatment can minimize glutamate toxicity by upregulating EAAT2 and activating astrocyte CB1R in the ischemic penumbra, thereby protecting neurons from cerebral ischemia. Furthermore, the GS-mediated glutamine synthesis helps astrocytes restore extracellular glutamate to normal levels and protect neurons from ischemia/reperfusion (I/R) injury (Stelmashook et al., 2011) (Figure 1).

3.2 Acupuncture regulates astrocytes to reduce inflammatory reactions

Astrocytes are critically involved in the brain's inflammatory network. After IS, pro-inflammatory factors rapidly induce pathophysiological changes in astrocytes, including reactive astrocyte hypertrophy and proliferation, vimentin and glial fibrillary acidic protein (GFAP) overexpression, and cytokine and chemokine production (IL-6, TNF- α, IL-1 α and β, IFN-γ, etc). Between 4 and 24 h post-IS, a multitude of astrocytes and glial scars appeared in the lesion's core area, peaking around the fourth day (Choudhury and Ding, 2015). From the neurotoxicity and neurotrophic perspectives, the glial scar has two characteristics. First, cytokines released from the glial scar can directly or indirectly induce neurotoxicity media [such as nitric oxide (NO)] and increase BBB permeability, thereby inducing neuronal death, promoting further cerebral infarction development, preventing inward axonal growth, and affecting nerve regeneration during recovery post-IS. Second, the scar can be used as a barrier to isolate damaged tissues from healthy tissues, preventing additional damage to surrounding tissues.

Acupuncture can upregulate GFAP in a cerebral ischemia astrocyte model and promote astrocyte activation (Tao et al., 2016). Paulina et al. found that EA preconditioning can downregulate the I/R injury-induced N-myc downstream-regulated gene 2 (NDRG2) (Vaitkiene et al., 2017), reduce stroke-induced astrocyte apoptosis, and exert neuroprotective effects. After 24 h of modeling, MCAO rats were treated with scalp acupuncture in combination with exercise therapy. According to the results, BDNF and GFAP were upregulated in the cortex and around the rats' striatum, which could have inhibited the inflammatory reaction and alleviated ischemic brain injury. Furthermore, Tao et al. found that treating MCAO rats with EA on the third day after modeling could promote the proliferation of GFAP+/vimentin+/nestin+reactive astrocytes, upregulate BDNF, and exert a neuroprotective effect. Cheng et al. (2014a,b) demonstrated that the combination of EA and induced pluripotent stem cell-derived extracellular vesicles could regulate the IL-33/ST2 axis and inhibit the activation of microglia and astrocytes 72 h after stroke, thereby exerting a neuroprotective effect.

4 Acupuncture can enhance the neuroprotective effect of microglia

As an intrinsic brain immune cell, microglia serve as the primary barrier against injury to the CNS (Colonna and Butovsky, 2017). Following ischemic brain injury, microglia rapidly migrate to the lesion site, eliminate cell debris, and produce anti-inflammatory/pro-inflammatory cytokines. It was previously assumed that microglia activation post-IS might aggravate brain injury. However, at present, growing evidence shows that microglia activation may reduce neuronal apoptosis and benefit the later nerve repair stages (Tian and Mao, 2022). Under normal physiological conditions, microglia exhibit a small cell body or branch shape and have a monitoring function (Bao et al., 2018; Souder et al., 2021). This condition is referred to as the "static microglia" state and disrupting brain homeostasis can induce microglia activation.

4.1 Acupuncture can downregulate inflammatory factors by modulating microglia

When activated by ischemia, microglia rapidly shift from a static to an active state. This transition is known as microglia polarization. Polarization is categorized into M1 and M2 phenotypes. On the one hand, M1 microglia secrete various inflammatory cytokines (including IL-1 β, IL-6, TNF-α, IFN-γ, and INOS) (Collmann et al., 2019) and neurotoxic mediators (MMP9 and MMP3), which cause BBB destruction and ECM degradation, resulting in nerve degeneration or death. The activation phenotypes in this process are CD11b (Liu et al., 2018), CD16 (Jiang et al., 2018), CD32 (Jin et al., 2014), and CD86 (Li et al., 2018). On the other hand, M2 microglia are reparative and can secrete anti-inflammatory factors (IL-10, IL-4, IL-13, IGF-1, and TGF-β), as well as the insulin-like growth factor (IGF) and the vascular endothelial growth factor (VEGF), to inhibit inflammation and promote angiogenesis and tissue repair (Zhu et al., 2019), with CD206 as the activated phenotype (Shu et al., 2016). Therefore, the M1/M2 change is the embodiment of microglia's dual characteristics in inflammatory responses, through which it critically influences stroke prognosis.

As a vital microglia-related inflammatory signaling pathway, NF-κ B can effectively induce inflammatory cytokine (TNF-α, IL-1 β, IL-6, etc.), chemokine (monocyte chemoattractant protein-1), adhesion molecule (ICAM-1 and VCAM-1), and other expressions to aggravate the inflammatory cascade reaction (Li et al., 2019; Meng et al., 2019; Xu et al., 2019). Some studies have shown that (Liu et al., 2002, 2016b) acupuncture can regulate the microglia activation direction and that EA can downregulate M1 microglia markers (Iba-1 and CD11b) in the ischemic focus of the cerebral ischemia model. They also found that inhibiting the NF-κ nuclear translocation of B p65 suppresses p38 mitogen-activated protein kinase (p38 MAPK) and myeloid differentiation factor 88 (MyD88) expression in the sensorimotor cortex around the infarction region and downregulates TNF-α, IL-1 β, and IL-6, thereby reducing the transformation from microglia to the M1 phenotype. Han et al. (2015) demonstrated that acupuncture at "Neiguan" and "Quchi" can suppress microglia activation and inhibit microglia involvement in the TLR4/NF-κ signaling pathway. Acupuncture-induced abnormal expression of the B signaling pathway factor downregulates TNF-α, IL-1 β, and IL-6, reducing the neurological function score of the MCAO model and necrosis of hippocampal neurons in the ischemic focus. In a bilateral carotid ischemia model, acupuncture can upregulate the nuclear translocation of Nrf2 in neurons and downstream target genes [NADPH quinone oxidoreductase 1 (NQO1) and heme oxygenase 1(HO1)] by reducing the activation of Nrf2-dependent microglia, which exerts a neuroprotective effect and improves bilateral carotid ischemia-induced cognitive impairment (Wang et al., 2015). Table A1 details the effect of acupuncture on microglia polarization post-IS.

5 Acupuncture can regulate homeostasis by enhancing cerebral blood flow or BBB and exert a neuroprotective effect

IS is often followed by brain network dysfunction, resulting in sudden nerve function defects. Therefore, to save the neurovascular unit, it is essential to restore blood flow perfusion. Blood flow recovery post-IS can be achieved through thrombolysis or mechanical recanalization. However, in some patients, reperfusion may aggravate the initial ischemia-induced injury, resulting in the so-called "brain reperfusion injury." According to research, acupuncture can maintain the integrity of the BBB, expand blood vessels, accelerate blood flow, enhance microcirculation, increase cerebral blood flow (CBF), relieve cerebral ischemia and hypoxia, promote cerebral collateral circulation (CCC), and reduce brain tissue damage.

5.1 Acupuncture can maintain the integrity of the BBB

The BBB is a multicellular vascular structure between the CNS and the peripheral blood circulation, which can limit the entry of pathogens, blood solutes, and macromolecules or hydrophilic molecules into the cerebrospinal fluid while maintaining the stability of the brain environment. Due to the distinct pathogenesis of IS, I/R injury causes BBB destruction and increases vascular permeability and brain edema, resulting in secondary brain injury (Moskowitz et al., 2010; Feng et al., 2022). Therefore, maintaining BBB integrity is one of the main objectives of brain protection post-IS.

Wu et al. (2001) found that EA can limit the area of Evans blue extravasation and reduce BBB damage post-I/R, implying that EA can regulate brain homeostasis after stroke by protecting BBB integrity and inhibiting NOX4 and ROS production. Xu et al. (2018) found that EA can downregulate AQP4 in the striatum of MCAO/R rats (AQP4 is the most abundant aquaporin in brain tissue and is expressed on the terminal foot around the BBB vessels wrapped by astrocytes), reduce brain edema and BBB damage, and exert a brain-protective effect. Additionally, Shen et al. (2009) showed that EA preconditioning can alleviate cerebral ischemia-induced brain edema and BBB dysfunction by downregulating matrix metalloproteinase-9 (MMP-9). Lin et al. also observed that EA treatment can inhibit MMP-2/MMP-9 expression in rats with cerebral ischemia, thus promoting brain protection. In this regard, because of its ability to maintain BBB integrity, acupuncture has become one of the key EBP measures.

Increasing oxygen and blood supply to brain tissue after stroke is crucial for preventing and treating brain damage. EA stimulation of "Zusanli" has been shown to increase CBF in rats with cerebral ischemia (Hsieh et al., 2006). Similarly, stimulating "Shuigou" with EA can also promote vascular endothelial cell proliferation and increase the local CBF of rats (Du et al., 2011). Additionally, stimulating "Fengfu" and "Shendao" with EA can significantly reduce ischemia-induced glutamate release and transient CBF increase during reperfusion to protect neurons from I/R injury, thereby exerting a neuroprotective effect (Pang et al., 2003). In conclusion, acupuncture is an exogenous therapeutic strategy that can improve microcirculation, regulate cerebral vascular reserve or increase CBF, mobilize brain tissue energy metabolism to reduce brain injury, and enhance the endogenous protective effect.

5.2 Acupuncture can regulate cerebrovascular reserve

Cerebrovascular reserve (CVR) is the ability to adjust the CBF stability to meet the demands of brain parenchymal metabolism via compensatory relaxation and contraction of intracranial arterioles and capillaries under physiological or pathological stimulation, which is the key mechanism of homeostasis regulation post-IS. Some domestic studies (Zhang and Tian, 2020) showed significantly improved clinical symptoms in acute cerebral infarction patients after intravenous thrombolysis by the "awakening the brain and opening the body" acupuncture method compared to the control group without acupuncture treatment and that CVR index improvement was found to be better in the treatment group than in the control group through transcranial Doppler ultrasound detection. Moreover, it is suggested that the "Xingnao Kaiqiao" acupuncture method can adjust CBF to realize its brain protection effect.

5.3 Acupuncture can enhance cerebral collateral circulation

As one of the endogenous compensatory mechanisms in the brain, collateral circulation (CC) is one of the important determinants of IS outcomes. However, within 3–5 days post-IS, brain edema continues to deepen, and CC establishment is also affected by the expansion of the infarction region (Campbell et al., 2013).

Shi et al. (2017) discovered that the number of blood vessels was significantly reduced around the infarction area in the MCAO group at the first, third, and sixth hours after replicating the MCAO model. Compared to the MCAO group, the number of blood vessels in the EA group was relatively higher, implying that EA intervention improved the CC around the infarction area, resulting in a corresponding increase in CBF. According to Sun et al. (2020) and other clinical studies, acupuncture intervention can expand cerebral blood vessels, increase CBF, reduce the infarction area, promote CCC formation, and improve motor function. The speculated mechanism underlying this process is that after EA stimulates the body's reflex center, bioelectric effects are transmitted to the cerebral cortex through nerves, changing the excitability of neurons in the cerebral cortex and accelerating CCC formation. Additionally, Qi et al. showed that acupuncture can improve the ischemia and hypoxia status of the ischemic focus, as well as promote CC formation and hematoma absorption. Therefore, after IS, acupuncture can promote the establishment of the vascular CCC, increase CBF, and improve the body's EBP.

6 Acupuncture mediates endogenous neuronal protection by enhancing T cell

Following a hemorrhagic stroke, the immune system adapts to the ischemic state to protect against post-stroke damage to the central nervous system. Once the blood–brain barrier is broken, immune cells including pro-inflammatory factors in the peripheral blood circulation can invade the central nervous system. Moreover, inflammatory cells [neutrophils (Cai et al., 2020), monocytes, macrophages, various types of T cells, and other inflammatory cells] in the peripheral blood can cross the blood–brain barrier to the ischemic area where they cause a systemic inflammatory response (Gan et al., 2014; Perez-de-Puig et al., 2015), thereby increasing the risk of secondary infarction and delaying neurological recovery (Magnus et al., 2012). Therefore, a strategy should be developed for reducing the damage caused by inflammatory cytokines after stroke. Within 24 h after the occurrence of ischemic stroke, T cells are released in the brain tissue where they infiltrate the tissues for a long time. Research has shown that compared with healthy individuals, stroke patients show increased expression of HLA-DR and CD25 on T cells, and the surviving T cells in peripheral blood are activated and secrete pro-inflammatory cytokines (Gill and Veltkamp, 2016). In an experimental mouse model of cerebral ischemic stroke, lymphocyte loss may occur which reduces the number of CD4+T cells, resulting in cell apoptosis, while the delayed recovery of CD4+T cell count indicates an increase in the risk of subsequent infection (Prass et al., 2003). Studies have shown that acupuncture can reduce the infiltration of inflammatory cells, downregulate the expression of CD4+in cells, improve nerve function scores, reduce the expression of Th17 cells (Lee et al., 2016), and inhibit the secretion of mouse pro-inflammatory cytokine IL-17 (Liu et al., 2013). This suggests that CD4+T cell count may be closely related to the degree of multiple immunosuppression induced by cerebral ischemia, that is, stroke is accompanied by T-cell activation, and this can be prevented by acupuncture treatment.

Regulatory T cells (Tregs) have been shown to inhibit excessive immune response and maintain immune tolerance and homeostasis. Tregs cells can prevent the expansion of secondary infarction by alleviating excessive production of pro-inflammatory cytokines and regulating the invasion and/or activation of lymphocytes and microglia in the ischemic brain. A study by Chamorro et al. (2012) showed that Tregs may exert an anti-inflammatory role by secreting the anti-inflammatory cytokine IL-10 and can cross the blood–brain barrier, and IL-10 gene knockout will promote an increase in infarction (Liesz et al., 2009). Serra et al. (2003) reported that Tregs inhibit inflammation by secreting the anti-inflammatory cytokine IL-10. IL-10 gene knockout will lead to an infarction increase (Liesz et al., 2009). Tregs can promote the expression of dendritic cells (DCs) and reduce the activation of DCs on effector T cells 9, which damages the blood–brain barrier and promotes leukocyte infiltration and brain injury. Within 24 h of cerebral ischemia, Treg cells demonstrate the capacity to suppress MMP-9 production. Moreover, they exert a protective effect against ischemic stroke by suppressing the expression of metalloproteinase-9 (MMP-9) (Li et al., 2013). However, when they are cultured in the extracellular pore, Tregs may lose their inhibitory effect on MMP-9. This indicates that Tregs can exert this inhibitory effect by interacting with neutrophils. Xu et al. (2014) showed that acupuncture or electroacupuncture stimulation of "Baihui" (GV20) and "Zusanli" (ST36) can significantly reduce inflammatory cell infiltration and the expression of pro-inflammatory metalloproteinase-2 (MMP-2) in rats with ischemia–reperfusion injury. It also significantly downregulates the expression of aquaporins AQP4 and AQP9 in the ischemic brain, thereby reducing inflammation and brain edema, demonstrating its brain-protective effect.

7 Acupuncture plays a neuroprotective role by enhancing the clearing function of the "brain lymphatic system"

The glymphatic system (GS) is a newly discovered system that clears waste from the central nervous system. Studies have found (Rasmussen et al., 2018; Chen et al., 2021; Lv et al., 2021; Ren et al., 2021; Zhou et al., 2021) that the "glymphatic system (GS) of the brain" closely regulates inflammation-related pathways and maintains the balance of clearing and accumulating harmful metabolic substances (such as Aβ and Tau) in the brain. This system facilitates the dynamic flow and exchange of materials between the cerebrospinal fluid (CSF) and interstitial fluid (ISF) through the polar distribution of aquaporin-4 (AQP4) on astrocytes. The GS is functionally connected to meningeal lymphatics (ML), CSF, and the BBB, collectively participating in the metabolic clearance process of Aβ, Tau, and other waste proteins. Imbalances in its functioning are implicated in stroke, Alzheimer's disease, and other neurological disorders. Moreover, recent studies have highlighted the role of the GS in melatonin-mediated neuroprotection and neuroinflammation characterized by pyroptosis (Li et al., 2020; Chen et al., 2021; Lv et al., 2021; Lyu et al., 2021a,b; Zhou et al., 2021). A recent study (Zhong et al., 2022) discovered that electroacupuncture at "Baihui and Shenting" can reduce cell pyroptosis by modulating the expression level of endogenous melatonin, thereby inhibiting neuroinflammation and activating plasma cells in the CA1 area of the hippocampus. This treatment approach also mitigates neurological and cognitive impairments in rats with cerebral ischemia–reperfusion injuries (Lin et al., 2015, 2016a,b, 2017).

Given the crucial role of astrocytes in the regulatory mechanism of the brain lymphoid system, along with the latest research advancements in melatonin, inflammatory factors, and other related regulatory pathways in the regulation of Aβ and Tau protein pathological metabolism, it is reasonable to hypothesize that electroacupuncture may have a neuroprotective effect in this process. However, it is important to note that there is currently a lack of experimental research to confirm its effectiveness.

8 Summary and outlook

Ischemic stroke is a multistep condition caused by the blockage of blood vessels in the brain. When the brain is deprived of oxygen for more than 60–90 s, the cells in the brain stop working, and irreversible damage occurs over a period of several hours, leading to the death of the brain tissue. Acute ischemic stroke triggers a series of reactions such as oxidative stress, excitotoxicity, and inflammation, and these pathological processes may significantly affect subsequent recovery. Therefore, it is important to mobilize endogenous protection against these pathological processes.

Ischemic stroke is caused by decreased blood and oxygen supply, which leads to neuronal energy metabolism disorders, including decreased intracellular ATP content, mitochondrial dysfunction, and oxidative stress. Disturbed energy metabolism triggers the release of large amounts of glutamate and excitation of glutamate receptors, which in turn increases intracellular calcium levels in neurons and causes excitotoxicity (Choi, 1988; Lipton and Rosenberg, 1994; Arundine and Tymianski, 2003); in addition, disrupted energy metabolism causes mitochondrial dysfunction and elevates oxidative stress (Li et al., 2022), which causes the release of large amounts of reactive oxygen radicals. Hypoxia and energy deficiency negatively affect the brain's antioxidant defense system, resulting in decreased activity of key enzymes such as glutathione, superoxide dismutase, and glutathione peroxidase. Consequently, this amplifies the degree of oxidative stress, ultimately exacerbating neuronal damage and cell death. Therefore, this review mainly explores the five aspects of astrocytes, microglia, cerebrovascular and cerebral blood flow changes, T-cell activation or not, as well as the clearing function of the cerebral lymphatic system. We synthesized findings from contemporary research to examine the pathological alterations in astrocytes following ischemic stroke. Based on the collation of relevant studies, it has been observed that acupuncture can potentially act as a neuroprotective agent by regulating astrocytes to reduce oxidative stress, inhibit excitotoxicity, and suppress inflammatory responses. Furthermore, acupuncture can modulate microglial polarization, thus reducing inflammatory reactions. Additionally, acupuncture has been found to maintain the integrity of the blood–brain barrier, improve microcirculation, optimize cerebrovascular reserve, enhance or establish collateral circulation, and increase cerebral blood flow. Moreover, it can inhibit T-cell activation or enhance the brain's lymphatic system clearance function, thereby exerting a protective effect on brain function.

Acupuncture is the most widely used traditional and complementary medicine, used in 113 of 120 countries according to a 2019 World Health Organization report (World Health Organization, 2019). Acupuncture was most commonly recommended for musculoskeletal and connective tissue diseases; neurological disorders; obstetrics, gynecology and women's health; oncology; and gastrointestinal disorders (Dobos et al., 2012; Cho et al., 2014; Birch et al., 2018). Acupuncture works by stimulating the microenvironment of acupoints, including processes that change the anatomical structure of acupoints, regulating the microenvironment of acupoints, modulating cellular functions, and releasing various bioactive substances. Studies (Chen et al., 2018; Ding et al., 2018) have shown that acupuncture can promote the degranulation of local mast cells at acupoints, accompanied by the generation of electrical signals and the secretion of biochemical substances, such as trypsin-like enzymes, 5-hydroxytryptamine (5-HT), substance P (SP), and histamine (HA), which have been shown to mediate the acupuncture effect. After the accumulation of acupoint sensitization effects, acupuncture signals are transmitted to the central nervous system where integration and regulation of the function and activity of the target organ occur. In addition to this, acupoints can sense mechanical stimuli from the outside world, which may be one of the most initial motivating factors for the effect of acupuncture (Berman et al., 2010). Acupuncture is considered to be a minimally invasive mechanical stimulus (Jin et al., 2019), which does not cause fracture or even necrosis of muscle fibers or accumulation of red blood cells, immune cells, and cellular debris in the muscle interstitial space. Some researchers examined the tissue adhering to acupuncture needles upon removal and identified fragments of collagen fibers, fibroblasts, and adipocytes. This provides additional evidence supporting the notion that acupuncture constitutes a minor traumatic stimulus (Kimura et al., 1992). Localized tissue injury will trigger an acute immune response (Eming et al., 2007), driven by infiltration by several cells including leukocyte and mast cells and the release of vasoactive substances such as HA, SP, and adenosine. Accordingly, this review collected and organized the changes of a series of biological indicators (biochemical measurements) after using acupuncture to stimulate different acupoints (as shown in Table A1). The present results provide a reference for further research into the biological mechanism of acupuncture in the management of ischemic stroke.

Author contributions

T-cF: writing—original draft, review and editing, and figure and table design. G-rW, Y-xL, and CW: data curation. Z-fX and YiG: writing—review and editing. R-cZ, Q-tM, and Y-jM: literature search. X-yD and YaG: writing—review and editing and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

Arundine, M., and Tymianski, M. (2003). Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calc.* 34, 325–337. doi: 10.1016/S0143-4160(03)00141-6

Auboire, L., Fouan, D., Grégoire, J.-M., Ossant, F., Plag, C., Escoffre, J.-M., et al. (2021). Acoustic and elastic properties of a blood clot during microbubble-enhanced sonothrombolysis: hardening of the clot with inertial cavitation. *Pharmaceutics* 13:1566. doi: 10.3390/pharmaceutics13101566

Bao, L.-H., Zhang, Y.-N., Zhang, J.-N., Gu, L., Yang, H.-M., and Huang, Y.-Y., et al. (2018). Urate inhibits microglia activation to protect neurons in an LPS-induced model of Parkinson's disease. *J. Neuroinflamm.* 15:131. doi: 10.1186/s12974-018-1175-8

Berman, B. M., Langevin, H. M., Witt, C. M., Dubner, R. (2010). Acupuncture for chronic low back pain. *N. Engl. J. Med.* 363, 454–461. doi: 10.1056/NEJMct0806114

Birch, S., Lee, M. S., Alraek, T., and Kim, T. H. (2018). Overview of treatment guidelines and clinical practical guidelines that recommend the use of acupuncture: a bibliometric analysis. *J. Altern. Complement. Med.* 24, 752–769. doi: 10.1089/acm.2018.0092

Cai, W., Liu, S., Hu, M., Huang, F., Zhu, Q., Qiu, W., et al. (2020). Functional dynamics of neutrophils after ischemic stroke. *Transl. Stroke Res.* 11, 108–121. doi: 10.1007/s12975-019-00694-y

Campbell, B. C., Christensen, S., Tress, B. M., Churilov, L., Desmond, P. M., Parsons, M. W., et al. (2013). Failure of collateral blood flow is associated with infarct growth in ischemic stroke. *J. Cereb. Blood Flow Metab.* 33, 1168–1172. doi: 10.1038/jcbfm.2013.77

Cao, B., Tan, F., Zhan, J., and Lai, P. (2021). Mechanism underlying treatment of ischemic stroke using acupuncture: transmission and regulation. *Neural Regener. Res.* 16:944. doi: 10.4103/1673-5374.297061

Chamorro, Á., Meisel, A., Planas, A. M., Urra, X., van de Beek, D., Veltkamp, R., et al. (2012). The immunology of acute stroke. *Nat. Rev. Neurol.* 8, 401–410. doi: 10.1038/nrneurol.2012.98

Chang, D., Wang, Y.-C., Bai, Y.-Y., Lu, C.-Q., Xu, T.-T., Zhu, L., et al. (2015). Role of P38 MAPK on MMP Activity in photothrombotic stroke mice as measured using an ultrafast MMP activatable probe. *Sci. Rep.* 5:16951. doi: 10.1038/srep16951

Chen, L.-Z., Kan, Y., Zhang, Z. Y., Wang, Y.-L., Zhang, X.-N., and Wang, X.-Y., et al. (2018). Neuropeptide initiated mast cell activation by transcutaneous electrical acupoint stimulation of acupoint LI4 in rats. *Sci. Rep.* 8:13921. doi: 10.1038/s41598-018-32048-3

Chen, S., Shao, L., and Ma, L. (2021). Cerebral edema formation after stroke: emphasis on blood-brain barrier and the lymphatic drainage system of the brain. *Front. Cell. Neurosci.*15:716825. doi: 10.3389/fncel.2021.716825

Cheng, C. Y., Lin, J. G., Su, S. Y., Tang, N. Y., Kao, S. T., Hsieh, C. L., et al. (2014a). Electroacupuncture-like stimulation at Baihui and Dazhui acupoints exerts neuroprotective effects through activation of the brain-derived neurotrophic factor-mediated MEK1/2/ERK1/2/p90RSK/bad signaling pathway in mild transient focal cerebral ischemia in rats. *BMC Compl. Altern. Med.* 14:92. doi: 10.1186/1472-6882-14-92

Cheng, C. Y., Lin, J. G., Tang, N. Y., Kao, S. T., and Hsieh, C. L. (2014b). Electroacupuncture-like stimulation at the Baihui (GV20) and Dazhui (GV14) acupoints protects rats against subacute-phase cerebral ischemia-reperfusion injuries by reducing S100B-mediated neurotoxicity. *PLoS ONE* 9:e91426. doi: 10.1371/journal.pone.0091426

Cheng, Y., and Wang, Y. J. (2020). Meningeal lymphatic vessels: a drain of the brain involved in neurodegeneration. *Neurosci. Bull.* 36, 557–560. doi: 10.1007/s12264-019-00456-8

Cho, H. W., Hwang, E. H., Lim, B., et al. (2014). How current clinical practice guidelines for low back pain reflect traditional medicine in East Asian countries: a systematic review of clinical practice guidelines and systematic reviews. *PLoS ONE* 9:e88027. doi: 10.1371/journal.pone.0088027

Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1, 623–634. doi: 10.1016/0896-6273(88)90162-6

Choudhury, G. R., and Ding, S. (2015). Reactive astrocytes and therapeutic potential in focal ischemic stroke. *Neurobiol. Dis.* (2016) 85, 234–244. doi: 10.1016/j.nbd.2015.05.003

Collmann, F. M., Pijnenburg, R., Hamzei-Taj, S., Minassian, A., Folz-Donahue, K., Kukat, C., et al. (2019). Individual *in vivo* profiles of microglia polarization after stroke, represented by the genes iNOS and Ym1. *Front. Immunol.* 10:1236. doi: 10.3389/fimmu.2019.01236

Colonna, M., and Butovsky, O. (2017). Microglia function in the central nervous system during health and neurodegeneration. *Annu. Rev. Immunol.* 35, 441–468. doi: 10.1146/annurev-immunol-051116-052358

Dai, M. (2016). *Effect and mechanism of the metabolic pathways about acupuncture Neiguan on MCAO rat astrocytes glutamate* (Master's thesis). Shandong University of Traditional Chinese Medicine. Available online at: https://kns.cnki.net/kcms2/article/abstract?v=wcPNn8Zia7NywCsfhMQnWLxyPgaEfNaDzBFNIQ-86Liz_WhccP76_867ycFdd73PpEktj-2Hqfq_4KhmUMXq54puyXjwnAB4HXAmZ45cMszd7UwEN-RZS70QmXlKtF-IYjOrCjmM61Mwo-94U7fgsw==uniplatform=NZKPTlanguage=CHS

Danbolt, N. C. (2001). Glutamate uptake. *Prog. Neurobiol.* 65, 1–105. doi: 10.1016/S0301-0082(00)00067-8

Datta, A., Sarmah, D., Mounica, L., Kaur, H., Kesharwani, R., Verma, G., et al. (2020). Cell death pathways in ischemic stroke and targeted pharmacotherapy. *Transl. Stroke Res.* 11, 1185–1202. doi: 10.1007/s12975-020-00806-z

de Pablo, Y., Nilsson, M., Pekna, M., and Pekny, M. (2013). Intermediate filaments are important for astrocyte response to oxidative stress induced by oxygen-glucose deprivation and reperfusion. *Histochem. Cell Biol.* 140, 81–91. doi: 10.1007/s00418-013-1110-0

Ding, N., Jiang, J., Qin, P., Wang, Q., and Hu, J. (2018). Mast cells are important regulator of acupoint sensitization via the secretion of tryptase, 5-hydroxytryptamine, and histamine. *PLoS ONE* 13:e0194022. doi: 10.1371/journal.pone.0194022

Dobos, G. J., Kirschbaum, B., and Choi, K. E. (2012). The western model of integrative oncology: the contribution of Chinese medicine. *Chin. J. Integr. Med.* 18, 643–651. doi: 10.1007/s11655-012-1200-1

Du, Y., Shi, L., Li, J., Xiong, J., Li, B., Fan, X., et al. (2011). Angiogenesis and improved cerebral blood flow in the ischemic boundary area were detected after electroacupuncture treatment to rats with ischemic stroke. *Neurol. Res.* 33, 101–107. doi: 10.1179/016164110X12714125204317

Eming, S. A., Krieg, T., and Davidson, J. M. (2007). Inflammation in wound repair: molecular and cellular mechanisms. *J. Invest. Dermatol.* 127, 514–525. doi: 10.1038/sj.jid.5700701

Feng, D., Zhou, J., Liu, H., Wu, X., Li, F., Zhao, J., et al. (2022). Astrocytic NDRG2-PPM1A interaction exacerbates blood-brain barrier disruption after subarachnoid hemorrhage. *Sci. Adv.* 8:eabq2423. doi: 10.1126/sciadv.abq2423

Franco-Bocanegra, D. K., McAuley, C., Nicoll, J., and Boche, D. (2019). Molecular mechanisms of microglial motility: changes in ageing and Alzheimer's disease. *Cells* 8:639. doi: 10.3390/cells8060639

Gan, Y., Liu, Q., Wu, W., Yin, J.-X., Bai, X-, F., Shen, R., et al. (2014). Ischemic neurons recruit natural killer cells that accelerate brain infarction. *Proc. Natl. Acad. Sci. U. S. A.* 111, 2704–2709. doi: 10.1073/pnas.1315943111

Gelderblom, M., Leypoldt, F., Steinbach, K., Behrens, D., Choe, C. U., Siler, D. A., et al. (2009). Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke* 40, 1849–1857. doi: 10.1161/STROKEAHA.108.534503

Gill, D., and Veltkamp, R. (2016). Dynamics of T cell responses after stroke. *Curr. Opin. Pharmacol.* 26, 26–32. doi: 10.1016/j.coph.2015.09.009

Han, B., Lu, Y., Zhao, H., Wang, Y., Li, L., Wang, T., et al. (2015). Electroacupuncture modulated the inflammatory reaction in MCAO rats via inhibiting the TLR4/NF-κB signaling pathway in microglia. *Int. J. Clin. Exp. Pathol.* 8, 11199–11205.

Hoxhaj, G., and Manning, B. D. (2020). The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat. Rev. Cancer.* 20, 74–88. doi: 10.1038/s41568-019-0216-7

Hsieh, C.-L., Chang, Q.-Y., Lin, I.-H, Lin, J.-G., Liu, C.-H., Tang, N.-Y., et al. (2006). The study of electroacupuncture on cerebral blood flow in rats with and without cerebral ischemia. *Am. J. Chin. Med.* 34, 351–361. doi: 10.1142/S0192415X06003886

Iadecola, C., and Anrather, J. (2011). Stroke research at a crossroad: asking the brain for directions. *Nat. Neurosci.* 14, 1363–1368. doi: 10.1038/nn.2953

Jelkmann, W. (2005). Effects of erythropoietin on brain function. *Curr. Pharm. Biotechnol.* 6, 65–79. doi: 10.2174/1389201053167257

Jeong, H.-K., Ji, K.-M., Min, K.-J., Choi, I., Choi, D.-J., Jou, I., et al. (2014). Astrogliosis is a possible player in preventing delayed neuronal death. *Mol. Cells* 37, 345–355. doi: 10.14348/molcells.2014.0046

Jia, H., He, J., Zhao, L., Hsu, C.-C., Zhao, X., Du, Y., et al. (2022). Combination of stem cell therapy and acupuncture to treat ischemic stroke: a prospective review. *Stem Cell Res. Ther.* 13:87. doi: 10.1186/s13287-022-02761-y

Jiang, M., Liu, X., Zhang, D., Wang, Y., Hu, X., Xu, F., et al. (2018). Celastrol treatment protects against acute ischemic stroke-induced brain injury by promoting an IL-33/ST2 axis-mediated microglia/macrophage M2 polarization. *J. Neuroinflammation.* 15:78. doi: 10.1186/s12974-018-1124-6

Jiang, P., Chen, C., Wang, R., Chechneva, O. V., Chung, S. H., Rao, M. S., et al. (2013). hESC-derived Olig2+ progenitors generate a subtype of astroglia with protective effects against ischaemic brain injury. *Nat. Commun.* 4:2196. doi: 10.1038/ncomms3196

Jin, Q., Cheng, J., Liu, Y., Wu, J., Wang, X., Wei, S., et al. (2014). Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. *Brain Behav. Immun.* 40, 131–142. doi: 10.1016/j.bbi.2014.03.003

Jin, X., Jin, L., and Jin, G. Y. (2019). The anti-inflammatory effect of acupuncture and its significance in analgesia. *World J. Acupunct. Moxibust.* 29, 1–6. doi: 10.1016/j.wjam.2019.03.003

Jin, X. L. (2017). *Study on the effect of Electroacupuncture on antioxidative stress in mice with cerebral ischemia reperfusioninjury based on Nrf2 pathway* (Master's thesis). Nanjing University of Traditional Chinese Medicine. doi: 10.27253/d.cnki.gnjzu.2017.000004

Kim, J., Kim, N., and Yenari, M. A. (2015). Mechanisms and potential therapeutic applications of microglial activation after brain injury. *CNS Neurosci. Ther.* 21, 309–319. doi: 10.1111/cns.12360

Kim, J.-T., Fonarow, G. C., Smith, E. E., Reeves, M. J., Navalkele, D. D., Grotta, J. C., et al. (2017). Treatment with tissue plasminogen activator in the golden hour and the shape of the 4.5-hour time-benefit curve in the National United States get with the guidelines-stroke population. *Circulation.* 135, 128–139. doi: 10.1161/CIRCULATIONAHA.116.023336

Kim, J. H., Jung, H. G., Kim, A., Shim, H. S., Hyeon, S. J., Lee, Y. S., et al. (2021). Hevin-calcyon interaction promotes synaptic reorganization after brain injury. *Cell Death Differ.* 28, 2571–2588. doi: 10.1038/s41418-021-00772-5

Kimura, M., Tohya, K., Kuroiwa, K., Oda, H., Gorawski, E. C., Hua, Z. X., et al. (1992). Electron microscopical and immunohistochemical studies on the induction of "Qi" employing needling manipulation. *Am. J. Chin. Med.* 20, 25–35. doi: 10.1142/S0192415X92000047

Kobayashi, M., Benakis, C., Anderson, C., Moore, M. J., Poon, C., Uekawa, K., et al. (2019). AGO CLIP reveals an activated network for acute regulation of brain glutamate homeostasis in ischemic stroke. *Cell Rep.* 28, 979–991.e6. doi: 10.1016/j.celrep.2019.06.075

Lee, M. J., Jang, M., Choi, J., Lee, G., Min, H. J., Chung, W.-S., et al. (2016). Bee venom acupuncture alleviates experimental autoimmune encephalomyelitis by upregulating regulatory T cells and suppressing Th1 and Th17 responses. *Mol. Neurobiol.* 53, 1419–1445. doi: 10.1007/s12035-014-9012-2

Li, N., Liu, T.-H., Yu, J.-Z., Li, C.-X., Liu, Y., Wu, Y.-Y., et al. (2019). Curcumin and curcumol inhibit NF-κB and TGF-β (1)/smads signaling pathways in CSE-treated RAW246.7 cells. *Evid. Based Complement. Alternat. Med.* 2019:3035125. doi: 10.1155/2019/3035125

Li, P., Gan, Y., Sun, B. L., Zhang, F., Lu, B., Gao, Y., et al. (2013). Adoptive regulatory T-cell therapy protects against cerebral ischemia. *Ann. Neurol.* 74, 458–471. doi: 10.1002/ana.23815

Li, R., Liu, W., Yin, J., Chen, Y., Guo, S., Fan, H., et al. (2018). TSG-6 attenuates inflammation-induced brain injury via modulation of microglial polarization in SAH rats through the SOCS3/STAT3 pathway. *J. Neuroinflammation.* 15:231. doi: 10.1186/s12974-018-1279-1

Li, Y., Zhang, J., Wan, J., Liu, A., and Sun, J. (2020). Melatonin regulates Abeta production/clearance balance and Abeta neurotoxicity: a potential therapeutic molecule for Alzheimer's disease. *Biomed. Pharmacother.* 132:110887. doi: 10.1016/j.biopha.2020.110887

Li, Z., Bi, R., Sun, S., Chen, S., Chen, J., Hu, B., et al. (2022). The role of oxidative stress in acute ischemic stroke-related thrombosis. *Oxid. Med. Cell. Longev.* 2022:8418820. doi: 10.1155/2022/8418820

Liesz, A., Suri-Payer, E., Veltkamp, C., Doerr, H., Sommer, C., Rivest, S., et al. (2009). Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat. Med.* 15, 192–199. doi: 10.1038/nm.1927

Lin, R., Chen, J., Li, X., Mao, J., Wu, Y., Zhuo, P., et al. (2016a). Electroacupuncture at the Baihui acupoint alleviates cognitive impairment and exerts neuroprotective effects by modulating the expression and processing of brain-derived neurotrophic factor in APP/PS1 transgenic mice. *Mol. Med. Rep*. 13, 1611–1617. doi: 10.3892/mmr.2015.4751

Lin, R., Li, X., Liu, W., Chen, W., Yu, K., Zhao, C., et al. (2017). Electro-acupuncture ameliorates cognitive impairment via improvement of brain-derived neurotropic factor-mediated hippocampal synaptic plasticity in cerebral ischemia-reperfusion injured rats. *Exp. Ther. Med*. 14, 2373–2379. doi: 10.3892/etm.2017.4750

Lin, R., Lin, Y., Tao, J., Chen, B., Yu, K., Chen, J., et al. (2015). Electroacupuncture ameliorates learning and memory in rats with cerebral ischemia-reperfusion injury by inhibiting oxidative stress and promoting p-CREB expression in the hippocampus. *Mol. Med. Rep*. 12, 6807–6814. doi: 10.3892/mmr.2015.4321

Lin, R., Wu, Y., Tao, J., Chen, B., Chen, J., Zhao, C., et al. (2016b). Electroacupuncture improves cognitive function through Rho GTPases and enhances dendritic spine plasticity in rats with cerebral ischemia-reperfusion. *Mol. Med. Rep*. 13, 2655–2660. doi: 10.3892/mmr.2016.4870

Lin, X., Song, F., Wu, Y., Xue, D., and Wang, Y (2022). Lycium barbarum polysaccharide attenuates Pseudomonas-aeruginosa pyocyanin-induced cellular injury in mice airway epithelial cells. *Food Nutr. Res*. 66. doi: 10.29219/fnr.v66.4585

Lipton, S. A., and Rosenberg, P. A. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *N. Engl. J. Med*. 330, 613–622. doi: 10.1056/NEJM199403033300907

Liu, A. J., Li, J. H., Li, H. Q., Fu, D. L., Lu, L., Bian, Z. X., et al. (2015). Electroacupuncture for acute ischemic stroke: a meta-analysis of randomized controlled trials. *Am. J. Chin. Med*. 43, 1541–1566. doi: 10.1142/S0192415X15500883

Liu, L. Q., Liu, X. R., Zhao, J. Y., Yan, F., Wang, R. L., Wen, S. H., et al. (2018). Brain-selective mild hypothermia promotes long-term white matter integrity after ischemic stroke in mice. *CNS Neurosci. Ther*. 24, 1275–1285. doi: 10.1111/cns.13061

Liu, R., Xu, N. G., Yi, W. (2002). Electroacupuncture attenuates inflammation after ischemic stroke by inhibiting NF-κB-mediated activation of microglia. *Evid. Based Complement. Alternat. Med*. 2020:8163052. doi: 10.1155/2020/8163052

Liu, W., Wang, X., Yang, S., Huang, J., Xue, X., Zheng, Y., et al. (2016a). Electroacupunctre improves motor impairment via inhibition of microglia-mediated neuroinflammation in the sensorimotor cortex after ischemic stroke. *Life Sci*. 151, 313–322. doi: 10.1016/j.lfs.2016.01.045

Liu, Y., Wang, H., Wang, X., Mu, L., Kong, Q., Wang, D., et al. (2013). The mechanism of effective electroacupuncture on T cell response in rats with experimental autoimmune encephalomyelitis. *PLoS ONE* 8:e51573. doi: 10.1371/journal.pone.0051573

Liu, Z., Yan, S., Wu, J., He, L., Li, N., Dong, G., et al. (2016b). Acupuncture for chronic severe functional constipation: a randomized trial. *Ann. Intern. Med*. 165, 761–769. doi: 10.7326/M15-3118

Lv, T., Zhao, B., Hu, Q., and Zhang, X. (2021). the glymphatic system: a novel therapeutic target for stroke treatment. *Front. Aging Neurosci*. 13:689098. doi: 10.3389/fnagi.2021.689098

Lyu, Z., Chan, Y., Li, Q., Zhang, Q., Liu, K., Xiang, J., et al. (2021b). Destructive effects of pyroptosis on homeostasis of neuron survival associated with the dysfunctional BBB-glymphatic system and amyloid-beta accumulation after cerebral ischemia/reperfusion in rats. *Neural Plas*. 2021:4504363. doi: 10.1155/2021/4504363

Lyu, Z., Li, Q., Yu, Z., Chan, Y., Fu, L., Li, Y., et al. (2021a). Yi-Zhi-Fang-Dai formula exerts neuroprotective effects against pyroptosis and blood-brain barrier-glymphatic dysfunctions to prevent amyloid-beta acute accumulation after cerebral ischemia and reperfusion in rats. *Front. Pharmacol*. 12:791059. doi: 10.3389/fphar.2021.791059

Ma, Y., Wang, J., Wang, Y., and Yang, G. Y. (2017). The biphasic function of microglia in ischemic stroke. *Prog. Neurobiol*. 157, 247–272. doi: 10.1016/j.pneurobio.2016.01.005

MacPherson, H., Richmond, S., Bland, M., Brealey, S., Gabe, R., Hopton, A., et al. (2013). Acupuncture and counselling for depression in primary care: a randomised controlled trial. *PLoS Med*. 10:e1001518. doi: 10.1371/journal.pmed.1001518

Magnus, T., Wiendl, H., and Kleinschnitz, C. (2012). Immune mechanisms of stroke. *Curr. Opin. Neurol*. 25, 334–340. doi: 10.1097/WCO.0b013e328352ede6

Meng, X. L., Zhang, D. L., and Sui, S. H. (2019). Acute remote ischemic preconditioning alleviates free radical injury and inflammatory response in cerebral ischemia/reperfusion rats. *Exp. Ther. Med*. 18, 1953–1960. doi: 10.3892/etm.2019.7797

Montaño, A., Staff, I., McCullough, L. D., and Fortunato, G. (2013). Community implementation of intravenous thrombolysis for acute ischemic stroke in the 3- to 4.5-hour window. *Am. J. Emerg. Med*. (2013) 31, 1707–1709. doi: 10.1016/j.ajem.2013.08.032

Moskowitz, M. A., Lo, E. H., and Iadecola, C. (2010). The science of stroke: mechanisms in search of treatments. *Neuron*. 67, 181–198. doi: 10.1016/j.neuron.2010.07.002

Neuhaus, A. A., Couch, Y., Hadley, G., and Buchan, A. M. (2017). Neuroprotection in stroke: the importance of collaboration and reproducibility. *Brain* 140, 2079–2092. doi: 10.1093/brain/awx126

Ornello, R., Degan, D., Tiseo, C., Di Carmine, C., Perciballi, L., Pistoia, F., et al. (2018). Distribution and temporal trends from 1993 to 2015 of ischemic stroke subtypes: a systematic review and meta-analysis. *Stroke* 49, 814–819. doi: 10.1161/STROKEAHA.117.020031

Pajarillo, E., Rizor, A., Lee, J., Aschner, M., and Lee, E. (2019). The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: potential targets for neurotherapeutics. *Neuropharmacology*. (2019) 161:107559. doi: 10.1016/j.neuropharm.2019.03.002

Pang, J., Itano, T., Sumitani, K., Negi, T., and Miyamoto, O. (2003). Electroacupuncture attenuates both glutamate release and hyperemia after transient ischemia in gerbils. *Am. J. Chin. Med*. 31, 295–303. doi: 10.1142/S0192415X03000977

Park, J., Park, H.-H., Choi, H., Kim, Y. S., Yu, H.-J., Lee, K.-Y., et al. (2012). Coenzyme Q10 protects neural stem cells against hypoxia by enhancing survival signals. *Brain Res*. (2012) 1478:64–73. doi: 10.1016/j.brainres.2012.08.025

Perez-de-Puig, I., Miró-Mur, F., Ferrer-Ferrer, M., Gelpi, E., Pedragosa, J., Justicia, C., et al. (2015). Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathol*. 129, 239–57. doi: 10.1007/s00401-014-1381-0

Prass, K., Meisel, C., Höflich, C., Braun, J., Halle, E., Wolf, T., et al. (2003). Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. *J. Exp. Med*. 198, 725–736. doi: 10.1084/jem.20021098

Qiu, Y. (2013). Clinical observation on scalp acupuncture combined with rehabilitation training for hemiplegia after stroke. *J. Acupunct. Tuina Sci*. 11, 226–229. doi: 10.1007/s11726-013-0696-z

Rasmussen, M. K., Mestre, H., and Nedergaard, M. (2018). The glymphatic pathway in neurological disorders. *Lancet Neurol*.17, 1016–1024. doi: 10.1016/S1474-4422(18)30318-1

Ren, X., Liu, S., Lian, C., Li, H., Li, K., Li, L., et al. (2021). Dysfunction of the glymphatic system as a potential mechanism of perioperative neurocognitive disorders. *Front. Aging Neurosci*. 13:659457. doi: 10.3389/fnagi.2021.659457

Roy Choudhury, G., Ryou, M. G., Poteet, E., Wen, Y., He, R., Sun, F., et al. (2014). Involvement of p38 MAPK in reactive astrogliosis induced by ischemic stroke. *Brain Res*. 1551, 45–58. doi: 10.1016/j.brainres.2014.01.013

Segawa, K., Blumenthal, Y., Yamawaki, Y., and Ohtsuki, G. A. (2021). Destruction model of the vascular and lymphatic systems in the emergence of psychiatric symptoms. *Biology* 10:34. doi: 10.3390/biology10010034

Serra, P., Amrani, A., Yamanouchi, J., Han, B., Thiessen, S., Utsugi, T., et al. (2003). CD40 ligation releases immature dendritic cells from the control of regulatory CD4+CD25+ T cells. *Immunity* 19, 877–889. doi: 10.1016/S1074-7613(03)00327-3

Shen, M. H., Li, Z. R., Xiang, X. R., and Niu, W. M. (2009). Effect of electroacupuncture on cerebral cortex ultrastructure in rats with cerebral ischemia-reperfusion injury. *Zhen Ci Yan Jiu*. 34, 167–170.

Shen, Y., Sun, A., Wang, Y., Cha, D., Wang, H., Wang, F., et al. (2012). Upregulation of mesencephalic astrocyte-derived neurotrophic factor in glial cells is associated with ischemia-induced glial activation. *J. Neuroinflamm*. 9:254. doi: 10.1186/1742-2094-9-254

Sherafat, A., Pfeiffer, F., Reiss, A. M., Wood, W. M., and Nishiyama, A. (2021). Microglial neuropilin-1 promotes oligodendrocyte expansion during development and remyelination by trans-activating platelet-derived growth factor receptor. *Nat. Commun*. 12:2265. doi: 10.1038/s41467-021-22532-2

Shi, L., Cao, H.-M., Li, Y., Xu, S-, X., Zhang, Y., Zhang, Y., et al. (2017). Electroacupuncture improves neurovascular unit reconstruction by promoting collateral circulation and angiogenesis. *Neural Regen Res*. 12, 2000–2006. doi: 10.4103/1673-5374.221156

Shu, Z. M., Shu, X. D., Li, H. Q., Sun, Y., Shan, H., Sun, X. Y., et al. (2016). Ginkgolide B protects against ischemic stroke via modulating microglia polarization in mice. *CNS Neurosci. Ther*. 22, 729–739. doi: 10.1111/cns.12577

Souder, D. C., Dreischmeier, I. A., Smith, A. B., Wright, S., Martin, S. A., Sagar, M. A. K., et al. (2021). Rhesus monkeys as a translational model for late-onset Alzheimer's disease. *Aging Cell* 20:e13374. doi: 10.1111/acel.13374

Stelmashook, E. V., Isaev, N. K., Lozier, E. R., Goryacheva, E. S., and Khaspekov, L. G. (2011). Role of glutamine in neuronal survival and death during brain ischemia and hypoglycemia. *Int. J. Neurosci*. 121, 415–422. doi: 10.3109/00207454.2011.570464

Sun, L., Fan, Y., Fan, W., Sun, J., Ai, X., Qiao, H., et al. (2020). Efficacy and safety of scalp acupuncture in improving neurological dysfunction after ischemic stroke: a protocol for systematic review and meta-analysis. *Medicine* 99:e21783. doi: 10.1097/MD.0000000000021783

Sun, L., Zhang, Y., Liu, E., Ma, Q., Anatol, M., Han, H., et al. (2019). The roles of astrocyte in the brain pathologies following ischemic stroke. *Brain Inj*. 33, 712–716. doi: 10.1080/02699052.2018.1531311

Sun, Y., Jiang, J., Zhang, Z., Yu, P., Wang, L., Xu, C., et al. (2008). Antioxidative and thrombolytic TMP nitrone for treatment of ischemic stroke. *Bioorg. Med. Chem*. 16, 8868–8874. doi: 10.1016/j.bmc.2008.08.075

Tanabe, N., Kuboyama, T., and Tohda, C. (2019). Matrine promotes neural circuit remodeling to regulate motor function in a mouse model of chronic spinal cord injury. *Neural Regen. Res*. 14, 1961–1967. doi: 10.4103/1673-5374.259625

Tao, J., Zheng, Y., Liu, W., Yang, S., Huang, J., Xue, X., et al. (2016). Electro-acupuncture at LI11 and ST36 acupoints exerts neuroprotective effects via reactive astrocyte proliferation after ischemia and reperfusion injury in rats. *Brain Res. Bull.* 120, 14–24. doi: 10.1016/j.brainresbull.2015.10.011

Terada, K., Murata, A., Toki, E., Goto, S., Yamakawa, H., Setoguchi, S., et al. (2020). Atypical antipsychotic drug ziprasidone protects against rotenone-induced neurotoxicity: an *in vitro* study. *Molecules* 25:4206. doi: 10.3390/molecules25184206

Tian, R., and Mao, G. (2022). Ghrelin reduces cerebral ischemic injury in rats by reducing M1 microglia/macrophages. *Eur. J. Histochem.* 66:3350. doi: 10.4081/ejh.2022.3350

Toro, C. A., Zhang, L., Cao, J., and Cai, D. (2019). Sex differences in Alzheimer's disease: Understanding the molecular impact. *Brain Res.* 1719, 194–207. doi: 10.1016/j.brainres.2019.05.031

Vaitkiene, P., Valiulyte, I., Glebauskiene, B., and Liutkeviciene, R. (2017). N-myc downstream-regulated gene 2 (NDRG2) promoter methylation and expression in pituitary adenoma. *Diagn. Pathol.* 12:33. doi: 10.1186/s13000-017-0622-7

Wang, X. (2005). Investigational anti-inflammatory agents for the treatment of ischaemic brain injury. *Expert Opin. Investig. Drugs.* 14, 393–409. doi: 10.1517/13543784.14.4.393

Wang, X.-R., Shi, G-, X., Yang, J.-W., Yan, C-, Q., Lin, L.-T., Du, S-, Q., et al. (2015). Acupuncture ameliorates cognitive impairment and hippocampus neuronal loss in experimental vascular dementia through Nrf2-mediated antioxidant response. *Free Radic. Biol. Med.* (2015) 89, 1077–1084. doi: 10.1016/j.freeradbiomed.2015.10.426

Wei, L., Chen, C., Ding, L., Mo, M., Zou, J., Lu, Z., et al. (2019). Wnt1 promotes EAAT2 expression and mediates the protective effects of astrocytes on dopaminergic cells in Parkinson's disease. *Neural Plast.* 2019:1247276. doi: 10.1155/2019/1247276

World Health Organization (2019). *WHO Global Report on Traditional and Complementary Medicine*. Beijing: People's Medical Publishing House.

Wu, X. D., Du, L. N., Wu, G. C., and Cao, X. D. (2001). Effects of electroacupuncture on blood-brain barrier after cerebral ischemia-reperfusion in rat. *Acupunct. Electrother. Res.* 26, 1–9. doi: 10.3727/036012901816356063

Xin, Z., Xue-Ting, L., and De-Ying, K. (2015). GRADE in systematic reviews of acupuncture for stroke rehabilitation: recommendations based on high-quality evidence. *Sci. Rep.* 5:16582. doi: 10.1038/srep16582

Xu, H., Zhang, Y., Sun, H., Chen, S., and Wang, F. (2014). Effects of acupuncture at GV20 and ST36 on the expression of matrix metalloproteinase 2, aquaporin 4, and aquaporin 9 in rats subjected to cerebral ischemia/reperfusion injury. *PLoS ONE* 9:e97488. doi: 10.1371/journal.pone.0097488

Xu, J., Yi, M., Ding, L., and He, S. A. (2019). Review of anti-inflammatory compounds from marine fungi, 2000-2018. *Mar. Drugs.* 17:636. doi: 10.3390/md17110636

Xu, Y., Guo, Y., Song, Y., Zhang, K., Zhang, Y., Li, Q., et al. (2018). A new theory for acupuncture: promoting robust regulation. *J Acupunct Meridian Stud.* 11, 39–43. doi: 10.1016/j.jams.2017.11.004

Yang, T., Sun, Y., Zhang, F. (2016). "The role of nonneuronal Nrf2 pathway in ischemic stroke: damage control and potential tissue repair," in *Non-Neuronal Mechanisms of Brain Damage and Repair After Stroke. Springer Series in Translational Stroke Research*, eds J. Chen, J. Zhang, and X. Hu (Cham: Springer).

Zhan, J., Pan, R., Zhou, M., Tan, F., Huang, Z., Dong, J., et al. (2018). Electroacupuncture as an adjunctive therapy for motor dysfunction in acute stroke survivors: a systematic review and meta-analyses. *BMJ Open* 8:e017153. doi: 10.1136/bmjopen-2017-017153

Zhang, Q., and Tian, Z. X. (2020). Effects of Xingnao Kaiqiao acupuncture combined with Alteplase on neurological impairment, lipid peroxidation and cerebral vascular reserve function in patients with acute cerebral infarction. *Shanghai J. Acupunct.* 39, 25–30. doi: 10.13460/j.issn.1005-0957.2020.01.0025

Zhang, S., Jin, T., Wang, L., Liu, W., Zhang, Y., Zheng, Y., et al. (2020). Electro-acupuncture promotes the differentiation of endogenous neural stem cells via exosomal microRNA 146b after ischemic stroke. *Front. Cell. Neurosci.* 14:223. doi: 10.3389/fncel.2020.00223

Zhao, L., Chen, J., Li, Y., Sun, X., Chang, X., Zheng, H., et al. (2017). The long-term effect of acupuncture for migraine prophylaxis: a randomized clinical trial. *JAMA Intern. Med.* 177, 508—515. doi: 10.1001/jamainternmed.2016.9378

Zhao, Q. Y., and Ma, X. D. (2018). Experimental study on the antioxidant effect of acupuncture Baihui and Sishencong on CI/RI model rats. *J. Liaoning Univ. Trad. Chin. Med.* 20, 66–69. doi: 10.13194/j.issn.1673-842x.2018.08.018

Zhong, X. Y., Ruan, S., Wang, F., et al. (2022). Mechanism of electroacupuncture alleviating cerebral ischemia reperfusion injury in rats by regulating endogenous melatonin secretion. *Acupunct Res.* 47, 39–49. doi: 10.3724/SP.J.1329.2022.01006

Zhou, X., Li, Y., Lenahan, C., Ou, Y., Wang, M., He, Y., et al. (2021). Glymphatic system in the central nervous system, a novel therapeutic direction against brain edema after stroke. *Front. Aging Neurosci.* 13:698036. doi: 10.3389/fnagi.2021.698036

Zhu, J., Cao, D., Guo, C., Liu, M., Tao, Y., Zhou, J., et al. (2019). Berberine facilitates angiogenesis against ischemic stroke through modulating microglial polarization via AMPK signaling. *Cell. Mol. Neurobiol.* 39, 751–768. doi: 10.1007/s10571-019-00675-7

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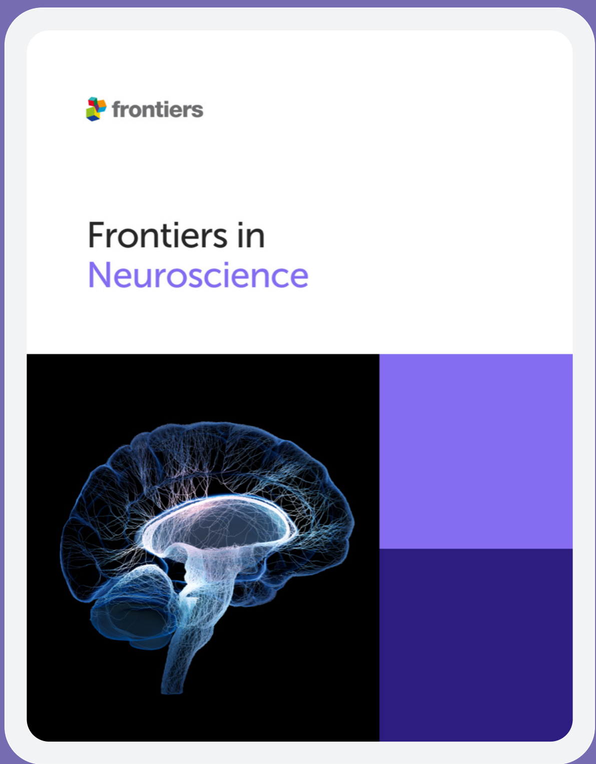

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