Cerebral Cortex, 2024, **34**, bhae127
https://doi.org/10.1093/cercor/bhae127
Advance access publication date 23 April 2024
Original Article

Multi-scale analysis of acupuncture mechanisms for motor and sensory cortex activity based on SEEG data

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Acupuncture, a traditional Chinese therapy, is gaining attention for its impact on the brain. While existing electroencephalogram and functional magnetic resonance image research has made significant contributions, this paper utilizes stereo-electroencephalography data for a comprehensive exploration of neurophysiological effects. Employing a multi-scale approach, channel-level analysis reveals notable δ-band activity changes during acupuncture. At the brain region level, acupuncture modulated connectivity between the paracentral lobule and the precentral gyrus. Whole-brain analysis indicates acupuncture's influence on network organization, and enhancing E_{glob} and increased interaction between the motor and sensory cortex. Brain functional reorganization is an important basis for functional recovery or compensation after central nervous system injury. The use of acupuncture to stimulate peripheral nerve trunks, muscle motor points, acupoints, etc., in clinical practice may contribute to the reorganization of brain function. This multi-scale perspective provides diverse insights into acupuncture's effects. Remarkably, this paper pioneers the introduction of stereo-electroencephalography data, advancing our understanding of acupuncture's mechanisms and potential therapeutic benefits in clinical settings.

Key words: acupuncture; sensory cortex; motor cortex; SEEG.

Introduction

Acupuncture, a traditional Chinese medical practice, has demonstrated efficacy in treating various diseases as reported earlier (Cheuk and Wong 2014; Da Silva 2015; Lin et al. 2016; Guo et al. 2020) including ischemic stroke, migraine, epilepsy, functional diarrhea, and Parkinson's disease. The therapeutic process of acupuncture in relation to the nervous system involves the stimulation of acupuncture points, transmission of stimulation to the brain to regulate or re-establish neural connections, and subsequent restoration of the relevant bodily functions. Experiments has demonstrated by Wang et al. (2014) utilized real-time dynamic (functional magnetic resonance image, fMRI) to compare patients with motor impairment caused by left central sulcus lesions to healthy individuals. Significant functional activation areas were observed when the group with motor impairment underwent acupuncture treatment. Notably, the effects of acupuncture appeared to be more pronounced in pathological conditions compared with healthy subjects. Additional investigations have reported that acupuncture at the GB34 (Yanglingquan) acupoint may enhance motor cognitive connectivity while reducing compensation in the unaffected motor cortex and ipsilateral synapses, thus facilitating recovery from hemiplegia and spasticity (Han et al. 2019). In clinical practice, the combination of GB34 and ST36 (Zusanli) acupoints was frequently used to treat various diseases. Similar findings were reported by Pan (2018), who examined diffusion tensor imaging and fMRI data in 22 stroke patients with subcortical infarction in the right hemisphere and 22 healthy controls. Multiple studies have demonstrated the immediate, after-effects, and cumulative effects of acupuncture (Huang et al. 2002; Zhang et al. 2010; Pan 2018), suggesting its impact on the nervous system. Nevertheless, the underlying mechanisms that explaining the therapeutic efficacy of acupuncture remained unclear, thereby arousing widespread skepticism and attracting significant attention.

Numerous studies employed bioimaging techniques, such as electroencephalogram (EEG) and fMRI (Onton et al. 2005; Tyvaert et al. 2009; Polikar et al. 2010; Cribben et al. 2013), to explore the impact of acupuncture point stimulation on the nervous system. The advantage of EEG with high temporal resolution allows us to explore brain activity stimulated by acupuncture at different time scales as reported earlier (Song et al. 2018). Hsu et al. (2011) revealed a substantial decrease in cortical power in response to acupuncture stimulation at a frequency of 100 times per minute. In the acupuncture state, statistically significant differences were investigated in the energy of electrodes T3, T4, O1, and O2. Qi et al. (2020) proposed the activation of brain regions beyond the occipital lobe induced by acupuncture, which showed alterations in the frontal lobe, anterior temporal lobe, and prefrontal regions. However, "No neuroan is an island" (Axer and Amunts 2022; Thiebaut et al. 2022). The key to the various functions of the brain is not that each brain region performs a

Received: January 8, 2024. **Revised:** March 8, 2024. **Accepted:** March 9, 2024

specific function independently, but rather the connections and communication between different regions. Different acupuncture states exhibit distinct patterns of brain networks as reported earlier (Yi et al. 2013). It has been observed that acupuncture increases long-distance connections between the left and the right hemisphere. Furthermore, in the study conducted by Lin et al. (2023), scalp acupuncture was found to exhibit bidirectional regulatory effects on functional connectivity between the cerebral cortex and basal ganglia in stroke patients, thereby promoting the restoration of balanced brain function. This finding is consistent with the results reported by Yu et al. (Haitao et al. 2018), who compared the synchronization degree in different acupuncture states and observed that δ and α bands exhibited significantly higher synchronization degrees during acupuncture compared with the pre-acupuncture state. Additionally, Zhang and Yang (2023) found that during acupuncture, the average characteristic path length increased compared with pre-acupuncture. EEG data has offerred the advantage of high temporal resolution for exploring cortical activity, but investigating deep brain regions remains tremendously challenging greater challenges.

Thus, several studies utilized fMRI data to examine the influence of acupuncture on deep brain regions, and it was discovered that acupuncture could enhance connectivity among cortices affected by impaired white matter tracts (Keinänen et al. 2018). Wang et al. (2024) explored the potential brain area for its brain effect mechanism, and found that acupuncture at GB34 could regulate the Per AF values in multiple brain regions of patients with right ischemic stroke. Furthermore, fMRI data not only allow exploration of connectivity between pairwise brain regions but also facilitate a deeper understanding of the interactions across the entire brain network. In the context of acupuncture research, Ren et al. (2010) applied a graph theory approach to investigate the modulatory effects of acupuncture on the reorganization of brain network functional connectivity across different acupuncture points. Specificity of acupuncture points has been revealed through whole-brain hierarchical analysis based on graph-theoretical features. For acupuncture at PC6, the nodule and uvula were identified as central hubs in the overall brain network. Acupuncture at PC7 highlighted the amygdala as an important node. Furthermore, acupuncture at GB37 showed the posterior cingulate cortex as a central hub. These findings elucidate the distinct effects of different acupuncture points on the overall brain network and provide crucial insights into the underlying mechanisms of acupuncture. Indeed, fMRI data are limited in its ability to observe high temporal resolution brain activity. However, this limitation can be overcome by employing Stereo-electroencephalography (SEEG) data.

SEEG possesses a unique advantage of both high temporal and spatial resolution, surpassing that of EEG and fMRI. SEEG (Guenot et al. 2001; Taussig et al. 2014; Bartolomei et al. 2017; Goodale et al. 2020; Khoo et al. 2020) is primarily employed for clinical purposes, specifically seizure localization and treatment in drug-resistant focal epilepsy. This invasive method allows for the recording of electrical signals from deep brain regions, facilitating three-dimensional and temporally accurate investigations of epileptic discharges, precise localization of brain regions, and exploration of deep brain structures. By utilizing SEEG data to explore intracranial deep electrodes in drug-resistant epilepsy patients, it has been discovered that their seizures originated from the inferolateral temporal lobe (Lentoiu et al. 2022). Furthermore, leveraging the high temporal resolution advantage of SEEG data, they conducted time-frequency analysis and connectivity analysis to identify brain regions crucially involved in expressing different emotions. In this study, we aim to utilize the advantages of SEEG data to explore the brain mechanisms involved in acupuncture at GB34. This paper is the first to study the neural mechanisms of acupuncture based on SEEG data. We considered multiple scales to conduct a comprehensive analysis of the acupuncture effects at GB34: (1) channel-level analysis examined changes in brain activity within individual recording channels. (2) Regional-level brain activity analysis explored activity changes within different brain regions and interactions between pairwise regions. (3) Whole-brain analysis investigated information exchange across the entire brain. Through this multi-scale analysis, our goal was to gain a more comprehensive understanding of the effects of acupuncture at GB34 and shed light on the underlying mechanisms that were still not fully understood.

Methods and materials

This chapter includes an introduction to the participants involved in the paper, description of the experimental paradigm employed, the steps involved in data preprocessing, and an overview of the three-level analysis approach. Figure 1 illustrates the research framework of this paper.

Participant

The patient with epilepsy included in this paper was selected from the neurosurgery department of a hospital, and has diagnosed with right hemiplegia. Acupuncture treatment was administered at the GB34 acupoint, situated below the knee in the slightly anterior depression of the small head of the fibula on the lateral side of the calf. Clinical studies have shown that needling at GB34 yields therapeutic benefits, including the treatment of liver and spleen pain, alleviation of knee pain, improvement of mood, and reduction of spasms (Xueyang and Li 2016). The SEEG data used in this experiment were recorded from the patient during a 7-day acupuncture treatment regimen. The subject provided informed consent for participation in the study, and the research protocol was conducted with approval from the hospital's Ethics Committee. Data acquisition of SEEG recordings was collected by Nicolet equipment, capturing signals from four electrodes that covered seven brain regions: precentral gyrus, paracentral lobule, superior postcentral gyrus, paracentral lobule, inferior precentral gyrus, anterior paracentral lobule, superior precentral gyrus, posterior posterolateral paracentral lobule. These recordings were acquired through a total of 38 channels, denoted as A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, C1, C2, C3, C4, C5, C6, C7, C8, C9, D1, D2, D3, D4, D5, D6, D7, D8, D9 as shown in Fig. 2.

Experimental paradigm

Acupuncture treatment was conducted four times a day, thewhole experimental process took approximately 40 minutes per day. In order to ensure consistency of the data, acupuncture sessions were conducted uniformly at noon. During these sessions, the patient experienced the clinical event known as deqi, characterized by sensations of soreness, numbness, and swelling, which are recognized phenomena in acupuncture therapy. The experimental procedure, as depicted in Fig. 3, entailed the following steps: Initially, the patient was allowed to rest briefly. Subsequently, the doctor inserted the acupuncture needle into the GB34 acupoint to a depth of 2–3 cm, continuing the needling process until the deqi response was achieved. This phase, characterized by the insertion of needles without manipulation, constituted

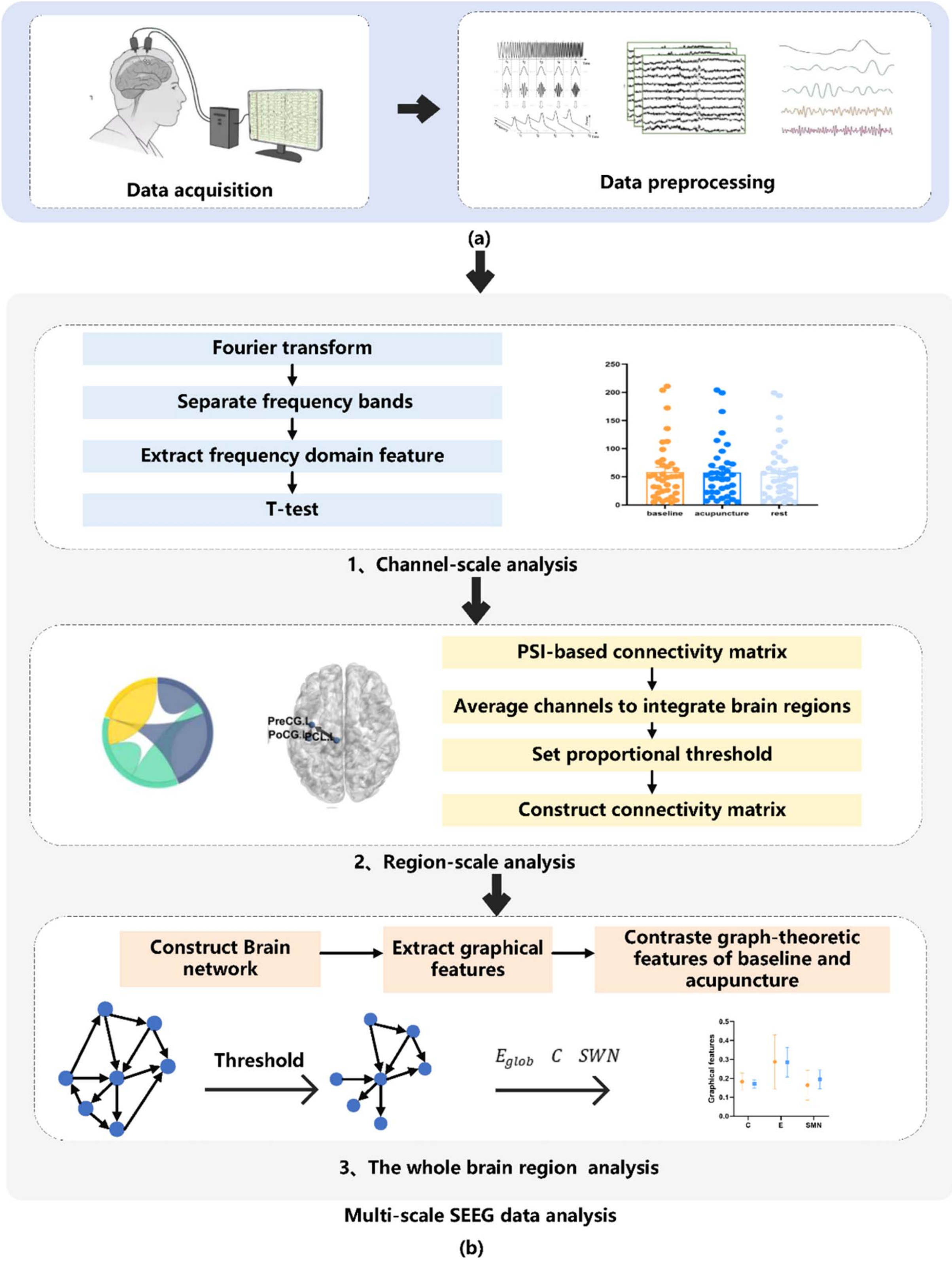

Fig. 1. The figure illustrates the research framework adopted in this study. The overall framework consists of three main steps: data acquisition, data preprocessing, and multi-scale analysis. (a) Data acquisition and data preprocessing. The data preprocessing step involves several procedures, including labeling, bandpass filtering, downsampling, and so on. (b) Multi-scale analysis. The multi-scale analysis is further divided into three components: channel-level analysis, brain region-level analysis, and whole-brain analysis. In the channel-level analysis, individual recording channels are examined to investigate changes in brain activity within specific regions. The brain region-level analysis explores the activity and connectivity patterns between different brain regions. Finally, the whole-brain analysis examines the global effects of acupuncture on brain connectivity.

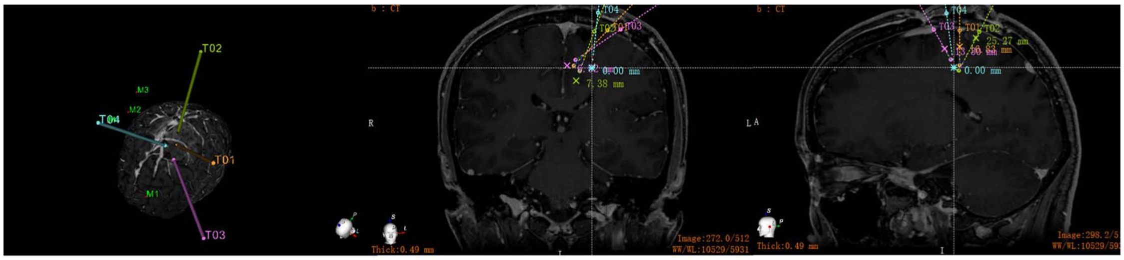

Fig. 2. The localization of SEEG electrodes. T01: A1–10. T02: B1–10. T03: C1–9. T04: D1–9.

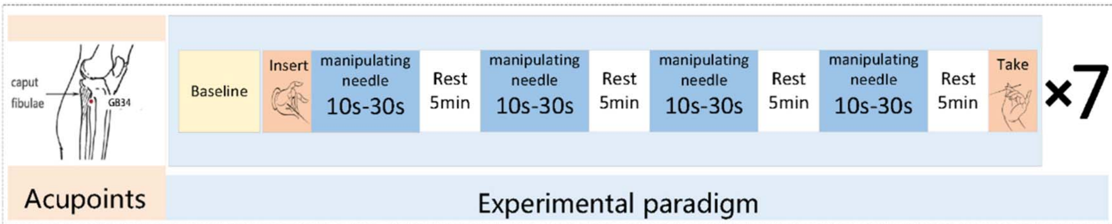

Fig. 3. Acupoints and acupuncture experimental paradigm. The acupuncture point targeted was GB34. The experimental protocol encompassed one measurement session, during which multiple phases were conducted. Initially, a baseline state collection was performed before the acupuncture intervention. Subsequently, acupuncture was administered to elicit the deqi response, with the duration of needle stimulation lasting between 10 and 30 s. After achieving deqi, the needles were retained, allowing the subject to rest for 5 min. This acupuncture procedure was repeated four times daily, consistently carried out over a total of seven consecutive days. This comprehensive experimental design facilitated the exploration of the cumulative effects of acupuncture at GB34 over an extended period.

the pre-acupuncture state or baseline. Following this, a twirling-rotating and lifting-thrusting manipulation lasting 10–30 s of the acupuncture needles was performed. After the manipulation, the patient was required to rest for 5 min before proceeding to the next repetition of the procedure. This entire procedure was conducted four times within each daily session. The course of treatment at the GB34 acupoint spanned a total of 7 days, with the same procedure repeated throughout this duration. Upon the completion of the final acupuncture session, the acupuncturist concluded the experiment.

Data preprocessing

The preprocessing steps are: labeling, segmentation, band-pass filtering, notch-filter, downsampling, and re-referencing. All preprocessing were implemented using EEGLAB 2021.1 in MATLAB. We selected the pre-needle lancing data segment to be labeled as the baseline state, the physician's row of needles to be labeled as the row of needles state, and the resting time segment after row of needles to be the inter-row interval. In the following analysis, the data segment from the initial 10 s prior to needle insertion serves as the baseline state. Needle manipulation corresponds to the data segment 10 s before receiving the needle, and the resting state refers to the period 20–10 s before the end of the rest. After labelling raw SEEG signals were subjected to 2-Hz high pass and 250-Hz low pass. In order to reduce line-noise-related artifacts, the voltage signals were notch-filtered at 50, 100, 150, 200 and 250 Hz. The signals were downsampled to 512 Hz and finally re-referencing using the average reference of all channels (Kirkby et al. 2018). Six frequency bands were transformed from the SEEG signals: δ(1–4 Hz), θ(5–8 Hz), α(9–12 Hz), β(13–30 Hz), low_gamma(31–70 Hz) and high_gamma(71–150 Hz).

Multi-scale SEEG data analysis: channel-scale analysis

Channel-scale analysis

Power spectrum density (PSD) refers to the distribution of power per unit of spectrum and serves as a fundamental metric in spectral analysis. One commonly employed method for PSD estimation is the multitaper technique (Babadi and Brown 2014), which utilizes a set of conical windows instead of a single window or spectral window. By calculating a windowed version of each time series, a collection of independent spectral components is obtained, enabling the computation of the complete power spectrum through their averaging. This approach yields robust and accurate results in spectral analysis, contributing to a comprehensive understanding of the frequency characteristics and dynamics within the SEEG signals under investigation. The spectrum of a discrete time mode $x[n]$ with sampling rate F_s, $n = 1, 2, \ldots, N$ is calculated as Equation 1:

$$PSD = \frac{1}{NF_S}\left|\sum_{n=1}^{N} x[n]w[n]^{-j2\pi fn/F_s}\right|^2, \tag{1}$$

where $w[n]$ represents the window function. We use the student-t analysis to examine whether there is a significant difference between these three states: baseline, acupuncture, and resting, and $P < 0.05$(*) is chosen as the threshold in statistical analysis, where $P < 0.001$(**) indicating a more distinct difference. This approach yields robust and accurate results in spectral analysis, contributing to a comprehensive understanding of the frequency characteristics and dynamics within the SEEG signals.

Region-scale analysis

To investigate the effects of acupuncture at the GB34 acupoint on brain connectivity, we employed a brain region-level analysis. Brain connectivity, an essential aspect of functional integration among brain regions, can be quantified by examining both structural and functional connections within the nervous system, as well as their causal interactions. In the field of SEEG connectivity analysis, several prevailing approaches have been utilized, such as phase slope index (PSI), phase locking value, phase lag index, synchronization likelihood, etc. Notably, PSI, as a measure of phase synchronization, initially introduced by Nolte et al. (Nolte et al. 2008), and has been employed to estimate causal direction between two signals, which is defined as

$$PSI = \widetilde{\varsigma}\left(\sum_{f\subseteq F} C_{xy}^{*}(f)\, C_{xy}(f+\delta f)\right), \tag{2}$$

where

$$C_{xy}(f) = \frac{S_{xy}}{\sqrt{S_{xx}S_{yy}}}, \tag{3}$$

is the coherency function between channel X and Y, S is the cross-spectral matrix, δf is frequency resolution, I refers to the imaginary component, and * denotes conjugate transpose.

The PSI metric encompasses values ranging from negative infinity to positive infinity, excluding the possibility of a zero value. By disregarding the sign of PSI values, larger absolute values indicate a more robust causal connection between two channels or brain regions. The positive or negative sign associated with PSI values signifies the directionality of the causal connection. Previous research (Guenot et al. 2001) has demonstrated that the network based on multichannel EEG can effectively reflect the state of the brain. To assess the strength of connections among channels, we calculated PSI for all time points in each epoch across all channels. Utilizing the PSI adjacency matrix as a foundation, we reconstructed brain networks by applying a threshold. This PSI-based adjacency matrix was subsequently normalized, and appropriate thresholds were set to reconstruct a binarized brain network. In the brain networks, each channel was represented as a node. Channels that exceeded a certain threshold were assigned a value of 1 to denote a connection between channels, while those falling below the threshold were assigned a value of 0 to indicate no connection between channels. This process resulted in the formation of binarized adjacency matrices, which allowed for the depiction of brain network connectivity. Furthermore, at the brain region level, we constructed an adjacency matrix by averaging the PSI values across channels within each brain region. This approach facilitated the examination of connectivity patterns at a broader level, taking into account the interactions between channels within specific brain regions. The brain region-level analysis has yielded valuable insights into the impact of acupuncture on connectivity patterns between different brain regions. By revealing the alterations in brain region connectivity induced by acupuncture, our research has advanced the current understanding of acupuncture's role in modulating brain function and communication pathways.

Whole-brain analysis

In order to investigate the global effects of acupuncture on brain activity, a comprehensive whole-brain analysis is performed. In this analysis, brain networks are employed to assess the distinct acupuncture states. To quantify the separation of brain networks, we utilize graph theory parameters, specifically focusing on the clustering coefficient C_i of each node. The clustering coefficient C_i of a node i serves as an effective index to evaluate the extent of local interconnectivity within a brain network. It is defined as the ratio of the number of edges present between the direct neighbors of node i to the total number of possible edges in the vicinity of node *i*. The formula for the clustering coefficient C_i can be expressed as

$$C_i = \frac{2E_i}{k_i(k_i-1)}, \tag{4}$$

where E_i represents the actual number of edges between the direct neighbors of node *i*, and k_i denotes the total number of neighbors of node *i*. The average clustering coefficient of nodes is generally the clustering coefficient of network, which is defined as

$$C = \frac{1}{N}\sum_{i=1}^{N} C_i, \tag{5}$$

where *N* is the quantity of nodes in the brain network. In our analysis, we considered both global and local efficiency metrics to assess the brain network properties. The global efficiency quantifies the ability of information transmission and integration across brain regions by calculating the average of the reciprocal of the shortest path lengths from a specific node to all other nodes in the network, which is defined as

$$E_i = \frac{1}{N(N-1)}\sum_{i,j=1}^{N}\frac{1}{d_{ij}}, \tag{6}$$

where d_{ij} is the shortest path from node i to node *j*. Higher values of global efficiency indicate superior information transmission and integration capabilities among brain regions within the network. By analyzing both global and local efficiency metrics, we gain a comprehensive understanding of the brain network's communication and organization during different acupuncture states at the GB34 acupoint. These metrics contribute to revealing the dynamic changes in brain network properties induced by acupuncture, shedding light on the mechanisms underlying the neurophysiological effects of acupuncture treatment. The small world network (*SWN*) index is a fundamental parameter used to assess whether a given graph G exhibits the characteristics of a small world network, which can be defined as

$$SWN = \frac{E_{glob}}{E_r}\cdot\frac{C}{C_r}, \tag{7}$$

where E_r and C_r are the efficiency and clustering coefficients, respectively, of random networks with the same number of nodes and degree as the original graph *G*. E_{glob} denotes the global efficiency and *C* represents the clustering coefficient of graph *G*. A small world network is characterized by a high *SWN* index, indicating that graph *G* exhibits both a high level of global integration (as reflected by its global efficiency) and a significant degree of local clustering (as captured by its clustering coefficient). These properties are distinctive features of small world networks, which efficiently balances local specialization and global communication. By calculating the *SWN* index for our brain networks during different acupuncture states at the GB34 acupoint, we can identify whether the brain networks exhibit small world characteristics, offering insights into the network dynamics and information processing efficiency during acupuncture treatment.

Results

Analysis of the channel-scale activity

The PSD of the SEEG signal data is initially conducted for the three states: baseline, acupuncture, and resting states. Figure 4(a) shows the PSD of the six frequency bands averaged over all channels. Our findings reveal that the average power values for the δ and β bands are notably higher than the other four bands, and there is consistency with the findings of Yu et al. (Haitao et al. 2018). Specifically, in the δ band, the mean power values across all days and channels are measured at 136.26, 125.01, and 127.38 for the baseline, acupuncture, and resting states, respectively. Compared with the baseline state, a significant power decrease was observed in the acupuncture state, indicating a weaker brain activity in this frequency band as shown in Fig.4(b). The difference between the two states was found to be statistically significant ($P = 0.0041 < 0.05(*)$). The θ, β, α, low_gamma, and high_gamma frequency bands exhibited exhibited no significant changes; therefore, we do not further analyze these aspects. In contrast, the mean power values for all days in the β band are recorded at 65.13, 69.41, and 64.77 for the three different states, respectively. Although there is an increase in power spectral for the acupuncture state compared with the baseline and resting states, indicating stronger brain activity in this band, no statistically significant difference was observed between the states. In the δ band, the significant p-values for seven days were 0.0013636, 0.000006, 0.000003, 0.079249, 0.0000007, and 0.0000001. The seventh day showed the greatest variability, suggesting that the effects of acupuncture over multiple days may be cumulative. The results demonstrate significant changes in SEEG power induced by acupuncture at the GB34 acupoint, with distinct trends observed in distinct frequency bands. Particularly, in the δ band, the power and brain activity decreased when acupuncture was performed. Given these observations, our subsequent statistical analysis focuses on this frequency band and aims to investigate the differences between the baseline and acupuncture states further.

To further analysis the changes in frequency band on different channels, we derive the power spectral values for each channel for both baseline and acupuncture states shown in Fig. 4(c). It represents that in the δ band, most of the channel power spectral is slightly reduced during the acupuncture state. Notably, there are significant changes in the mean power in the δ band for channels A1, B2, B9, B10, D3, and D4 ($P < 0.05(*)$), while more pronounced changes are found for channels A2, A3, A4, A5, A8, A9, B2, B3, B4, B5, B6, B10, D1, and D2 ($P < 0.001(**)$). In Fig. 4(d), the power spectra of the four channels in the paracentral lobule are presented, highlighting distinct differences in the EEG activity before and during acupuncture. Upon investigating the brain regions corresponding to these channels, we find significant changes in four out of the seven brain regions, namely, the paracentral lobule, precentral gyrus, superior postcentral gyrus, and posterolateral paracentral lobule. Functionally, the paracentral lobule, precentral gyrus, superior postcentral gyrus, and posterior lateral paracentral lobule are associated with the somatomotor and somatosensory area. Our analysis demonstrate that acupuncture has significant modulatory effects on both the somatomotor and somatosensory areas, as evident from the comparison of average power values across channels in different states. Furthermore, we observe a consistent trend of change in channels within the same brain region during the same state, while substantial differences are observed between different brain regions.

Analysis of the regional scale

The brain regions showing significant effects included the paracentral lobule, precentral gyrus, superior postcentral gyrus, and posterior lateral paracentral lobule, all of which are known for their roles in somatosensory and somatomotor functions. Thus, in our subsequent connectivity analysis and graph theory analysis, we will prioritize these frequency bands and brain regions for further investigation. In this study, we observed significant modulatory effects of acupuncture on specific brain regions. To gain deeper insights into brain region interactions, we conducted a more in-depth investigation based on connectivity analysis. At channel scale, we explored the interregional connectivity in the δ band. For brain region-level analysis, we calculated the average values for each brain region by averaging the connectivity values across all channels within that region. These average values served as indicators of functional connectivity between brain regions. To determine meaningful connections between brain regions, we applied a threshold of 30%. Connections exceeding this threshold were considered meaningful and indicative of functional connectivity between the corresponding brain regions. Figure 5(a) provides a visual representation of these connectivity patterns. During the 7-day acupuncture intervention, distinct patterns of connectivity emerged among specific brain regions as shown in Figure 5(b). Notably, the paracentral lobule exhibited connectivity with the precentral gyrus, the anterior paracentral lobule displayed connectivity with the superior precentral gyrus, and the posterolateral paracentral lobule demonstrated connectivity with the superior precentral gyrus. Additionally, the precentral gyrus showed connectivity with the inferior precentral gyrus, while the superior postcentral gyrus exhibited connectivity with the superior precentral gyrus. Furthermore, connections were observed between the posterolateral paracentral lobule and the superior frontal gyrus, as well as between the posterolateral paracentral lobule and the paracentral lobule. The identified interregional connectivity patterns, as depicted in Fig. 5(c), lay the groundwork for further investigations into the effects of acupuncture at GB34. Motivated by these findings, we proceeded to perform a comprehensive analysis of the whole-brain connectivity patterns. This analysis aims to provide a comprehensive understanding of the global effects of acupuncture on brain activity and connectivity, shedding further light on the underlying mechanisms of acupuncture's therapeutic effects.

Analysis of the whole-brain activity

In this section, we further explored the mechanisms of acupuncture from a whole-brain network perspective. By examining the C, E_{glob}, and SWN properties in Figure 6, we gained a comprehensive understanding of how acupuncture influences brain network properties. Firstly, we investigated the long-term effects of 7 days of acupuncture. In the baseline state, both the C and E_{glob} showed a significant increase. The C increased from 0.151 to 0.186, while the E_{glob} increased from 0.152 to 0.164. In the acupuncture state, both the C and E_{glob} showed a significant decrease. This suggests that 7 days of acupuncture may have attenuated the inter-connectivity between the seven brain regions, leading to more effective interactions between specific brain regions. These findings indicate that the effective brain networks in the somatosensory and somatomotor areas exhibited small-world characteristics and are highly efficient. Comparing the same features between different states on the same day allows us to investigate the immediate effects of acupuncture. On the first day, the immediate effects of acupuncture at GB34 resulted in

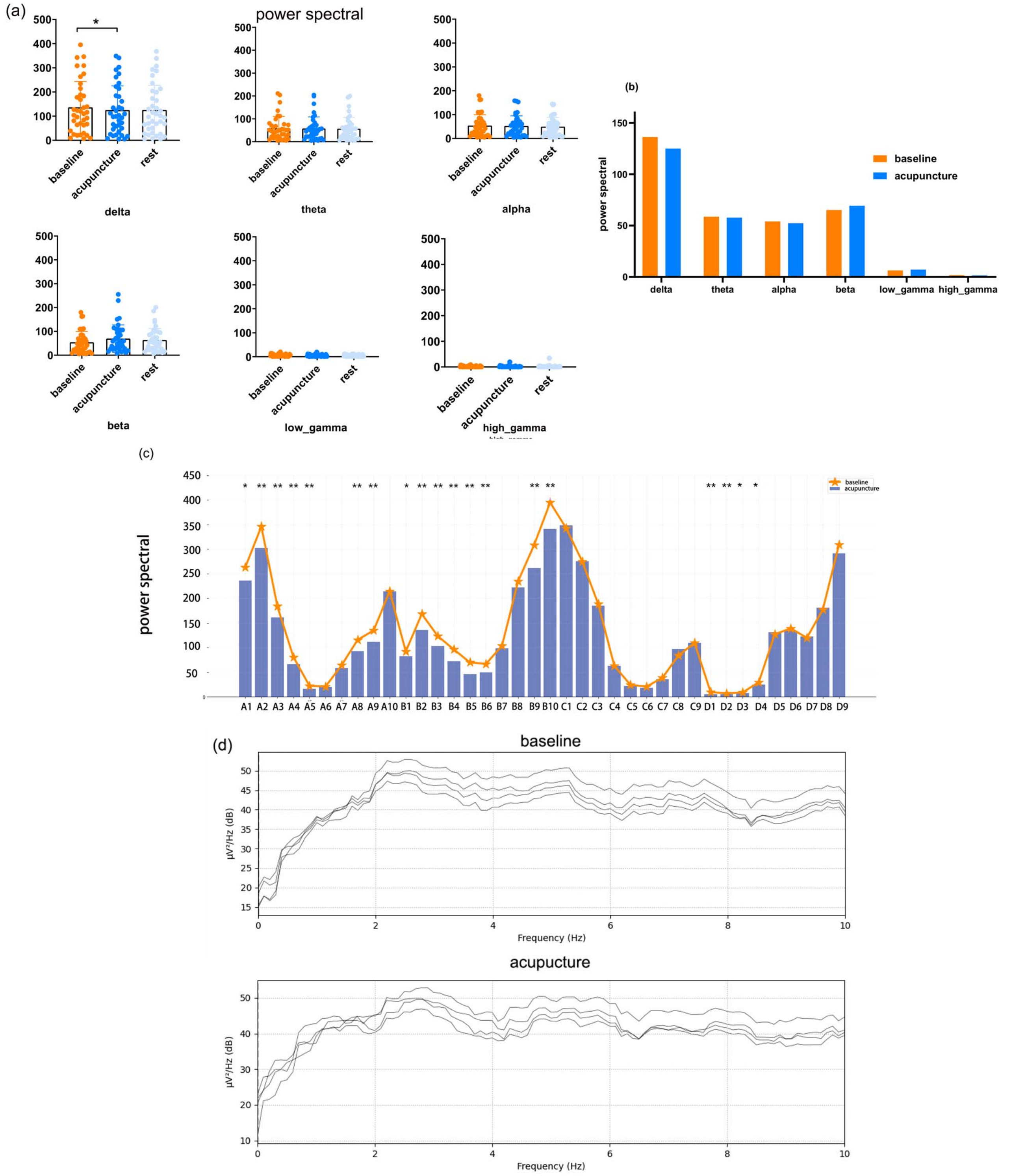

Fig. 4. Power spectral density. (a) The power spectral in the six frequency bands for the three states before, during, and after acupuncture are shown in bar plots, *represents $P < 0.05$, and **represents $P < 0.001$. (b) Power spectral for the two states before and during acupuncture. (c) In the δ band, the power spectral values of each channel in the acupuncture and baseline states. (d) In the δ band, the power spectra of the four channels with significant changes.

an enhancement of the C and E_{glob} throughout the whole brain, and there exhibited significant changes. On the last day, the C decreased and the E_{glob} increased. E_{glob} exhibited significant change, while C did not.

The immediate effects of acupuncture exhibited a notable enhancement in the efficiency of brain region interactions, indicating improved communication and information transfer between different brain regions. This increased efficiency suggests that acupuncture may facilitate more effective coordination and integration of neural activity across the brain. However, it is important to highlight that while the immediate effects improved the efficiency of brain region interactions, they did not necessarily

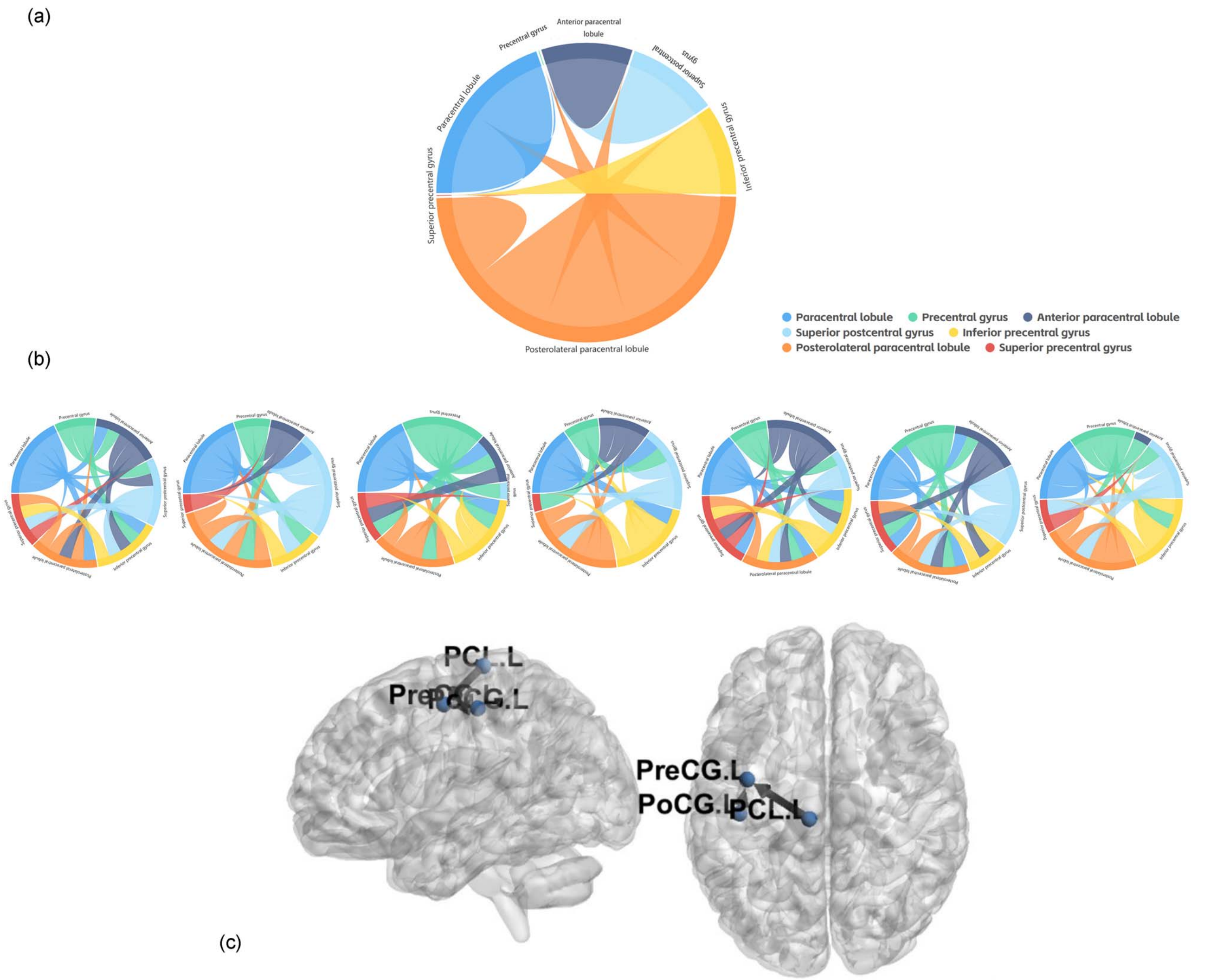

Fig. 5. (a) PSI-based effective brain region network connectivity in the δ band. Chords represent connectivity between brain regions. Each color represents the specified brain region. Common connectivity between brain regions found in the acupuncture across 7 days. (b) Seven days of brain regional connectivity activity in the acupuncture state. Each chordogram consisted of four acupuncture brain region connectivity pairs per day, from left to right for days 1–7. (c) Brain region connectivity during acupuncture state. PreCG.L: precentral gyrus, PoCG.L: postcentral gyrus, PCL.L: paracentral lobule.

result in a concurrent increase in the clustering coefficient. The clustering coefficient reflects the extent to which nodes within a network tend to cluster together, forming local functional modules. In the context of brain networks, a higher clustering coefficient signifies a higher degree of local specialization and segregation of information processing. The lack of significant change in the clustering coefficient suggests that acupuncture's immediate effects may not induce substantial alterations in the local functional specialization of brain regions. Instead, the focus of these effects appears to be on enhancing the overall efficiency of brain region interactions, enabling effective communication and integration of information across the whole-brain network.

These findings contribute valuable insights into the immediate effects of acupuncture on brain network properties, shedding light on its complex and diverse impact on brain activity and connectivity. Understanding how acupuncture modulates brain network properties at different temporal scales is crucial for comprehending its therapeutic potential and advancing our knowledge of its underlying mechanisms. The observed changes in C, E_{glob}, and *SWN* indicate that acupuncture has the potential to modulate the functional organization and efficiency of brain networks in the somatosensory and somatomotor areas.

Discussion

To comprehensively investigate the neuroregulatory effects of GB34 acupuncture, we conducted analyses at three different scales: channel-, regional-, and whole-brain level. The key findings are as follows: (1) acupuncture at GB34 modulates the activity of deep brain regions in the δ band and exhibits long-term effects. (2) The acupuncture treatment mainly modulates the connectivity between the paracentral lobule and the precentral gyrus. (3) The cumulative effects of acupuncture may reorganize the connectivity between the somatomotor and somatosensory areas.

To explore how GB34 acupuncture modulates deep brain activity, we first conducted a channel-level analysis. Significant differences in the δ-band activity were observed between the baseline and acupuncture states. Specifically, acupuncture led to a decrease in brain activity in the δ band compared with the baseline state. This finding is consistent with existing research

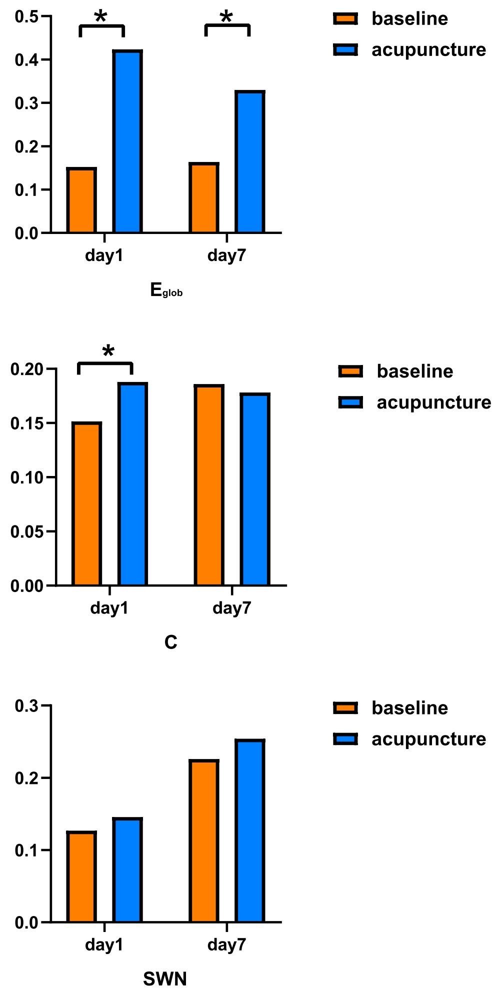

Fig. 6. PSI-based effective brain network connectivity. The figure presents the effective brain network connectivity using the PSI measure, with a threshold of 30% applied to remove insignificant connections. Graph theory features, including E_{glob}, C, SWN are examined to explore the cumulative and immediate effects of acupuncture. Bar charts are used to visualize these features, representing the cumulative and immediate effects of acupuncture. To examine the cumulative effects, we compared the features on the first day (baseline state) and the last day (acupuncture state) of the treatment period. In the acupuncture state, the eigenvalues of the effective brain network connectivity are averaged over the four daily sessions. This averaging process ensures a representative measurement of the acupuncture state, capturing the overall effects of acupuncture throughout the treatment period.

that has demonstrated by Yu et al. (Haitao et al. 2018) the significant modulatory effects of acupuncture on brain activity in the δ and α frequency bands. Mao et al. (2021) find that the EEG power spectrum of the affected side during active exercise was significantly higher than that of the unaffected side. Acupuncture (Nan 2023) can reduce the content of inflammatory factor IL-6 in the blood, inhibit inflammatory reaction, relieve pain, and accelerate nerve repair construction. Therefore, the changes observed in the δ frequency band at acupuncture point GB34 may effectively suppress neuroinflammatory responses in the motor and sensory cortices, consequently improving motor function in patients. The consistency between our findings and prior research adds credibility to our results, and suggests a consistent pattern of acupuncture's impact on brain activity in specific frequency bands. Furthermore, a trend analysis of the average power changes in the δ band over 7 days revealed that the baseline state exhibited higher power on the last day compared with the first day. This suggests that the needles may enhance brain activity in the normal state. Notably, the largest significant difference in brain activity was observed on the seventh day, indicating the cumulative effect of multiple days of acupuncture treatment. This suggests that the neurophysiological effects of acupuncture at GB34 may gradually build over time, resulting in more pronounced changes in brain activity after prolonged intervention. These findings provide valuable insights into the temporal dynamics of acupuncture-induced changes in deep brain activity and underscore the importance of considering the duration of acupuncture treatment when investigating its neurophysiological effects. The observed cumulative effects of acupuncture over seven days highlight its potential as a therapeutic intervention for modulating brain activity and warrant further investigation into the long-term clinical applications of acupuncture at GB34 in various medical conditions.

Additionally, we identified specific and more deeper brain regions that were significantly modulated by acupuncture in the δ band. This provided more detailed findings compared with previous EEG-based studies. EEG-based studies primarily focused on overall brain activity due to data limitations. Earlier research only explored reduced brain activity pre- and post-acupuncture in the δ and α frequency bands by Lin et al. (2021). We found that channels within the paracentral lobule, precentral gyrus, superior postcentral gyrus, and posterolateral paracentral lobule exhibited significant changes in power spectral density. These brain regions are known to be involved in somatomotor and somatosensory functions. Previous studies (Betzel et al. 2012) have shown that acupuncture at GB34 can modulate cortical activity in the somatomotor area. Furthermore, Wang et al. (2022) demonstrated that GB34 acupuncture can also modulate the somatosensory cortex. The observed modulatory effects of acupuncture on these brain regions suggest its potential in modulating somatomotor and somatosensory areas. The identification of specific brain regions influenced by acupuncture provides valuable insights into the neuroanatomical basis of acupuncture's effects. These findings contribute to our understanding of how acupuncture modulates brain activity in targeted regions involved in somatomotor and somatosensory functions. The convergence of our results with previous research adds further support to the notion that GB34 acupuncture has selective effects on brain areas responsible for motor and sensory processing. These findings have potential implications for the clinical application of acupuncture in treating conditions related to motor and sensory dysfunction. Acupuncture at GB34 may be considered as a therapeutic intervention to modulate brain activity and potentially improve motor and sensory function in patients with neurological disorders. However, further investigations are necessary to elucidate the specific neurophysiological mechanisms underlying acupuncture's effects on these brain regions and to explore the clinical applications of acupuncture in diverse patient populations.

Given the significant changes in δ-band activity, we conducted regional-level analyses based on this frequency band. In the regional-level analysis, we examined the interregional connectivity using the PSI metric. By considering the functional properties of brain regions, we investigated the neurobiological mechanisms underlying acupuncture-induced connectivity changes. Previous research by Sasabayashi et al. (2021) highlighted the role of the paracentral lobule in both motor and sensory functions, with a larger representation of motor regions. The precentral gyrus is responsible for the primary motor cortex and serves as the origin of multiple motor pathways as reported earlier (Banker and Tadi 2019). Studies have indicated that unilateral acupuncture can stimulate bilateral regions (Liu et al. 2009) and enhance connectivity in the sensory-motor network in humans (Liu et al. 2016). Acupuncture at GB34 may promote hemiplegic recovery by regulating motor related networks (Chen et al. 2022). When acupuncture at GB34, there are specific target effects in the functional connectivity of brain regions between bilateral supplementary motor area (SMA), dorsal premotor area (PMd), ventral premotor area (PMv), and primary motor cortex (M1), which are of great significance for the reorganization of brain tissue and the remodeling of brain function in stroke hemiplegia (Si et al. 2013). Brain functional reorganization is an important basis for functional recovery or compensation after central nervous system injury, mainly occurring in the cerebral cortex and subcortical structures; The activation of synapses and the utilization of synapses formed by lateral branching have significant implications, as they play a crucial role in the recovery of brain function. The use of acupuncture to stimulate peripheral nerve trunks, muscle motor points, etc., in clinical practice may contribute to the reorganization of brain function. For example, limb motor and sensory disorders caused by encephalopathy (Yuanhao 2024) can be treated by stimulating the brachial plexus nerve stimulation points in the upper limbs, and stimulating the peroneal and sciatic nerves in the lower limbs. At present, research has suggested that GB34 can play anti-inflammatory, analgesic, and promoting synaptic remodeling effects in the peripheral and central regions, respectively. In our study, we found that the main connectivity modulated by acupuncture was between the paracentral lobule and the precentral gyrus, indicating a potential modulation of motor-sensory connections by acupuncture. The observed connectivity changes between the paracentral lobule and precentral gyrus suggest that acupuncture may influence the communication and integration of motor and sensory information within the motor-sensory network. This finding aligns with previous studies demonstrating the role of acupuncture in modulating motor and sensory functions in both healthy individuals and patients with neurological disorders. The specific interaction between the paracentral lobule and precentral gyrus may contribute to the observed changes in δ-band activity and reflect the neurophysiological mechanisms underlying acupuncture-induced effects on motor-sensory processing.

The whole-brain analysis further revealed alterations in network properties induced by acupuncture. By examining the C, E_{glob}, and SWN, we gained a comprehensive understanding of how acupuncture influences brain network properties. The long-term effects of 7 days of acupuncture intervention were evident, leading to changes in the clustering coefficient and global efficiency. The enhanced efficiency of brain region interactions indicates improved communication and information transfer between different brain regions, suggesting that acupuncture may facilitate more effective coordination and integration of neural activity across the brain. Although the immediate effects of acupuncture did not induce substantial alterations in the local functional specialization of brain regions, the focus of these effects appears to be on enhancing the overall efficiency of brain region interactions. Network efficiency is an assessment of information exchange within a network (Rosted et al. 2001). Optimal brain function is achieved through a dynamic balance between global integration and local specialization, characterized by relatively high global and local efficiency (Tononi et al. 1998). Previous studies have revealed reduced communication efficiency at both global and local levels in brain regions following stroke (Siegel et al. 2016). Brain functional reorganization is an important basis for functional recovery or compensation after central nervous system injury. The use of acupuncture to stimulate peripheral nerve trunks, muscle motor points, acupoints, etc., in clinical practice may contribute to the reorganization of brain function. In the whole-brain analysis, we examined the clustering coefficients and information transfer efficiency of the brain network under acupuncture. The immediate effects of acupuncture resulted in decreased clustering coefficients and information transfer efficiency in the whole brain, as well as reduced long-range effects. However, the baseline state exhibited increased clustering coefficients. Given the cumulative effects of acupuncture, multiple days of treatment may enhance the connectivity within the somatomotor network in the normal state. These findings highlight the dynamic and adaptive nature of brain network efficiency under the influence of acupuncture. The immediate effects appear to modulate the efficiency of brain region interactions, promoting effective communication and information transfer across the whole-brain network. However, the observed decrease in clustering coefficients during the immediate effects suggests a potential reorganization of local functional modules, promoting a more globally interconnected network. On the other hand, the cumulative effects of acupuncture, evident after multiple days of treatment, may lead to increased clustering coefficients, indicating enhanced local specialization and segregation of information processing within specific brain regions.

In conclusion, our study provides valuable insights into the neuroregulatory effects of GB34 acupuncture, highlighting its impact on brain activity, connectivity patterns, and network properties. The observed modulatory effects on specific brain regions involved in motor and sensory functions suggest potential therapeutic implications for acupuncture in treating conditions related to motor and sensory dysfunction. The network efficiency sheds light on the neurophysiological effects of acupuncture on brain connectivity and highlights the intricate balance between global integration and local specialization in brain function. The comprehensive analysis at different scales sheds light on the underlying mechanisms of acupuncture and contributes to our understanding of its therapeutic benefits in clinical populations. Further research is warranted to explore the clinical applications of acupuncture and elucidate the specific physiological mechanisms underlying its effects on the brain.

Conclusion

In conclusion, our study provides a comprehensive analysis of the neuroregulatory effects of GB34 acupuncture at multiple scales based on SEEG data. The channel-level analysis revealed significant changes in δ-band activity, which further guided the regional-level and whole-brain analyses. Acupuncture at GB34 exhibited notable modulatory effects on the activity and connectivity of deep brain regions, particularly within the somatomotor and somatosensory areas. The findings provide valuable insights into the potential of acupuncture to modulate brain function and connectivity. The multi-scale approach utilized in this study enabled a comprehensive understanding of the neuroregulatory effects of acupuncture, providing insights into brain activity, interregional connectivity, and whole-brain network properties. Future studies should further investigate the clinical implications and underlying physiological mechanisms of these findings in the context of acupuncture. Exploring the effects of acupuncture in clinical populations and elucidating the specific neural circuits and pathways influenced by acupuncture will advance our understanding of its therapeutic benefits and optimize its integration into clinical practice.

Author contributions

Xiaoyu Chang (Conceptualization, Formal analysis, Investigation, Methodology, Software, Visualization, Writing—original draft, Writing—review & editing), Pengliang Hao (Methodology, Resources), Shuhua Zhang (Investigation, Methodology, Resources), Yunayuan Dang (Methodology, Resources), Aijun Liu (Methodology), Nan Zheng (Conceptualization, Methodology, Writing—original draft), Zhao Dong (Methodology), Hulin Zhao (Conceptualization, Writing—original draft).

Funding

This work was supported by the National Key Research and Development Program of China (Grant 2022YFB4700805).

Conflict of Interests statement: None declared.

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