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Electroacupuncture at HT5 + GB20 produces stronger activation effect on swallowing cortex and muscle than single points

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ABSTRACT

Introduction: This study aimed to investigate the effects of electroacupuncture on cortical activation and swallowing muscle groups. The study examined brain activation in healthy subjects performing swallowing tasks during electroacupuncture. Additionally, the study analyzed electromyographic signals of swallowing muscle groups after electroacupuncture.

Methods: Twenty-seven healthy subjects were randomly separated into three groups. They underwent electroacupuncture at HT5 acupoint (HT5 group), or GB20 acupoint (GB20 group), or HT5 + GB20 acupoint (HT5 + GB20 group) for 30 min of intervention. Subjects performed a swallowing task while receiving electroacupuncture. Functional near-infrared spectroscopy (fNIRS) was used to detect cortical activation and functional connectivity (FC). The mean amplitude values of the swallowing muscle groups after electroacupuncture were also measured. Statistical analysis was used to investigate the differences between the three groups. The protocol was registered with the China Clinical Trials Registry with the registration number ChiCTR2300067457.

Results: Compared with the HT5 group, the HT5 + GB20 group showed higher cortical activation in the LM1 (t = 2.842, P < 0.05) and a tighter FC in the RM1 and LM1 (t = 2.4629, P < 0.05) with considerably increased mean amplitude values of the swallowing muscle groups (t = 5.2474, P < 0.0001). Increased FC was found in the HT5 + GB20 group compared to the GB20 group between the RM1 and RS1 (t = 2.9997, P < 0.01), RM1 and RPM (t = 2.2116, P < 0.05), RM1 and LM1 (t = 3.2078, P < 0.01), RPM and LM1 (t = 2.7440, P < 0.05). However, there were no statistically significant differences in cortical activation or mean amplitude values of swallowing muscle groups.

Conclusion: This study showed that electroacupuncture at HT5 + GB20 acupoints particularly engaged the cerebral cortex related to swallowing, resulting in tighter functional connectivity and higher amplitude values of swallowing muscle groups than electroacupuncture at single acupoints. The results may reveal the mechanism of electroacupuncture for post-stroke swallowing dysphagia.

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1. Introduction

Stroke is a chronic disease that seriously affects the health of people all over the world. According to the Global Burden of Disease Study 2019 [1], there were about 3.94 million new stroke patients in China, and most of them suffered from dysphagia [2]. Dysphagia can lead to aspiration, bronchospasm, dehydration, and malnutrition, and it is associated with a bad prognosis [3]. Currently, there is no specific treatment for dysphagia, but clinical practice confirms that acupuncture is effective [4], and it may be used with electrical stimulation to get even greater outcomes. Acupuncture therapy is safe and effective in the treatment of post-stroke dysphagia (PSD) [5], but few studies have been conducted to investigate the therapeutic mechanisms of electroacupuncture at various acupuncture points for PSD, and the lack of sufficient high-quality evidence has hindered its use [6].

In the theory of Traditional Chinese Medicine, the treatment of dysphagia caused by stroke should based on the principle of waking up the brain, dispelling wind, and opening the orifices to benefit the pharynx. The HT5 acupoint can clear the heart and help the tongue, treating tongue stiffness and speech [7]. The GB20 point is a key point for dispersing all internal and external winds, and acupuncture may dispel the winds and open the orifice to assist the throat [8]. The combination of the HT5 acupoints and the GB20 acupoints can treat post-stroke dysphagia from both central and peripheral perspectives [9]. To investigate the particular impacts of the acupuncture points, the activation effect of HT5 and GB20 alone was compared to the combination of HT5 + GB20.

Non-invasive neuroimaging techniques such as functional magnetic resonance imaging (fMRI) [10,11] are frequently used in current clinical trials to investigate the activation mechanisms of electrical brain targeting. But limitations of fMRI restrict its application, like having to be examined inside a magnetic cavity. For this reason, fMRI can only be used to study resting states or simpler task states, and it is not possible to detect changes in the cerebral cortex during walking, acupuncture, etc. As a noninvasive imaging technique, functional near-infrared spectroscopy (fNIRS) can utilize near-infrared light ($650 \sim 950$ nm) to penetrate the cerebral cortex by $2 \sim 3$ cm [12]. There are two main absorbing chromophores in biological tissues: oxygenated hemoglobin (HbO₂) and deoxy-hemoglobin (HbR), which absorb different kinds of near-infrared light [13]. Therefore, we can use fNIRS to detect changes in the concentrations of HbO₂ and HbR to investigate the functional activities of the cerebral cortex. The fNIRS has benefits such as little restriction on test site or subject's body posture; strong noise resistance; simple pinning operation [14]; it can be used for examination in complex tasks; and its results are highly consistent with fMRI [15]. Currently, it is mainly used to investigate brain remodeling after stroke and less frequently to detect activation changes in the brain during swallowing [16].

In this study, we propose to use fNIRS to investigate brain activation in healthy subjects during electroacupuncture and swallowing tasks. We aim to examine electromyographic signals of swallowing muscle groups after electroacupuncture [17], to reveal electroacupuncture therapeutic mechanisms, and to investigate differences in the effects of different acupuncture points. We hypothesize that the HT5 + GB20 group activates the swallowing-related cerebral cortex more highly and has tighter functional connectivity (FC) than the other two groups. We also hypothesize that nerve impulses descend to the swallowing muscle group, increasing its average amplitude. This study will provide a theoretical basis for the clinical application of electroacupuncture in post-stroke dysphagia treatment.



Fig. 1. | (A) Activation maps of the mean HbO₂ changes under the three conditions. (B) Average changes in HbO₂ concentration of ROIs for three conditions. Comparisons of execution-related HbO₂ changes of ROIs for the HT5, the GB20, and the HT5 + GB20. HbO₂, oxy-hemoglobin; ROIs, regions of interest; R, right; L, left; PFC, prefrontal cortex; M1, primary motor cortex; S1, primary somatosensory cortex PM, premotor area and supplementary motor cortex; *P < 0.05.

2. Results

This study included 27 healthy subjects who underwent electroacupuncture therapies at various acupuncture points. We examined eight brain regions, which are the right and left prefrontal (RPFC/LPFC), primary motor (RM1/LM1), primary somatosensory (RS1/LS1), and premotor and supplementary motor (RPM/LPM) cortices. All subjects completed fNIRS and surface electromyography (sEMG) detection, and no adverse effects were reported. There was no significant statistical difference between the fNIRS and sEMG baseline data of the three groups.

2.1. Cortical activation

The graphic in Fig. 1A depicts the activation maps of mean HbO₂ changes under the three conditions.

One-way repeated measures ANOVA indicated significant differences in mean HbO₂ increases among conditions in one ROI (Fig. 1B): LM1(F = 4.043, P < 0.05). The HT5 + GB20 group had higher cortical activation in the LM1 than the HT5 group (t = 2.842, P < 0.05). The HT5 + GB20 group showed no statistically significant difference in cortical activation compared to the GB20 group. There was no statistically significant difference in mean HbR changes among conditions (Fig. 2).

2.2. Functional connectivity

The FC of the three groups is displayed in Fig. 3. The Pearson correlation coefficient was used to examine the FC in the TS. One-way repeated measures ANOVA indicated a significant difference in FC between RM1 and RS1 (F = 4.1183, P < 0.05), RM1 and LM1 (F = 5.5761, P < 0.05), RM1 and LS1 (F = 4.1601, P < 0.05), and RPM and LM1(F = 3.7978, P < 0.05). The detailed data are shown in Table 1. The FC between RM1 and LM1 is tighter in the HT5 + GB20 group (t = 2.4629, P < 0.05) than in the HT5 group (Fig. 4A). Increased FC was found in the HT5 + GB20 group compared to the GB20 group between RM1 and RS1 (t = 2.9997, P < 0.01), RM1 and RPM (t = 2.2116, P < 0.05), RM1 and LM1 (t = 3.2078, P < 0.01), and RPM and LM1 (t = 2.7440, P < 0.05) (Fig. 4B). There was no statistically significant difference in FC between the HT5 group and the GB20 group.

2.3. Swallowing amplitude

One-way repeated measures ANOVA revealed a statistical difference among the three groups in the swallowing amplitude of the subglottis muscle group (F = 13.7820, P < 0.0001). The HT5 + GB20 group was significantly stronger than the HT5 group (t = 5.2474, P < 0.0001) and the GB20 group was stronger than the HT5 group (t = 2.7719, P < 0.05), while there was no statistically significant difference between the HT5 + GB20 and GB20 groups (Fig. 5).

3. Discussion

The purpose of this study was to observe the activation effect on brain swallowing-related cortices, the FC between brain regions, and the mean amplitude values of swallowing muscle groups after electroacupuncture at the HT5 point, the GB20 point, and the HT5 + GB20 point during electroacupuncture under physiological conditions. The differences between the three groups were analyzed to investigate the potential mechanism of electroacupuncture for PSD and the specificity of the acupuncture point groupings.

The results revealed that the HT5 + GB20 group exhibited increased activation in the LM1 region than the HT5 group. In the comparison of FC, the HT5 + GB20 group was stronger than the HT5 group between the RM1 and LM1. The FC of the HT5 + GB20 group was also stronger than that of the GB20 group, as shown between the RM1 and RS1, the RM1 and RPM, the RM1 and LM1, and the RPM and LM1. Differences in the amplitude values of the swallowing muscle groups were also observed in the three groups after electroacupuncture, indicating that the HT5 + GB20 group was stronger than both other groups.



Fig. 2. | Average changes in HbR concentration of ROIs for three conditions. HbR, deoxy-hemoglobin; ROIs, regions of interest; R, right; L, left; PFC, prefrontal cortex; M1, primary motor cortex; S1, primary somatosensory cortex PM, premotor area and supplementary motor cortex.



Fig. 3. | Functional connectivity maps of the brain generated using NirSpark software for all subjects. The FC has been shown through a color bar from blue to red. The blue color means no connectivity whereas the red color shows maximum connectivity. FC, functional connectivity.

 Table 1

 Comparison of functional connectivity among the three groups.

	HT5 (mean \pm SD)	GB20 (mean \pm SD)	HT5 + GB20 (mean \pm SD)	F-value	p-value
RM1 and RS1	0.8593 ± 0.5075	0.6883 ± 0.4657	1.2799 ± 0.3648	4.1183	0.0289
RM1 and LM1	0.7478 ± 0.4482	0.7265 ± 0.2331	1.2706 ± 0.4523	5.5761	0.0103
RM1 and LS1	0.6487 ± 0.4696	0.5188 ± 0.2804	1.0336 ± 0.4074	4.1601	0.0281
RPM and LM1	0.6823 ± 0.3242	0.6387 ± 0.2195	0.9786 ± 0.2999	3.7978	0.0369

R, right; L, left; M1, primary motor cortex; S1, primary somatosensory cortex; PM, premotor area and supplementary motor cortex.



Fig. 4. | (A) Channels with differences in FC among ROIs in the HT5 + GB20 and HT5 groups. (B) Channels with differences in FC among ROIs in the HT5 + GB20 and GB20 groups (Pearson correlation). FC, functional connectivity; ROIs, regions of interest.



Fig. 5. | Comparison of sEMG values of swallowing muscle groups among the three groups. sEMG, surface electromyography; *P < 0.05; ****P < 0.0001.

There were discrepancies in the activation effects of electroacupuncture on the brain and swallowing muscle groups in this study, which may be related to the anatomical location of the acupuncture sites. The GB20 point is located in the collar, below the occipital bone, between the sternocleidomastoid and trapezius muscles, according to the WHO Standard Acupuncture Point Locations [18]. Some fibers of the sternocleidomastoid muscle descend to the pharynx from the anatomical location, affecting the swallowing muscles; there is a vertebral artery entering the foramen magnum via the posterior atlantooccipital membrane deep in the GB20 point, allowing for deep acupuncture to improve circulation [19]. In addition, as the GB20 point is located close to the medulla oblongata, it is on both sides of the glossopharyngeal and hypoglossal nerves, etc. Thus, electroacupuncture can indirectly stimulate the medulla oblongata or the cerebral cortex, thereby enhancing the blood flow to the cerebral cortex.

Electroacupuncture at GB20 point promotes the expression of transforming growth factor-beta 1 to reduce brain ischemia-induced injury [20]. In a conscious migraine rat model, the electroacupuncture group showed a significant increase in locomotor and feeding/drinking behaviors after electroacupuncture intervention at the GB20 point as compared to the model group. In addition, the mean number of *c*-Fos neurons in the periaqueductal grey, raphe magnus nucleus, and trigeminal nucleus caudalis was significantly reduced in the electroacupuncture group, which showed reduced migraine symptoms [21]. Furthermore, electroacupuncture increases myosin light chain kinase activity in the middle meningeal artery, controlling intracerebral blood flow and confirming its efficacy in the prevention and treatment of acute migraine attacks [22]. The HT5 point is located on the palmar side of the forearm, at the radial edge of the ulnar carpal flexor tendon. It has been found that electroacupuncture of HT5 enhances the activity of the hippocampal-soleus nucleus-vagus pathway, which is associated with swallowing [23]. Shi et al. [24] discovered that acupoint specificity exists for CV23 and GV16 with significantly higher *c*-fos expression than other groups.

The meridian routes of both points may explain why HT5 + GB20 group's activation impact is stronger than that of a single point. HT5 is part of Hand-Shaolin Heart Meridian and branches up from heart conveying pharynx upward and connecting to eye. The GB20

point is a Gallbladder Meridian of Foot-Shaoyang that stretches from outer corner of eye to frontal horn and then downhill to GB20 point behind ear. The HT5 point stimulates linguopharyngeal swallowing muscles whereas GB20 point activates swallowing-related cerebral cortex. When two acupoints are electroacupunctured their effects superimpose resulting in more substantial central and peripheral activation impact. However, further research is needed to determine whether similar benefits occur with coupled electroacupuncture of non-acupuncture locations.

The higher cortical swallowing center network includes several brain regions such as the caudal sensorimotor cortex, anterior insula, premotor cortex, frontal operculum, anterior cingulate and prefrontal cortex, anterolateral and posterior parietal cortex, and precuneus and superomedial temporal cortex [25]. The results of this study showed that the activation of the M1 area was stronger bilaterally in the HT5 + GB20 group than in the HT5 group, but only the LM1 area showed a statistically significant difference, and the connectivity between RM1 and LM1 was stronger. While M1 is actively responsible for initiating downstream motor nerve conduction for voluntary swallowing, and activating and modulating peripheral nerves to control swallowing [26,27]. This suggests that electroacupuncture of HT5 + GB20 is more conducive to activate the M1 area and enhance the nerve conduction in the M1 area bilaterally, thus indirectly raising the mean amplitude of the swallowing muscle groups, and facilitating the initiation stage of swallowing. In a study on mice, Yao et al. [26] discovered a cluster of excitatory neurons in layer V of M1. They were able to improve swallowing performance by regulating the subglottis muscle groups, which is consistent with our findings.

Functional recovery from stroke is based on brain remodeling. Li et al. [28] reported a substantial reduction in infarct volume along with motor function recovery in mice treated with electroacupuncture for cerebral ischemia. In the mice, this was accompanied by increased FC between several brain regions such as the left motor cortex and the left posterior cerebellar lobe, as well as the right motor cortex, the left striatum, and the bilateral sensory cortex. During the rehabilitation of patients with cerebral hemisphere stroke, Huang et al. [29] observed in fMRI that changes in the functional oral intake scale were associated with changes in FC associated with several brain regions including the precuneus ventral default mode network. In this study, we found that the connectivity between RM1 and RS1, RM1 and RPM, RM1 and LM1, and RPM and LM1 was stronger in the HT5+GB20 group than in the GB20 group, but the difference in brain region activation was not statistically significant. This suggests that brain regions in the GB20 group, although activated by electroacupuncture, were not synergistically involved in the swallowing process, and thus there was less neurotransmission and weaker connectivity between brain regions. In contrast, the electroacupuncture HT5+GB20 group was more conducive to the initiation, regulation and completion of the whole swallowing process. This suggests that electroacupuncture HT5+GB20 treatment of PSD patients may more significantly enhance the FC of brain regions and promote brain remodeling in patients, resulting in an improvement of the contractile function of the swallowing muscles.

This study has some limitations such as limited number of fNIRS probes which did not cover all superficial layers of the brain. Furthermore, restricted depth of fNIRS collection makes it difficult to analyze changes in deep brain regions such as cerebellum and brainstem. Electroacupuncture activation effects at GB20 and HT5 points indicate differences in effects of different acupoints on body and combining acupoints can provide better results. In future investigations, fNIRS instrument may be utilized with additional probes and conjunction with other devices to completely investigate therapeutic processes of different acupoints.

4. Conclusion

Our study found that the HT5 + GB20 group specifically activated LM1 and had stronger FC between RM1 and LM1 with higher amplitude values of swallowing muscle groups compared to the HT5 group. The FC between M1 and other brain regions was stronger in the HT5 + GB20 group compared with the GB20 group. These findings suggest that the acupuncture point group is specific and may disclose the mechanism of electroacupuncture for PSD. However, additional research is needed to confirm the remodeling impact after several treatments.

5. Materials and methods

5.1. Participants

This study consisted of 27 healthy subjects (15 males and 12 females), all right-handed and aged 22.4 ± 1.1 years. The subjects were divided into three groups (HT5, GB20, and HT5 + GB20) in a 1:1:1 ratio. The general data of gender and mean age of the subjects in the three groups were statistically evaluated, and there were no statistically significant differences between the groups (P > 0.05). Exclusion criteria included severe pharyngitis, rhinitis, and other organic nasopharyngeal lesions, as well as throat or swallowing disorders; and diagnosis of brain damage, mental illness, or neck injury. All subjects volunteered for this study and completed an informed consent form. The Medical Ethics Committee of Panyu District Central Hospital in Guangzhou, China accepted this study with approval number (PYRC-2022-070).

5.2. Task conditions

The subjects were examined in a peaceful and pleasant atmosphere with a room temperature of 26 °C and a humidity level of less than 80 %. The subjects were instructed to stay calm throughout the test, follow the movements as indicated, and report any signs of discomfort to the investigator. They were also advised not to swallow, cough or vocalize at will.

Electroacupuncture stimulation was delivered to all three groups. For the HT5 group, the subject was seated, the HT5 on both sides were regularly selected and sanitized, and 1 cun milli-needle was used to obtain Qi by stabbing 0.5 cun directly, and 0.5 cun was

stabbed directly at the proximal end 0.2 cun close to the main needle to assist in the energization. The electroacupuncture device was linked to two needles on the same side with a frequency of 10 Hz and a continuous wave, and the intensity was adjusted between 0.2–0.6 mA, which was enough to produce sensation in subjects, and the needles were left in place for 30min. For the GB20 group, the subject was seated, and the GB20 points on both sides were routinely selected and disinfected. The 1.5 cun milli-needle tip was stabbed obliquely 1 cun in the direction of the pharynx to obtain Qi, and 0.5 cun was stabbed directly 0.2 cun from the proximal end of the main needle, which was only used to assist the electroacupuncture instrument to energize. The treatment settings were identical to those used in the HT5 group. For the HT5 + GB20 group, the subject was seated, and the HT5 + GB20 acupoints on both sides were routinely identified and disinfected, followed by acupuncture to obtain Qi, and the two needles on the same side were linked. The treatment settings were identical to those used in the HT5 group.

5.3. Task procedure

The task was selected to be swallowing 3 mL of warm water. The computer would deliver the instruction "Please start swallowing" during the task state (TS), and the experimenter would feed the subject 3 mL of warm water three times, with the subject swallowing normally each time. The computer would give the command "rest" in the resting state (RS), during which the subject will remain quiet and avoid thinking and moving. The baseline was fNIRS data from the first 10 s of resting state. Following the commencement of the test, a cycle of "TS - RS" was done, with each cycle lasting 35 s, for a total of 4 cycles of 150 s. In other words, the task data of swallowing warm water for the first 15 s of each cycle, as well as the rest data for the final 20 s, were collected, with electroacupuncture stimulation throughout. The specific paradigm is shown in Fig. 6.

5.4. fNIRS data acquisition and analysis

The NirSmart-6000 A equipment (Danyang Huichuang Medical Equipment Co., Ltd., China) was used in this study to measure the changes in HbO_2 and HbR concentrations with two types of near-infrared light, including 730 nm and 850 nm, and the data were sampled at 11 Hz. 14 light sources and 14 detectors were used in this equipment, with each source-detector link being a channel, and a total of 35 channels. These channels cover the subjects' cerebral cortex and include bilateral PFC, bilateral M1, bilateralS1, and bilateral PM (Fig. 7). Integration of the region of interest (ROI) channel set was accomplished based on the Brodmann area (BA) and cortical location of each subject. In addition, a black shell was covered over each optical node to insulate it from external light.

Both HbO₂ and HbR were utilized as indicators of hemodynamic changes during local cortical activation [30]. HbO₂ is more responsive to signs of altered cerebral blood flow at the regional level than HbR is, as has been established in prior research [31]. However, Näsi et al. [32] showed that HbO₂ signals are much more influenced by global processes in both the extracerebral and intracerebral compartments than HbR. Therefore, the findings would be more reliable if changes in HbO₂ and HbR were reported.

Preprocessing fNIRS data, as done in prior experiments [33], was accomplished using the NirSpark software package (Danyang Huichuang Medical Instrument Co., Ltd., China). A first review of the raw data was undertaken by an expert who flagged and disregarded signals of low quality. Second, to correct motion artifacts per channel, we utilized a spline interpolation approach on the final signals. One typical technique of adjustment is spline interpolation. One benefit is that it only fixes issues that have already been locally isolated. The raw data were band-pass filtered between 0.01 and 0.2 Hz to eliminate physiological noise. Then, the HbO₂ and HbR data of channels encompassing functionally important locations, namely the eight ROIs, were analyzed further. The relative variations in HbO₂ and HbR concentrations were then calculated using the modified Beer-Lambert equation [34]. The "-10-0 s" was used as a baseline, and then the cycle was repeated four times, with each cycle lasting 35 s, for a total of 140 s. A block average was calculated by averaging the hemodynamic response function from each block paradigm. The HbO₂ and HbR time series data from each pre-treated experimental dataset were analyzed using a generalized linear model. Finally, each subject's hemoglobin time series was retrieved, spanning a consistent 150 s.

Pearson's correlation analysis was performed in NirSpark on the oxygenation time series for each channel to generate the FC matrix. We constructed a correlation matrix with 35 columns and 35 rows for each participant. Due to its normalcy properties, we then



Electroacupuncture Stimulation

Fig. 6. | Experimental procedure. There are three states of fNIRS testing, namely, the baseline, the task state (TS) and the resting state (RS). The first 10 s before the test were taken as the baseline. Then start four tests, each including a 15 s task and a 20 s resting. fNIRS, functional near-infrared spectroscopy.



Fig. 7. | Regions of interest and the channel setting. The probes were located in the RPFC, LPFC, RM1, LM1, RS1, LS1, RPM and LPM. fNIRS, functional near-infrared spectroscopy, R, right; L, left; PFC, prefrontal cortex; PM, premotor area and supplementary motor cortex; M1, primary motor cortex; S1, primary somatosensory cortex.

employed the z matrix for further calculations after having transformed the r-values of the correlation coefficients(r) using Fisher's r-to-z transformation. Then, according to BA, we divided all channels into eight regions of interest: bilateral PFC, bilateral M1, bilateralS1, and bilateral PM. Finally, an 8×8 matrix was created by averaging the z values of the FC matrix independently to assess the functional linkages between and within networks.

5.5. Myoelectric signal of swallowing muscle group

After electroacupuncture, surface electromyography (sEMG) analysis equipment (Thought Technology, Canada) was employed to identify the myoelectric signals of the pharyngeal swallowing muscles. The subject was seated; the skin of the neck was disinfected with alcohol; each electrode was covered with conductive paste; and two electrodes were put bilaterally to the subglottis muscle belly, 2 cm apart. To acquire the average value, the test was repeated three times. The researchers gathered and extracted mean amplitude values; generated filtered and rectified signals; recorded root mean square values of swallowing amplitudes; and compared mean values of two muscle groups.

5.6. Statistical analysis

We used SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) to perform all statistical analyses. The mean and standard deviation of measurement data were used. The Shapiro-Wilk test was used to determine data normality. Changes in HbO₂ and HbR of the three acupuncture groups were utilized to analyze brain activation. The FC was described using Pearson's correlation coefficient.

We used a one-way repeated measures analysis of variance (ANOVA) to compare the cortical activation effect, swallowing muscle group sEMG values, and FC among the three groups. If statistically significant, we performed the Bonferroni-t test to compare the two groups. To rectify the statistical results for numerous comparisons across channels, we used the false discovery rate. In this investigation, the statistically significant threshold was 0.05.

Data availability statement

Data included in article/supp. Material/referenced in article.

Ethics statement

This study was reviewed and approved by the Ethics Committee of Guangzhou Panyu Central Hospital, with the approval number: PYRC-2022-070. The protocol was registered with the China Clinical Trials Registry with the registration number ChiCTR2300067457. All participants provided informed consent to participate in the study.

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Credit author statement

Xuefeng Fu: Performed the experiments; Wrote the paper.Hao Li, Wen Yang, Zhehao Wu: Contributed reagents, materials, analysis tools or data. Xuezheng Li, Lijun Lu: Performed the experiments. Hua Guo, Haoming Xu: Analyzed and interpreted the data. Kaifeng Guo, Zhen Huang: Conceived and designed the experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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