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*Original Article*

# Modulation of subjective and objective indicators in college students with subthreshold depression by TEAS combined with five-tone music therapy

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**Abstract:** Objective: To observe the multimodal regulatory effects of transcutaneous electrical acupoint stimulation (TEAS) combined with Traditional Chinese Medicine (TCM) five-tone music therapy (FTT) on cerebral oxygenation and digital tongue-pulse parameters in college students with subthreshold depression (SD) of the liver stagnation and spleen deficiency type. Methods: Ninety eligible college students with SD were randomized into control, TEAS, and TEAS+FTT group (n = 30 each). The control group received general mental health education. The latter two groups received TEAS, with the TEAS+FTT group additionally listening to TCM liver-soothing and spleen-fortifying music (30 minutes/session, twice weekly for 4 weeks). Outcome measures included Hamilton Depression Rating Scale-17 (HAMD-17) scores, TCM syndrome scores, prefrontal Oxy-Hb levels, and digitalized tongue and pulse parameters. Results: No baseline differences existed ($P>0.05$). After treatment, compared with the control group, the TEAS+FTT group showed greater pre-post difference in HAMD-17 and TCM syndrome scores ($P<0.05$), and significant increases in the left prefrontal Oxy-Hb slope (S17-D13, S20-D13 channels), tongue coating RGB values, left-hand H1 and right-hand Ass pulse wave value ($P<0.05$). Compared with the TEAS group, the TEAS+FTT group exhibited lower TCM syndrome scores ($P<0.05$) and significantly higher left prefrontal Oxy-Hb slope (S17-D13, S20-D13), tongue coating R-value, and right-hand Ass pulse wave value ($P<0.05$). Conclusion: TEAS plus five-tone music therapy more effectively improves cerebral oxygenation, enhance visceral functional status, and alleviate depressive symptoms in college students with SD of the liver stagnation and spleen deficiency type compared to TEAS alone.

**Keywords:** Subthreshold depression, transcutaneous electrical acupoint stimulation, traditional Chinese medicine five-tone music therapy, functional near-infrared spectroscopy, digital tongue and pulse diagnosis

## Introduction

Subthreshold depression (SD) refers to a pre-depressive state characterized by core depressive symptoms such as low mood and loss of interest, lasting for more than two weeks, but with an insufficient number of symptoms to meet the diagnostic criteria for depressive disorder [1]. Reportedly, at least 11.02% of the global population suffers from SD, with a prevalence rate as high as 14% among young adults [2]. Among these individuals, 12% are reported to develop depressive disorder within two years, a condition associated with low remission rates and high mortality risk [3, 4]. Over the past few years, increasing research on depressive disorder and SD has revealed that patients with depressive disorder exhibit reduced functional connectivity and gray matter volume in the dorsolateral prefrontal cortex [5], while no irreversible brain damage has been observed in individuals with SD [6]. Therefore, the SD phase has emerged as a critical window for preventing the progression from SD to depressive disorder.

Regarding the evaluation and diagnosis of SD, clinical practice still primarily relies on depres-

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sion scales, which presents strong subjectivity and insufficient consistency. Functional near-infrared spectroscopy (fNIRS), a technique capable of monitoring cerebral cortical hemodynamics changes induced by neural activity, may serve as a novel auxiliary tool for assessing the status of SD. Current clinical treatments for SD often include cognitive behavioral therapy and anti-depressant medication. However, ensuring both the effectiveness of and adherence to these treatments remains challenging. In traditional Chinese medicine (TCM), SD is categorized under the "depression syndrome" (yu zheng), with the core pathogenesis identified as liver stagnation and spleen deficiency. Previous studies have revealed that transcutaneous electrical acupoint stimulation (TEAS) and TCM five-tone music therapy (FTT) can, to some extent, alleviate depressive symptoms [7-9]. However, the synergistic regulatory effects of TEAS plus TCM five-tone music on SD of the liver stagnation and spleen deficiency type, as well as their impact on cerebral neurophysiology, remain unclear. This study employed commonly used depression scales and TCM syndrome scores for evaluating SD of the liver stagnation and spleen deficiency type, in conjunction with fNIRS, and integrated digital tongue and pulse diagnosis. It aimed to compare and investigate the intervention effects of TEAS alone versus TEAS combined with five-tone music therapy on SD of the liver stagnation and spleen deficiency type, as well as their regulatory effects on cerebral neurophysiology, so as to provide a multimodal objective evaluation basis and effective intervention strategies for SD.

## General data

### *Study population*

Participants were recruited from Beijing University of Chinese Medicine. Ninety eligible college students with SD were randomly divided into three groups (n = 30 each): control group, TEAS group, and TEAS+five-tone music therapy group (TEAS+FTT group).

### *Ethical approval and consent to participate*

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Medical Ethics Committee of Beijing University of Chinese Medicine (Approval No.: 2023BZYLL-1412) and has been registered with the International Traditional Medicine Clinical Trial Registry (Registration No.: ITMCTR2024000-1760). Prior to participation in this trial, all participants were informed of the study procedures, potential risks, and their rights. Written informed consent was obtained from each participant prior to any study-related procedure. Participants were assured of their right to withdraw from the study at any time without penalty.

### *Diagnostic criteria*

The Western diagnostic criteria for SD were based on the *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.) (DSM-5) [10]. Participants were required to present with the core depressive symptoms including depressed mood and diminished interest or pleasure for most of the day, nearly every day, for at least two weeks. This had to be accompanied by at least two of the following symptoms: changes in appetite, sleep disturbances, fatigue or loss of energy, diminished ability to think or concentrate, feelings of worthlessness or hopelessness. Crucially, the symptom profile did not meet the full diagnostic criteria for depressive disorder as outlined in the DSM.

The TCM diagnosis of depression syndrome was based on the diagnostic criteria outlined in the *Guidelines for the Diagnosis and Treatment of Depressive Disorders by Integrating Chinese and Western Medicine* [11]. Participants were required to present with the primary symptoms of depression syndrome, including melancholy, distress, and emotional restlessness, for most of the day, nearly every day, for at least two weeks. This had to be accompanied by at least two of the concurrent symptom characteristics of the liver stagnation and spleen deficiency, including chest tightness or oppression, excessive worry and suspicion, frequent sighing, loss of appetite, weight loss, fatigue, epigastric stuffiness and belching, or irregular bowel movements.

### *Inclusion criteria*

(1) Aged 18 to 30 years, any gender, right-handed; (2) Meeting the aforementioned diagnostic criteria of both Western medicine and TCM; (3) Not meeting the diagnostic criteria for depressive disorder according to the DSM-5, as determined by the Mini-International Neuropsychiat-

ric Interview (MINI version 5.0), and having no suicidal tendencies; (4) With Hamilton Depression Rating Scale (HAMD-17) scores >7 and <17; (5) Had not received any depression-related treatment in the past 6 months; (6) Providing informed consent and voluntarily participating.

*Exclusion criteria*

(1) Meeting DSM-5 criteria with mood disorders, bipolar depression, psychotic disorders, etc.; (2) Having a history of mental illness, organic mental disorders, or depression caused by psychoactive substances or non-addictive substances; (3) Having any significant medical or neurological condition, history of head trauma, or pregnancy; (4) Currently participating in other clinical trials that could influence the results of this study.

*Discontinuation criteria*

(1) Having poor compliance and failing to receive the intervention as prescribed; (2) Occurrence of special physiological or pathological changes that make it inappropriate to continue the trial.

## Methods

*Sample size calculation*

Based on the results of preliminary experiments for this study, the post-treatment depression scale scores for the TEAS+FTT and TEAS groups, expressed as mean ± standard deviation, were 5.63 ± 2.615 and 7.79 ± 3.240, respectively. The sample size was calculated using the superiority test formula for comparing two means. A two-sample two-sided t-test assuming equal variance was employed, with a Type I error rate (α) of 0.025 (one-sided). To detect a difference of μ1 - μ2 = 2.16 with 90% power, it was determined that 25 participants were required per group. Accounting for a 20% dropout rate, a total of 90 participants (30 per group) are needed.

$$n = \frac{(Z_{\alpha} + Z_{\beta}) * 2\sigma^2}{\delta^2}$$

*Randomization, allocation, and blinding*

This study employed a block randomization design. Participants were randomly assigned to three groups in a 1:1:1 ratio, with a block size of 6. The random sequence was generated using SPSS 24.0. Due to the nature of the TCM five-tone music therapy used in this study, blinding of the participants and treating physicians was not feasible. However, blinding was implemented for the data collectors and statistical analysts.

*Intervention methods*

*Control group:* The control group received general mental health education, including the provision of basic knowledge about depressive disorder and SD to enhance treatment confidence, analysis of the predisposing factors for SD of the liver stagnation and spleen deficiency type, guidance on preventing disease aggravation, recommendations on diet and psychological adjustment, encouragement of emotional expression, and reinforcement of positive behaviours and emotional feedback. Each participant received one session lasting 10 minutes.

*TEAS group:* Following the enrolment, the participants received general mental health education as described above. Additionally, they underwent TEAS using a YINGDI KWD-808I electronic pulse therapy device. Circular electrode pads (2 cm in diameter) were bilaterally attached to the following acupoints: Neiguan (PC6), Shenmen (HT7), Zusanli (ST36), and Taichong (LR3).

Neiguan (PC6): On the palmar aspect of the forearm, 2 cun proximal to the wrist crease, between the tendons of palmaris longus and flexor carpi radialis.

Shenmen (HT7): On the wrist, at the ulnar end of the transverse crease of the wrist, in the depression on the radial side of the flexor carpi ulnaris tendon.

Zusanli (ST36): On the anterior aspect of the lower leg, 3 cun inferior to the lower border of the patella, one finger-width (middle finger) lateral from the anterior crest of the tibia.

Taichong (LR3): On the dorsum of the foot, in the depression distal to the junction of the first and second metatarsal bones.

Stimulation was delivered using a dense-disperse wave pattern at a frequency of 2/100 Hz, with an intensity of 10 mA [12], adjusted to each participant's tolerance level. Each treatment session lasted 30 minutes and was administered twice weekly for 4 weeks in total.

*TEAS+FTT group:* Participants received general mental health education, TEAS (identical to the TEAS group protocol), and TCM five-tone music therapy. The music was delivered using a spatial field playback device (Beijing Wuyin Inheritance Culture Co., Ltd.) consisting of six speakers positioned around the participant according to the five-element directional correspondences, ensuring balanced whole-body sound exposure and resonance. The specific tracks were Caomu Qingxin (mode: Jue, for the liver) and Chunhui Dadi (mode: Gong, for the spleen). Each track lasts 30 minutes, and the two tracks were used alternately per session. The volume was set at approximately 60 dB, adjusted to a level at which the participant could feel slight vibration on the back.

Each treatment session lasted 30 minutes and was administered twice weekly for 4 weeks in total.

*Outcome measures*

*Primary outcome measure:* The primary outcome measure of this study was the HAMD-17 score from baseline to post-treatment (pre-post difference). The scale employs a five-level scoring method: none (0 points), mild (1 point), moderate (2 points), severe (3 points), and extremely severe (4 points), with a total score ranging from 0 to 68.

*Secondary outcome measures:* (1) TCM syndrome score: The TCM syndrome score utilizes a four-level scoring method: none (1 point), mild (2 points), moderate (3 points), and severe (4 points), with a total score ranging from 23 to 92.

(2) fNIRS measurement indicators: A NirSmart-6000A portable 63-channel fNIRS device (Danyang Huichuang Medical Equipment Co., Ltd.) was used to assess participants. The verbal fluency task (VFT) paradigm was employed to measure and record oxygenated hemoglobin (Oxy-Hb) levels in the prefrontal cortical channels. The total acquisition duration was 5 minutes, with wavelengths of 760 nm and 850 nm and a sampling rate ≥70 Hz. A total of 14 source-detector (S-D) channels were configured in the frontal probe.

Verbal Fluency Task (VFT) paradigm: A block design (rest-task alternation) was used. During the baseline (rest) period, participants repeatedly counted "1, 2, 3, 4, 5" for 30 seconds while remaining relaxed and awake. The task period required participants to form words using given Chinese characters for 20 seconds per trial. One practice cycle was used to familiarize participants and reduce anxiety. After a 30-second rest, the formal test began, consisting of a 30-second baseline, a 60-second task (three consecutive word-formation trials, each for 20 seconds), and a 30-second post-task recovery. Total acquisition time was 5 minutes. Participants were seated and instructed to avoid excessive head or body movement.

(3) Objective tongue diagnosis indicators: A Daosh TCM Smart Screen device (Shanghai Daosh Medical Technology Co., LTD.) was used to capture and extract the values of each color channel in the RGB color space for the tongue body and tongue coating of the subjects before and after treatment.

(4) Objective pulse diagnosis indicators: A DS01-C Pulse Diagnosis Information Acquisition System (Shanghai Daosh Medical Technology Co., LTD.) was employed. The time-domain analysis method was used to segment the pulse graph. Bilateral pulse diagnosis information was collected from the subjects before and after treatment to extract characteristic data, and the characteristic values of the pulse manifestations were analyzed among the three groups.

*Statistical analysis methods*

fNIRS data processing: Raw fNIRS signals were converted to optical density using standard preprocessing. A dual-correction strategy was applied: baseline drift was compensated using cubic spline interpolation, and motion artifacts were detected and corrected with a channel-specific Savitzky-Golay smoothing algorithm. A bandpass filter (0.01-0.2 Hz) was used to remove interference from heartbeat, respiration, and body movement. The modified Beer-Lambert law was then applied to convert optical signals into oxygenated hemoglobin (Oxy-Hb) parameters. Three features were extracted: baseline mean Oxy-Hb, task-induced change, and the slope of concentration change.

Normally distributed data are presented as mean ± standard deviation ($\bar{x} \pm sd$), while

**Table 1.** Comparison of general information among the three groups (*n* = 30)

| | TEAS+FTT group | TEAS group | Control group | $F/\chi^2$ | *P* |
|---|---|---|---|---|---|
| Cases | 30 | 30 | 30 | | |
| Sex (male/female) | 6/24 | 5/25 | 5/25 | 0.152 | 0.927 |
| Age | 22.33 ± 2.70 | 22.60 ± 2.28 | 23.50 ± 2.62 | 1.737 | 0.182 |

**Table 2.** Comparison of total HAMD scores among the three groups before and after treatment (points, $\bar{x}$ ± sd; *n* = 30)

| | TEAS+FTT group | TEAS group | Control group | *F* | *P* |
|---|---|---|---|---|---|
| Before treatment | 13.60 ± 2.13 | 12.47 ± 2.37 | 12.23 ± 2.92 | 2.573 | 0.082 |
| After treatment | 5.77 ± 2.50$^{**}$ | 6.57 ± 2.70$^{**}$ | 10.23 ± 2.56 | 25.435 | <0.001 |
| pre-post difference | 7.83 ± 2.48$^{*,\#\#}$ | 5.90 ± 2.64$^{*}$ | 1.00 ± 2.10 | 63.636 | <0.001 |

Notes: $^{*}P<0.05$ *vs*. the control group at the same time point; $^{**}P<0.01$ *vs*. the control group at the same time point; $^{\#\#}P<0.01$ *vs*. the TEAS group at the same time point.

those not conforming to a normal distribution are described by median (quartiles) [*M* ($P_{25}$, $P_{75}$)]. Counting data are presented as frequencies.

SPSS 26.0 software was adopted for analyzing the scale data and the objective digital tongue and pulse diagnosis data. All continuous data were tested for normality using the Shapiro-Wilk test, and all variables were found to be normally distributed (P>0.05). Accordingly, one-way ANOVA was used for intergroup comparisons. For variables with significant differences, post-hoc pairwise comparisons were conducted using the Least Significant Difference (LSD) method. Considering the relatively small sample size, the LSD method was used for post-hoc pairwise comparisons. To further control for Type I error, the Bonferroni correction was also applied, and the pattern of significance remained consistent. The Pearson Chi-square test was performed. fNIRS data were preprocessed using NirSpark 1.8.1 software to ex tract blood oxygen activation parameters for each channel. Subsequently, using each channel as a node, a correlation analysis was performed between the brain functional activation levels of all channel pairs. For all statistical analyses, $\alpha = 0.05$ was set as the significance level, and $P<0.05$ indicates a notable difference.

## Results

### *Comparison of general information among the three groups*

No participants withdrew from the study. No notable difference was found in sex and age among the three groups ($P>0.05$), indicating comparability (**Table 1**).

### *Comparison of HAMD scores among the three groups*

Before treatment, no notable difference existed in HAMD scores among the three groups ($P>0.05$), indicating comparability. After a 4-week treatment, the TEAS+FTT and TEAS groups showed notably decreased HAMD scores (pre-post difference) in contrast to the control group ($P<0.001$), with a significantly greater decrease in the TEAS+FTT group than the TEAS group ($P<0.001$, **Table 2**).

### *Comparison of TCM syndrome scores among the three groups before and after treatment*

Before treatment, the three groups showed no notable difference in TCM syndrome scores ($P>0.05$), indicating comparability. After treatment, the TEAS+FTT group showed notably lower TCM syndrome scores than the control and TEAS groups ($P<0.05$), while no notable difference was found between the latter two groups ($P>0.05$). The TEAS+FTT group showed more notable decrease in scores than the TEAS and control groups ($P<0.001$), with no notable difference between the latter two groups ($P>0.05$, **Table 3**).

### *Comparison of fNIRS results among the three groups*

According to observation of average brain oxygenation activation levels in the three groups after hemoglobin conversion, before treatment,

**Table 3.** Comparison of TCM syndrome scores among the three groups before and after treatment (score, $\bar{x}$ ± sd; *n* = 30)

| | TEAS+FTT group | TEAS group | Control group | *F* | *P* |
|---|---|---|---|---|---|
| Before treatment | 40.80 ± 4.49 | 38.63 ± 4.06 | 36.80 ± 3.38 | 2.201 | 0.117 |
| After treatment | 32.40 ± 2.51$^{**,\#\#}$ | 33.27 ± 3.97 | 35.33 ± 3.72 | 6.745 | 0.002 |
| pre-post difference | 8.40 ± 3.91$^{**,\#\#}$ | 4.73 ± 3.91 | 4.47 ± 3.33 | 10.416 | <0.001 |

Notes: $^{**}$*P*<0.01 *vs*. the control group at the same time point; $^{\#\#}$*P*<0.01 *vs*. the TEAS group at the same time point.

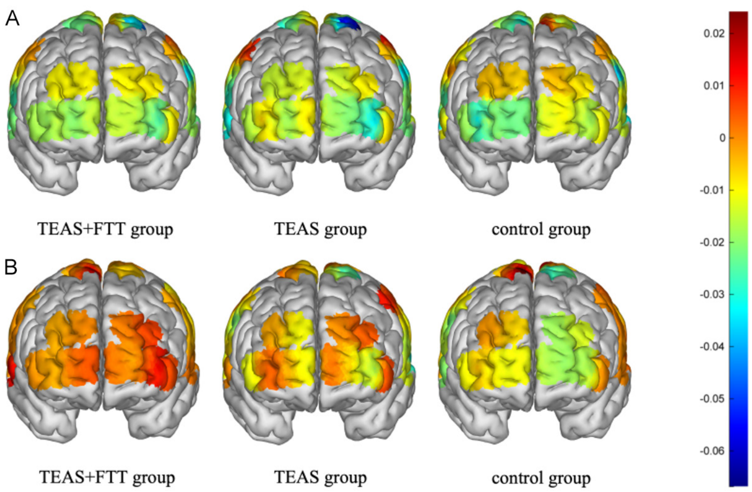


**Figure 1.** Comparison of fNIRS brain activation maps before and after treatment in the three groups. A: Pre-treatment; B: Post-treatment. The mean concentration of oxygenated hemoglobin across the brain is reflected by the color scale. Warm hues (yellow to red) indicate increased neuronal activity accompanied by a rise in oxygenated hemoglobin; cool hues (green to blue to purple) indicate inhibition of neuronal activity.

the activation levels across the brain were relatively low in all three groups, while after treatment, the TEAS+FTT and TEAS groups showed relatively higher average prefrontal oxygenation activation levels than the control group, with the TEAS+FTT group showing higher overall activation levels than the TEAS group (**Figure 1**).

Before treatment, Oxy-Hb slopes in cortical venous blood flow across all channels showed no notable difference among the three groups (*P*>0.05), indicating comparability. After treatment, the TEAS+FTT group presented notably higher Oxy-Hb slopes on channels S17-D13 and S20-D13 during task performance than the TEAS and control groups (*P*<0.05), while no notable differences were found in the Oxy-Hb slopes of the remaining channels. The differential indicators of Oxy-Hb slopes among the three groups after treatment are displayed in **Table 4** and **Figure 2**.

Visual comparison of the whole-brain functional connectivity maps (**Figure 3**) showed that connectivity was relatively low in all groups before treatment. After treatment, both the TEAS+FTT and TEAS groups exhibited increased connectivity, particularly in frontotemporal regions, with the TEAS+FTT group showing a visually higher overall connectivity than the TEAS group. However, statistical analyses of network property metrics revealed no significant differences between the two treatment groups. Therefore, **Figure 3** is presented as a descriptive illustration rather than a statistically supported finding.

*Comparison of objective tongue diagnosis indicators among the three groups*

Before treatment, no notable difference was found in the RGB values of tongue color and coating color among the three groups (*P*>0.05), indicating comparability. After treatment, in contrast to the control group, the TEAS+FTT and TEAS groups presented notably increased R, G, and B values of coating color (*P*<0.001); compared with the TEAS group, the TEAS+FTT group presented notably increased R value (*P*<0.05); there were no notable differences in the R, G, and B values of tongue color among the three groups before and after treatment. The comparison of coating color R, G, and B values among the three groups is displayed in **Table 5**, and typical tongue manifestations of the two treatment groups before and after treatment are presented in **Figure 4** (within-group comparisons were based on images from the same subject).

**Table 4.** Comparison of Oxy-Hb slopes in differential frontal lobe channels among the three groups before and after treatment ($\bar{x}$ ± sd; *n* = 30)

| | S-D channel | TEAS+FTT group | TEAS group | Control group | *F* | *P* |
|---|---|---|---|---|---|---|
| Before treatment | S17-D13 | 0.000 ± 0.004 | -0.001 ± 0.003 | -0.001 ± 0.004 | 1.366 | 0.557 |
| | S20-D13 | 0.000 ± 0.003 | -0.002 ± 0.003 | -0.001 ± 0.003 | 2.953 | 0.382 |
| After treatment | S17-D13 | 0.000 ± 0.003*,# | -0.001 ± 0.003 | -0.001 ± 0.002 | 8.202 | 0.011 |
| | S20-D13 | 0.002 ± 0.004*,# | -0.001 ± 0.004 | -0.001 ± 0.003 | 8.668 | 0.011 |

Notes: *$P<0.05$ *vs*. the control group after treatment; #$P<0.05$ *vs*. the TEAS group after treatment.

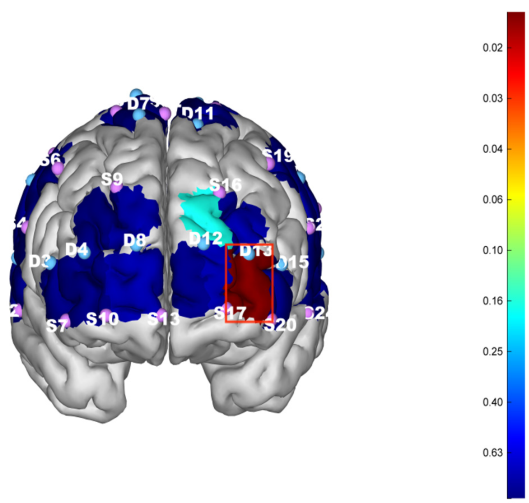


**Figure 2.** One-way ANOVA P-value map for inter-group comparison of post-treatment frontal Oxy-Hb slopes. Color scale denotes channel-wise *P*-values from one-way ANOVA examining Oxy-Hb slope differences among the three groups post-treatment. The color bar on the right provides the *P*-value scale. Red colors in the red square indicate significant inter-group differences in Oxy-Hb slope for a given channel ($P<0.05$), while cooler colors (green to blue to purple) indicate no statistically significant difference.

*Comparison of objective pulse diagnosis indicators among the three groups*

Parameters of pulse waveform height (H1-H5), width (T), and area (Ass, Ad) were compared among the three groups. Before treatment, there existed no notable differences in the characteristic values of left and right hand pulses across all groups ($P>0.05$). After treatment, the TEAS+FTT and TEAS groups showed notably higher left hand H1 values than the control group ($P<0.05$); the TEAS+FTT group presented notably higher right hand Ass value than the control and TEAS groups ($P<0.05$). No notable differences were found in the remaining indicators (H2-H5, T, Ad) among the three groups. The differences in characteristic values of pulse waveforms are shown in **Table 6**. Typical pulse waveforms for within-group comparison before and after treatment are shown in **Figure 5** (within-group comparisons were based on images from the same subject).

## Discussion

SD falls within the category of "depression syndrome" in TCM. A study analyzing the distribution patterns of TCM syndrome elements in depression found that liver stagnation and spleen deficiency are the most common syndrome of this disease [13]. Previous evaluations of SD have predominantly relied on subjective scale assessments. TCM offers certain advantages in treating SD. In particular, TEAS and TCM five-tone music therapy have demonstrated efficacy in treating depression and are clinically convenient to administer. In this study, we applied TEAS at four acupoints (Neiguan, Shenmen, Zusanli, Taichong) - commonly used to soothe the liver, harmonize the spleen and stomach, promote the generation and transformation of qi and blood, and calm the mind [14] - either alone or combined with five-tone music therapy. We observed that both interventions alleviated depressive symptoms, and the combined therapy produced additional benefits in TCM syndrome scores, prefrontal oxygenation, and objective tongue-pulse parameters, suggesting a broader regulatory effect on functional status.

TEAS can stimulate the aforementioned acupoints to activate the circulation of meridian qi and blood [15], regulate brain neural activity, and thereby produce a mitigating effect on depressive mood [16, 17]. The TCM five-tone music therapy, based on the principle of "resonance with the same tone", regulates the functions of the five viscera through the vibration of scale frequencies [18], and has been shown to alleviate depressive and anxiety symptoms [19-21]. In this study, after receiving TEAS or TEAS plus five-tone music therapy, college stu-

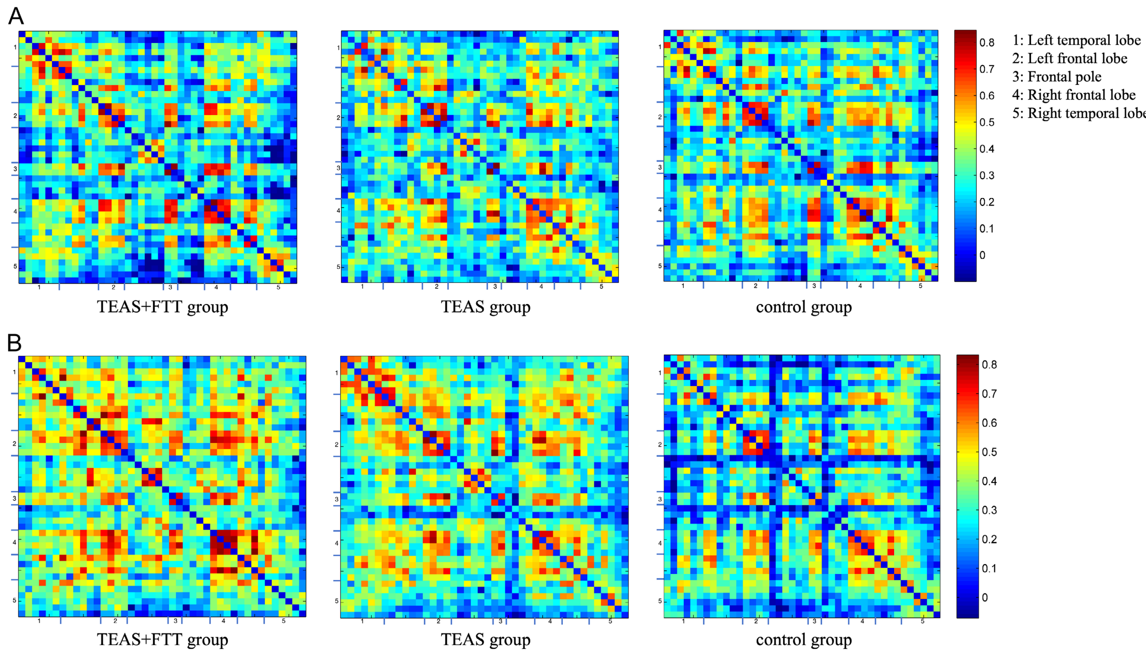


**Figure 3.** Comparison of pre- and post-treatment fNIRS brain network connectivity maps across the three groups. A: PRe-treatment; B: Post-treatment. Each square's color scale reflects the functional connectivity strength between the two channels indicated on the horizontal and vertical axes. The positions of channel clusters corresponding to different brain regions are marked with numbers 1, 2, 3, 4, and 5 on the axes. Warm colors indicate enhanced connectivity, and cool colors indicate reduced connectivity, reflecting the level of interregional collaboration. This figure is for visual comparison only; statistical analyses of network metrics do not show significant differences between the TEAS+FTT and TEAS groups.

**Table 5.** Comparison of objective tongue diagnosis indicators among the three groups before and after treatment ($\bar{x}$ ± sd; *n* = 30)

| | Indexes | | TEAS+FTT group | TEAS group | Control group | *F* | *P* |
|---|---|---|---|---|---|---|---|
| Before treatment | Coating color | R | 178.53 ± 28.28 | 171.40 ± 20.67 | 180.47 ± 27.19 | 1.043 | 0.357 |
| | | G | 160.13 ± 27.33 | 156.40 ± 23.15 | 159.47 ± 26.65 | 0.179 | 0.836 |
| | | B | 165.07 ± 25.58 | 161.00 ± 20.04 | 166.93 ± 27.31 | 0.460 | 0.633 |
| After treatment | Coating color | R | 201.60 ± 24.83$^{**,\#\#}$ | 179.13 ± 20.26$^{*}$ | 170.67 ± 30.86 | 20.612 | <0.001 |
| | | G | 187.20 ± 23.83$^{**}$ | 161.63 ± 22.25$^{**}$ | 155.87 ± 33.98 | 16.430 | <0.001 |
| | | B | 191.73 ± 25.34$^{**}$ | 168.00 ± 25.75$^{**}$ | 161.13 ± 33.40 | 15.627 | <0.001 |

Notes: $^{*}$*P*<0.05 *vs*. the control group after treatment; $^{**}$*P*<0.01 *vs*. the control group after treatment; $^{\#\#}$*P*<0.01 *vs*. the TEAS group after treatment.

dents with SD exhibited reduced HAMD scores, indicating a certain regulatory effect of both therapeutic approaches on depressive symptoms such as low mood and loss of interest. Further analysis showed that TEAS plus five-tone music therapy was significantly superior to TEAS alone in improving TCM syndrome scores for SD of the liver stagnation and spleen deficiency type. These findings suggest that TEAS plus five-tone music therapy can ameliorate liver stagnation and spleen deficiency symptoms, such as chest tightness, epigastric stuffiness, and belching, in college students with this syndrome type, thereby enhancing their physical functional status.

fNIRS enables the quantification of dynamic changes in cortical Oxy-Hb and allows for the reverse inference of cortical neuronal activation patterns based on neurovascular coupling mechanisms. To a certain extent, this technique can provide relatively objective evidence for assessing depressive states [22, 23]. According to previous studies, patients with

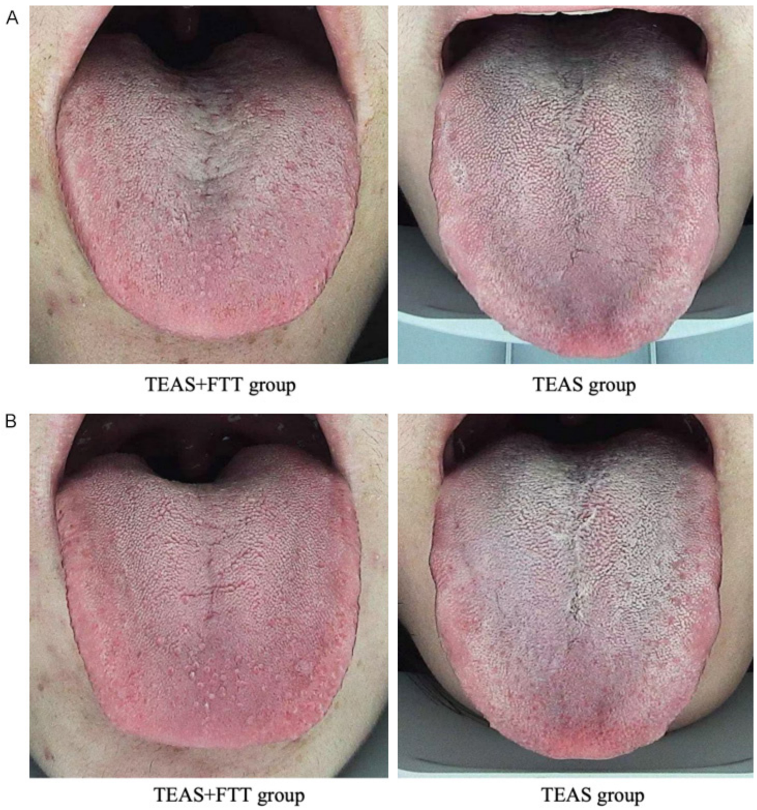


**Figure 4.** Comparison of typical tongue manifestations before and after treatment between the two treatment groups. A: Pre-treatment; B: Post-treatment. The images for within-group comparison were from the same subject.

depressive states exhibit decreased blood oxygen level-dependent signal intensity in the left prefrontal cortex and reduced functional connectivity density during working memory tasks [24, 25]. The slope method, which analyzes the temporal rate of change in Oxy-Hb concentration, is capable of sensitively capturing the initiation and dynamics of time-dependent neural activity [26, 27]. This sensitivity is clinically and experimentally important, and the application of fNIRS in detecting delayed initiation of neural function has also been validated [28, 29]. The present study found that TEAS plus five-tone music therapy enhanced the degree of hemodynamic activation in the left prefrontal cortex channels, as evidenced by increased slope values. This suggests that TEAS combined with five-tone music therapy may improve depressive states in college students with SD by increasing the response efficiency of neural activity in the left prefrontal brain region, enhancing the initiation rate of neural activity, and improving frontotemporal functional coordination.

Objective tongue and pulse diagnostic technologies reflect the functional status of the viscera by objectively quantifying the color characteristics of the tongue body and coating, as well as performing time-domain segmentation and feature parameter extraction of pulse waveforms [30, 31]. Studies have found that the tongue manifestations of liver stagnation and spleen deficiency syndrome are predominantly pale white and pale red tongues with white greasy or pale yellow greasy coating, characterized by parameters showing weak color saturation and attenuated values. In individuals with liver stagnation and spleen deficiency, inadequate nourishment of the blood and vessel walls, along with diminished myocardial contractility, frequently results in wiry and deep pulse patterns. In the present study, in college students with SD of the liver stagnation and spleen deficiency type, the RGB values of tongue coating color increased after receiving either TEAS alone or TEAS combined with five-tone music therapy, with the combined treatment demonstrating a more significant improvement in the R value of coating color compared to TEAS alone. In contrast, the control group, which received no active treatment, showed a slight decrease in RGB values after 4 weeks relative to baseline - a finding consistent with TCM theory: persistent liver stagnation and spleen deficiency impair the upward transportation of qi and blood to the tongue, resulting in a darker and less saturated tongue coating. Together, these observations suggest that both therapeutic approaches promote the generation and transformation of qi and blood, thereby enriching stomach qi. However, TEAS combined with five-tone music therapy more effectively restores the transportation and transformation function of the spleen, facilitating the upward nourishment of the tongue surface by stomach qi. Regarding pulse manifestations, both treatment methods increased the H1 value (height of the main wave) of the

**Table 6.** Comparison of characteristic values of pulse waveforms among the three groups before and after treatment ($\bar{x} \pm sd$; $n = 30$)

| | Indexes | TEAS+FTT group | TEAS group | Control group | *F* | *P* |
|---|---|---|---|---|---|---|
| Before treatment | Left hand-H1 | 13.42 ± 8.78 | 13.98 ± 6.08 | 13.42 ± 5.23 | 0.066 | 0.936 |
| | Right hand -Ass | 80.70 ± 5.79 | 77.59 ± 6.00 | 75.23 ± 5.48 | 0.057 | 0.945 |
| After treatment | Left hand-H1 | 18.09 ± 8.78* | 17.89 ± 3.92* | 14.21 ± 6.25 | 3.337 | 0.040 |
| | Right hand -Ass | 92.62 ± 6.17*,# | 74.13 ± 4.84 | 74.79 ± 6.25 | 3.225 | 0.045 |

Notes: *$P<0.05$ *vs*. the control group after treatment; #$P<0.05$ *vs*. the TEAS group after treatment.

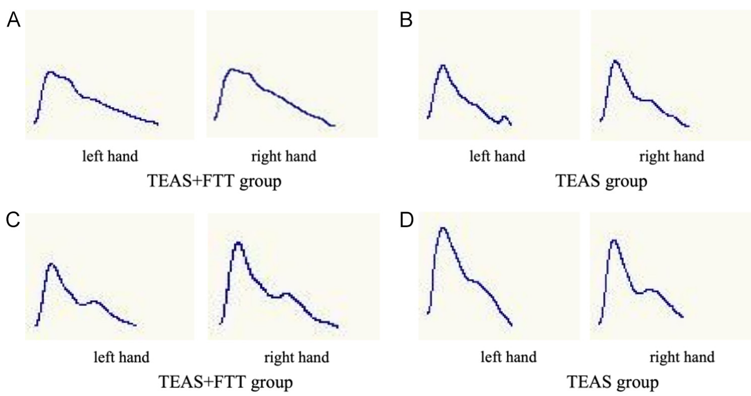


**Figure 5.** Comparison of typical pulse waveforms before and after treatment between the two treatment groups. A, B: Pre-treatment; C, D: Post-treatment. The images for within-group comparison were from the same subject.

pulse waveform, and TEAS combined with five-tone music therapy substantially increased the Ass value (systolic area) of the pulse waveform. These findings indicate that both TEAS alone and TEAS plus five-tone music therapy can promote the smooth flow of liver qi and the healthy movement of spleen qi, thereby enhancing overall myocardial systolic function. Moreover, TEAS combined with five-tone music therapy is superior to TEAS alone in increasing stroke volume and nourishing the blood vessels.

The superior effects of TEAS combined with five-tone music therapy over TEAS alone can be further understood by integrating the fNIRS, tongue, and pulse findings. The combined therapy significantly increased Oxy-Hb slopes specifically in the left prefrontal channels. This region is critical for emotion regulation and cognitive control, and its dysfunction is linked to both depressive symptoms and visceral disturbances in TCM liver stagnation pattern. The five-tone music used in this study may promote the smooth flow of liver qi and strengthen spleen transport through acoustic resonance, complementing the direct meridian stimulation of TEAS. The concurrent improvements in tongue coating RGB values indicating upward nourishment by stomach qi and pulse Ass values reflecting enhanced cardiac output suggest that the combined therapy facilitates "brain-viscera axis" regulation: enhanced left prefrontal activation may modulate hypothalamic and autonomic functions, thereby improving both mood and digestive functions. In contrast, TEAS alone, while effective in reducing depressive symptoms, showed less pronounced effects on these objective functional indicators. Therefore, the superiority of the combined therapy likely arises from the synergistic integration of peripheral electroacupuncture stimulation and central auditory-visceral resonance, leading to more comprehensive restoration of qi and blood dynamics.

In summary, this study employed a multimodal evaluation approach integrating scale assessments, fNIRS brain functional imaging, and digital tongue and pulse diagnostics to preliminarily observe the regulatory effects and potential mechanisms of TEAS or TEAS combined with five-tone music therapy on depressive mood in college students with SD. The findings indicated that: 1. Both TEAS alone and TEAS combined with five-tone music therapy effectively alleviated depressive symptoms such as low mood and loss of interest in college students with SD of the liver stagnation and spleen deficiency type; 2. The overall regulatory effect of TEAS combined with five-tone music therapy on liver stagnation and spleen deficiency syndrome in college students with SD was superior to that of TEAS alone; 3. TEAS combined with five-tone music therapy enhanced the response efficiency of neural activity in the left prefrontal cortex, thereby alleviating depressive mood in college students with SD. Limitations of this study are as follows: 1. Due to the nature of the five-element music therapy and TEAS, blinding of participants and treating practitioners was not feasible. Although data collectors and statistical analysts were blinded, the lack of participant blinding may have introduced a risk of bias, particularly for subjective outcome measures such as HAMD-17 and TCM syndrome scores; 2. The intervention period was relatively short (4 weeks), which may not be sufficient to fully capture the therapeutic potential or to observe sustained effects; 3. No follow-up assessments were conducted after the 4-week treatment period; therefore, the long-term durability of the observed improvements remains unknown. Future studies should recruit a more general population and develop appropriate sham controls to better isolate specific treatment effects, extending the treatment duration, and including follow-up visits to evaluate long-term efficacy. Despite these limitations, the multimodal objective assessments (fNIRS, digital tongue and pulse diagnosis) provide valuable preliminary evidence for the synergistic effects of TEAS combined with five-element music therapy in subthreshold depression.

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## Disclosure of conflict of interest

None.

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