

Details of the acupuncture manipulation

The acupuncture method used was the “Ping Bu Ping Xie” technique, with light to moderate stimulation, aiming to produce a sensation known as “De Qi” (soreness, numbness, distention, and pain), which is considered an effective acupuncture response. All procedures were performed by licensed acupuncturists with more than 5 years of experience.

Details of the Rs-fMRI scanning

The subjects were scanned with a MAGNETOM Prisma 3.0T magnetic resonance imaging system (Siemens, Germany), with a standard 64-channel cranial coil used as the signal receiving coil. Before enrollment, prospective participants (all healthy volunteers) were asked whether they had a fear of silence and being alone. Prior to scanning, the subjects were allowed to observe the experimental process and were informed about the entire scan procedure, duration, and precautions to alleviate any feelings of nervousness or anxiety. Additionally, technicians were present throughout the scanning process to monitor the participants and prevent any adverse effects. Post-assessment counseling has not revealed any significant emotional fluctuations in the participants. The subjects were instructed to relax, wear special earplugs to reduce noise interference, lie flat on the examination bed in a supine position, and place their hands at their sides naturally. Foam pads were placed on either side of the subject's head to reduce head movement and prevent image artifacts. FLAIR, T2WI, and T1WI sequence scans were performed first to rule out brain disorders. Functional sequences included the 3D-T1 and SMS-BOLD sequences. The specific scan sequence and parameters were as follows: T1WI: repetition time (TR) = 2500ms, echo time (TE) = 24ms; T2WI: TR = 3650ms, TE = 92ms; T2FLAIR: TR = 9000ms, TE = 120ms, flip angle = 90°, slice thickness = 5mm, slice interval = 1.5mm. The 3D-T1WI images were acquired by T1 FLAIR sequence scanning with the following parameters: TR = 2530 ms, TE = 2.98 ms, flip angle = 7°, slice thickness = 1mm, slice interval = 0mm, FOV = 256×256mm, spatial resolution 0.5mm×0.5mm×1mm, number of slices = 192, and scanning time = 5min58s. SMS-BOLD images were acquired with the following parameters: TR = 500, TE = 30 ms, FOV = 224×224 mm, spatial resolution 3.5×3.5×3.5 mm, slice thickness = 3.5 mm, slice interval = 0, acquisition time point = 960, number of slices = 35, flip angle = 60°. The BOLD sequence adopts the multi-layer acquisition technology (4 times acceleration), the acceleration factor AF = 1, and scanning time = 8min07s.

Clinical safety assessment

A clinical safety assessment was conducted based on unexpected incidents occurring during the acupuncture procedure (such as needle breakage, needle retention, needle bending, or needle residue) and the subjects' adverse reactions (including fainting, local hematoma, local infection, unbearable needle pain, post-needle pain, fatigue, palpitations, dizziness, headache, insomnia, or other discomfort). The severity of adverse reactions was evaluated on a scale of 0-10, and the duration of adverse reactions was recorded (Supplementary Table S3).

Results

Clinical safety assessment

In this study, the acupuncture procedure performed by the acupuncturists was not associated with any issues such as needle breakage, needle retention, needle bending, or needle residue. The participants also did not experience needle-induced fainting, local hematoma, local infection, intolerable needle pain, post-needle pain, fatigue, palpitations, dizziness, headache, insomnia, or other discomfort.

Supplementary Table S1 Clinical safety assessment of acupuncture

Symptom code/name* (for 01-15)	Number of occurrences (for 01-15)	Average degree of “other discomfort after acupuncture” (VAS average score) (10-15 is applicable)	Average duration of “other discomfort after acupuncture” (for 10-15)
/			h min
/			h min

* Symptom code: 01 = broken needle; 02 = stuck needle; 03 = needle sickness[†]; 04 = retained needle; 05 = bent needle; 06 = local hematoma; 07 = local infection; 08 = unbearable acupuncture pain (VAS ≥ 8); 09 = severe post-acupuncture pain (VAS ≥ 4 , lasting > 2 h).

The following are other uncomfortable feelings after acupuncture. Please select the symptoms with VAS ≥ 4 and fill in the corresponding VAS average score and average duration: 10 = fatigue, 11 = palpitation, 12 = dizziness, 13 = headache, 14 = insomnia, 15 = others. Please describe: _____.

[†] Needle sickness is characterized by symptoms representative of acupuncture-associated vasovagal response. (Christensen KA, Gosse BJ, Hildebrand C, Gershon LA. Acupuncture-associated vasovagal response: revised terminology and hospital experience. *Med Acupunct*. 2017;29:366-76).

Supplementary Table S2 Functional connection of the three core regions of the DMN

FC value	Pre-needle insertion scan	Acupuncture needle-in-place scan	Post-needle removal scan
ROI1-ROI2	0.48 $\pm$ 0.23	0.42 $\pm$ 0.20	0.52 $\pm$ 0.20
ROI1 and ROI3	0.59 $\pm$ 0.21	0.53 $\pm$ 0.21	0.48 $\pm$ 0.24
ROI2 and ROI3	0.38 $\pm$ 0.24	0.42 $\pm$ 0.20	0.43 $\pm$ 0.24

Data are expressed as mean $\pm$ SD.

ROI 1 was set as the mPFC, ROI 2 as the PCC/PCU, and ROI 3 as the ANG.

Supplementary Table S3 Functional connection between the seed regions and whole-brain voxels

FC value	Pre-needle insertion scan	Acupuncture needle-in-place scan	Post-needle removal scan
PCC/PCU-Cerebellum_8_L	0.21 $\pm$ 0.16	0.38 $\pm$ 0.15	0.37 $\pm$ 0.16
ANG- Frontal_Sup_Medial_L	0.48 $\pm$ 0.28	0.30 $\pm$ 0.22	0.27 $\pm$ 0.23

Data are expressed as mean $\pm$ SD.