Effect of acupuncture 'dose' on modulation of the default mode network of the brain

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ABSTRACT

Objective Recent functional MRI (fMRI) studies show that brain activity, including the default mode network (DMN), can be modulated by acupuncture. Conventional means to enhance the neurophysiological 'dose' of acupuncture, including an increased number of needles and manual needle manipulation, are expected to enhance its physiological effects. The aim of this study was to compare the effects of both methods on brain activity.

Methods 58 healthy volunteers were randomly assigned into four groups that received single needle acupuncture (SNA, n=15) or transcutaneous electrical nerve stimulation (TENS, n=13) as active controls, or enhanced acupuncture by way of three needle acupuncture (TNA, n=17) or SNA plus manual stimulation (SNA+MS, n=13). Treatment-associated sensations were evaluated using a visual analogue scale. Central responses were recorded before, during, and after treatment at LI4 on the left hand using resting state fMRI.

Results TNA and SNA+MS induced DMN-insula activity and extensive DMN activity compared to SNA, despite comparable levels of *de qi* sensation. The TNA and SNA+MS groups exhibited a delayed and enhanced modulation of the DMN, which was not observed followed SNA and TENS. Furthermore, TNA increased precuneus activity and increased the DMN-related activity of the cuneus and left insula, while SNA+MS increased activity in the right insula.

Conclusions The results showed that conventional methods to enhance the acupuncture dose induce different DMN modulatory effects. TNA induces the most extensive DMN modulation, compared with other methods. Conventional methods of enhancing the acupuncture dose could potentially be applied as a means of modulating brain activity.

INTRODUCTION

Functional MRI (fMRI) has been extensively applied as a non-invasive methodology to image brain functions, including the brain regions associated with pain processing,[1] and regional cerebral blood flow changes corresponding to acupuncture stimulation.[2] Recently, numerous studies have used fMRI to elucidate the central mechanisms of acupuncture analgesia, and to explore regional brain responses to acupuncture manipulation.[3] For example, acupuncture with manipulation at LI4 (*Hegu*), which is the most commonly used traditional acupuncture point for analgesia, has been shown to reduce activity in the limbic system and subcortical structures,[4] and electroacupuncture (EA) at LI4 has been shown to decrease activity in the anterior cingulate gyrus and increase activity in the hypothalamus, primary sensory cortex, and motor cortex.[5] Another recent study revealed that EA at LI4 activated the bilateral thalamus, basal ganglia, cerebellum and left putamen.[6] Transcutaneous electrical nerve stimulation (TENS) at LI4 has been found to induce activation of regions including the bilateral secondary somatosensory area, insula, contralateral anterior cingulate cortex and thalamus.[7] Furthermore, resting-state MRI provides a new approach for acupuncture research, especially to observe the effect of acupuncture on the default mode network (DMN), which is the spatial coherence of resting brain activities and plays an important physiological role in introspection, enabling individuals to remain alert to unexpected environmental events.[8] The hubs of the DMN include the ventral medial prefrontal cortex, posterior cingulate

(PC), precuneus, inferior parietal lobule, lateral temporal cortex, dorsal medial prefrontal cortex and hippocampal formation.[9] The DMN is related to the central modulation of chronic pain,[10] low back pain[11] and fibromyalgia,[12] and DMN pattern modulation has been demonstrated during acupuncture at traditional points including ST36, PC6 and GB37.[3 13–15]

Many factors may contribute to acupuncture-induced modulation of the DMN and its clinical effects, including needling and non-needling effects, and even the placebo effect.[16–18] The aim of this study was to explore the effects of subtle variations in neurophysiological 'dose' of acupuncture on DMN modulation. We varied two factors conventionally expected to enhance the dose of the acupuncture treatment: (1) number of needles; and (2) the degree of stimulation achieved by twirling (manipulation) of the needle. More specifically, regarding variation in the number of needles, we aimed to evaluate the 'three needle acupuncture' (TNA) technique, which involves inserting one needle at a given traditional acupuncture point and two needles nearby. TNA has been used with the intention of amplifying therapeutic effects in chronic musculoskeletal pain, pyriformis syndrome and tennis elbow,[19 20] and is closely related to the Dao-Ma technique described in *Tung's Acupuncture*.[21] We were also interested in the effect of needle manipulation, which is the most frequently used technique to increase the dose of stimulation. Acupuncturists frequently twirl the needles to try and enhance therapeutic effects in clinical practice. The specific objectives of this study were to compare the effects on the DMN of: (1) TNA versus single needle acupuncture (SNA); and (2) SNA with manual stimulation (MS) versus SNA alone. We additionally included a TENS group as an active (non-penetrating) control, despite the fact that TENS is known to have some central effects.[22] We aimed to assess the effects of the various treatments on the DMN via fMRI.

METHODS
Subjects and ethics statement
This study was approved by the Institutional Review Board of National Yang-Ming University (IRB reference no. 1000001) and a total of 58 healthy volunteers (32 females and 26 males) were included. Subjects were randomly assigned to one of four groups: SNA (n=15); TNA (n=17); SNA+MS (n=13); and TENS (n=13). All subjects were right-handed. Subjects with a history of seizure, neuro-psychiatric disease, pregnancy, claustrophobia or metallic implants were excluded.

Stimulation protocol
Subjects were asked to avoid staying up late on the night before the experiments. Subjects were reconfirmed as having no contraindications (as above) before starting the experimental procedures. All

acupuncture and TENS interventions were performed by a single acupuncturist with 10 years of experience. Stainless steel needles (0.27 mm diameter, 1.7 cm length) and plastic stick handles were used, and the depth of insertion was 1 cm. In the SNA group, one needle was inserted at LI4 on the left and retained for 15 min without any manipulation throughout the whole procedure (ie, minimal acupuncture). In the TNA group, three needles were inserted at LI4 plus two other sites 1.5 cm distal and proximal to this point, parallel to the second metacarpal bone, and retained for 15 min. In the SNA+MS group, one needle was inserted at LI4 and gently twirled/rotated through 180° at a frequency of 1 Hz for a total of 20 s. The same 20 s manipulation was repeated nine times at 20 s intervals, until the needles were removed after 15 min. In the TENS group, two surface pads were applied to the left LI4 and adjacent skin area. Electrical stimulation was applied intermittently for periods of 20 s using pulse waves of 1 Hz frequency and pulse width 0.1 ms for periods at 20 s intervals over a 15 min period. The strength of stimulation remained below the motor threshold and produced a non-noxious tingling sensation. A schematic representation of the methodology is provided in figure 1.

Visual analogue scale for stimulation sensation and anxiety index
The sensations associated with acupuncture and TENS were quantified using the Massachusetts General Hospital Acupuncture Sensation Scale (MASS), which is a visual analogue scale (VAS) that includes soreness, aching, deep pressure, heaviness, fullness, tingling, numbness, sharp pain, dull pain, warmth, cold, throbbing and anxiety.[23 24] The anxiety index of the subjects was measured using the Chinese version of the Beck Anxiety Inventory (BAI).[25]

fMRI acquisition
All fMRI data were obtained using a Siemens Trio 3.0 T scanner (Magnetom Trio, A Tim system 3T, Siemens, Germany) at the National Yang-Ming University, which was equipped with an actively shielded gradient coil and a multichannel head coil. During the scan, subjects were placed in a supine position in the scanner with their head immobilised by foam pads, and were instructed to maintain a resting state with their eyes open and focused on a black cross in the centre of their visual field. All subjects were asked to relax and reduce mental processes but stay awake during the examination. To ensure wakefulness, a brief communication was conducted before every 6 min fMRI scan. As detailed in figure 1, three sessions of fMRI scans were acquired: before needling (session 1), 9 min after needling (session 2), and after needle removal (session 3).

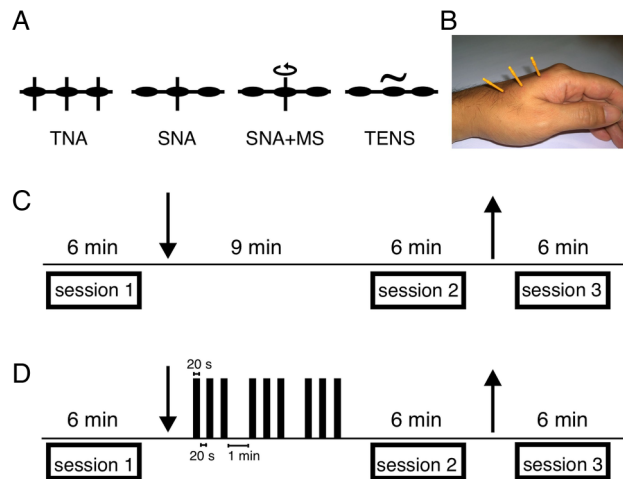Forty-five continuous slices (no gap) were acquired using a gradient echoplanar (EPI) sequence with the

Figure 1 (A) Schematic representation of experimental protocols for three needle acupuncture (TNA), single needle acupuncture (SNA) with or without manual stimulation (MS) and transcutaneous electrical nerve stimulation (TENS). Vertical black lines indicate needles. Ovals indicate acupuncture points, with the middle one representing LI4. The black circle with an arrow indicates MS. '∼' indicates TENS. (B) Photograph of participant receiving TNA. (C) In TNA and SNA groups, needles were retained for 15 mins. (D) In SNA+MS and TENS groups, nine blocks of manipulation/stimulation were applied. In all groups, three fMRI scans were acquired, specifically a first scan (session 1) that was carried out before treatment, a second scan (session 2) that was carried out 9 min into treatment, and a third scan (session 3) after needle or electrode removal.

following parameters: 2800 ms repetition time (TR); 30 ms echo time (TE); flip angle 50°, orientation by anterior cingulate to PC (AC-PC) line; 220 mm field of view (FOV), 3.4 mm slice thickness; voxel size 3.4_3.4_3.4 mm. Anatomical images were acquired using the 3D Magnetization Prepared Rapid Gradient Echo Imaging (MPRAGE) protocol with the following parameters: 2730 ms TR; 3.02 ms TE; 256 mm FOV; voxel size 1_1_1.3 mm.

Data analysis and image processing

We used the SPM8 (Statistical Parametric Mapping software, Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk) for data analysis. Preliminary raw data processing began with realigning and unwarping of the images, performing co-registration of functional images to the anatomical images. Anatomical images were then normalised to stereotactic space (Montreal Neurological Institute, Quebec, Canada) and were smoothed with a Gaussian kernel of 8 mm.

Connectivity analysis

An independent component analysis (ICA) was carried out for fMRI data analysis using the 'group ICA' function of the fMRI toolbox, called GIFT (V.1.3h; http://icatb.sourceforge.net). Data from individual subjects were concatenated across time, and calculated for subject-specific components using the MDL (minimum description length) rule. Best-fit DMN components were selected manually using the DMN template in GIFT. Scans with problems related to best-fit DMN component selection were excluded. The time-series of the best-fit DMN components of each session were used as the regressor in SPM8 to construct the statistical functional map of DMN. Central modulations induced by the acupuncture stimulation were recorded and defined as follows: the 'during treatment effect' and 'post-treatment effect' were derived by subtracting images from sessions 1 and 2, and 1 and 3, respectively. The effects were analysed statistically by paired t-test. The threshold was set at $p=0.001$ (uncorrected) with a cluster extent threshold of 10 voxels.

RESULTS

The average ages of patients in the TNA, SNA, SNA+MS and TENS groups were 25.3 ± 4.7, 26.5 ± 3.64, 24.3 ± 3.11 and 27.0 ± 4.93 (mean±SD) years, respectively. The scores for the Chinese version of the BAI of the TNA, SNA, SNA+MS and TENS groups were 5.1 ± 5.5, 4.9 ± 3.8, 7.9 ± 6 and 5.3 ± 5.4, respectively, and were all within the normal range (<10).[25][26] There were no significant differences between these four groups in terms of age or BAI score.

Intensity of *de qi* sensation

Sensation scores for soreness and aching in the three acupuncture groups were all significantly higher than those in the TENS group (one-way analysis of variance (ANOVA), $p<0.05$). There were no statistically significant differences for any individual items related to *de qi* sensation between the TNA, SNA and SNA+MS groups (figure 2).

DMN-related activity distribution across brain regions

Table 1 and figure 3 detail the specific DMN-related brain regions demonstrating significant changes in activity during and/or after treatment in the four different groups. In the TNA group, DMN activity during
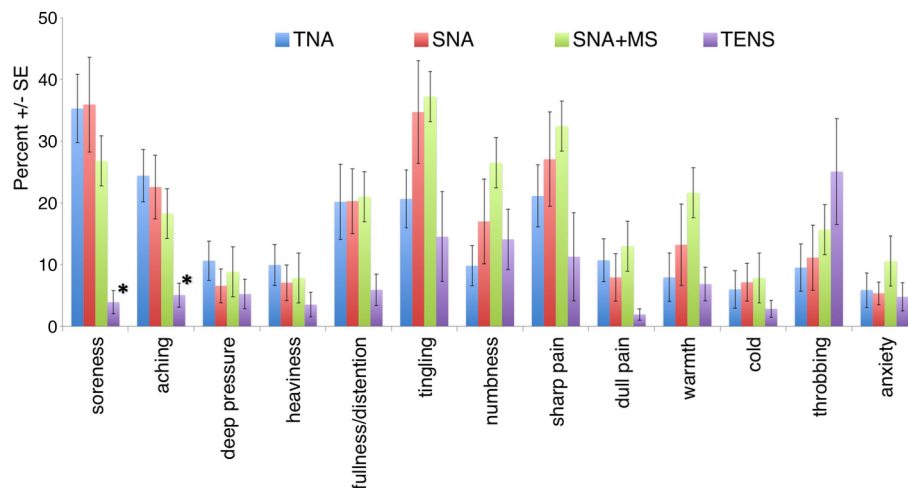
Figure 2 Subjective sensations during three needle acupuncture (TNA), single needle acupuncture (SNA) with or without manual stimulation (MS) and transcutaneous electrical nerve stimulation (TENS). The sensations experienced during mechanical or electrical stimulation were reported using a visual analogue scale rating from 0 to 100. Data are presented as mean±SD. *p<0.05: TENS versus TNA, SNA and SNA+MS groups (one-way analysis of variance (ANOVA) plus Bonferroni post hoc test).

acupuncture showed the presence of significantly more positive clusters with large numbers of active voxels in the left calcarine, PC, insula and inferior frontal gyrus. Areas with increased DMN-related activity included the right medial frontal gyrus, insula and precuneus. Several areas showed persistent activation after acupuncture including the bilateral cuneus and precuneus, and right middle occipital gyrus, where a large cluster of 1799 voxels was recorded, while negative DMN-related activity was observed in the right precuneus and corpus callosum.

In the SNA group, increased DMN activity was found during acupuncture in the right limbic lobe, precentral gyrus and uncus, and left medial frontal gyrus, middle occipital gyrus and insula. After acupuncture, DMN activity returned to a state similar to that before acupuncture treatment began, that is, no voxels passed the threshold of p=0.001 (uncorrected).

In the SNA+MS group, there were no voxels with signal higher than threshold during acupuncture. After acupuncture, areas with positive DMN activities with large numbers of active voxels were the right insula and supplementary motor area, and left lateral frontal-occipital lobe, superior temporal gyrus and lentiform nucleus.

In the TENS group, which was included as a sensory stimulation control, several brain areas showed positive DMN activities during treatment including the left angular gyrus and right inferior frontal-orbital lobe, and the middle and medial frontal gyri. On the other hand, negative DMN activity was observed in the subgyrus of the left frontal lobe. After TENS, an increase in DMN activity was observed together in the bilateral inferior parietal lobes and right precuneus, while negative activity was recorded in the subgyrus of the right frontal lobe.

DISCUSSION

The effects of acupuncture on brain activity have been extensively studied using fMRI. Our previous studies have demonstrated that acupuncture at different classical acupuncture points induce specific activations of the central nervous system,[27] and we have shown that the hypothalamic response is enhanced by EA.[28] Although numerous studies have examined the differential effects of various acupuncture modalities at different points on region-specific brain activities and brain networks,[29] the potential impact of acupuncture 'dose' has rarely been explored.[30]

To our knowledge, this is the first study to compare modalities that increase the acupuncture dose in terms of central neural responses. We found that, despite comparable measures of *de qi* sensation, significant DMN activity with large numbers of active voxels were observed in both the TNA and SNA+MS groups, but not the SNA group, suggesting that both increasing the number of needles and enhancing the stimulation via manipulation are effective at modulating DMN activity.

Both acupuncture groups with increased dose exhibited increased activity between the DMN and insula. DMN-insula connectivity was found to be prominently increased during treatment in the TNA group and after needles were removed in the SNA+MS group. Furthermore, TNA induced a significant response in the right insula, while SNA+MS induced responses in the left insula. The insular cortex is related to pain processing, representation and the modulation of pain, and plays an important role in the mechanism of descending pain control in acupuncture anaesthesia.[31] DMN-insula connectivity is associated with spontaneous pain in patients with fibromyalgia[12] and chronic pain.[32] Moreover, the activity of the posterior hub of the DMN (precuneus)

Table 1 Default mode network-related brain regions exhibiting statistically significant changes in activity during acupuncture or TENS

Structure	Hemisphere	MNI (mm)			Peak T value	Voxel numbers
		x	y	z		
TNA: during treatment effect						
Calcarine	L	−24	−66	12	6.08	312
Posterior cingulate	L	−10	−66	8	5.06	
Insula	L	−44	12	14	6.74	193
IFG	L	−42	18	6	4.74	
MeFG	R	12	−24	56	5.42	16
Insula	R	44	−10	4	4.85	15
Precuneus	R	22	−74	18	4.77	16
TNA: post-treatment effect						
Cuneus	L	−16	−82	18	5.68	
MiOG	R	32	−72	14	4.82	
Precuneus	R	26	−74	16	4.77	1799
Precuneus	L	−26	−78	16	4.71	
Cuneus	R	10	−74	4	4.01	
Precentral gyrus	R	44	4	10	4.84	34
Precentral gyrus	L	−48	−2	10	4.14	11
MiOG	L	−36	−82	6	4.65	76
Corpus callosum	R	2	−30	10	−6.55	91
Precuneus	R	6	−66	38	−4.80	44
SNA: during treatment effect						
Limbic lobe	R	20	4	36	5.05	21
SG-FL	R	20	4	38	6.06	
Limbic lobe	R	30	2	20	5.34	12
Precentral gyrus	R	66	0	34	4.01	20
Uncus	R	18	6	−34	5.1	19
MeFG	L	−14	0	54	4.77	13
MiOG	L	−32	−94	2	4.46	21
Insula	L	−30	−10	16	4.46	14
SNA+MS: post-treatment effect						
Lateral FOG	L	−34	44	−16	6.07	15
Superior TG	L	−44	8	−14	5.68	49
SMA	R	14	−10	64	5.6	36
Insula	R	40	6	12	5.22	146
Superior TG	L	−66	−42	16	3.53	13
Lentiform nucleus	L	−18	−6	−4	3.5	14
TENS: during treatment effect						
SG-FL	R	24	22	−12	5.75	55
IFG	R	30	36	−10	4.84	
MeFG	R	18	4	52	5.07	12
SG-FL	L	−30	4	36	−5.33	18
TENS: post-treatment effect						
IPL	R	52	−26	28	5.22	58
IPL	L	−44	−40	26	4.46	16
Precuneus	R	20	−64	42	4.35	14
SG-FL	R	22	−42	28	−4.67	26

The 'during treatment' and 'post-treatment' effects were derived from the subtraction of images from session 1 and 2, and 1 and 3, respectively. p<0.001 (uncorrected) and voxel numbers >10. The MNI coordinates and peak T values were taken from the voxel with maximal signal change for each structure. FOG, frontal-orbital gyrus; IFG, inferior frontal gyrus; IFG, inferior frontal gyrus; IPL, inferior parietal lobe; MeFG, medial frontal gyrus; MiOG, middle occipital gyrus; MNI, Montreal Neurological Institute; SG-FL, subgyrus of frontal lobe; SMA, supplementary motor area; SNA, single needle acupuncture; SNA+MS, single needle acupuncture with manual stimulation; TENS, transcutaneous electrical nerve stimulation; TG, temporal gyrus; TNA, three needle acupuncture.
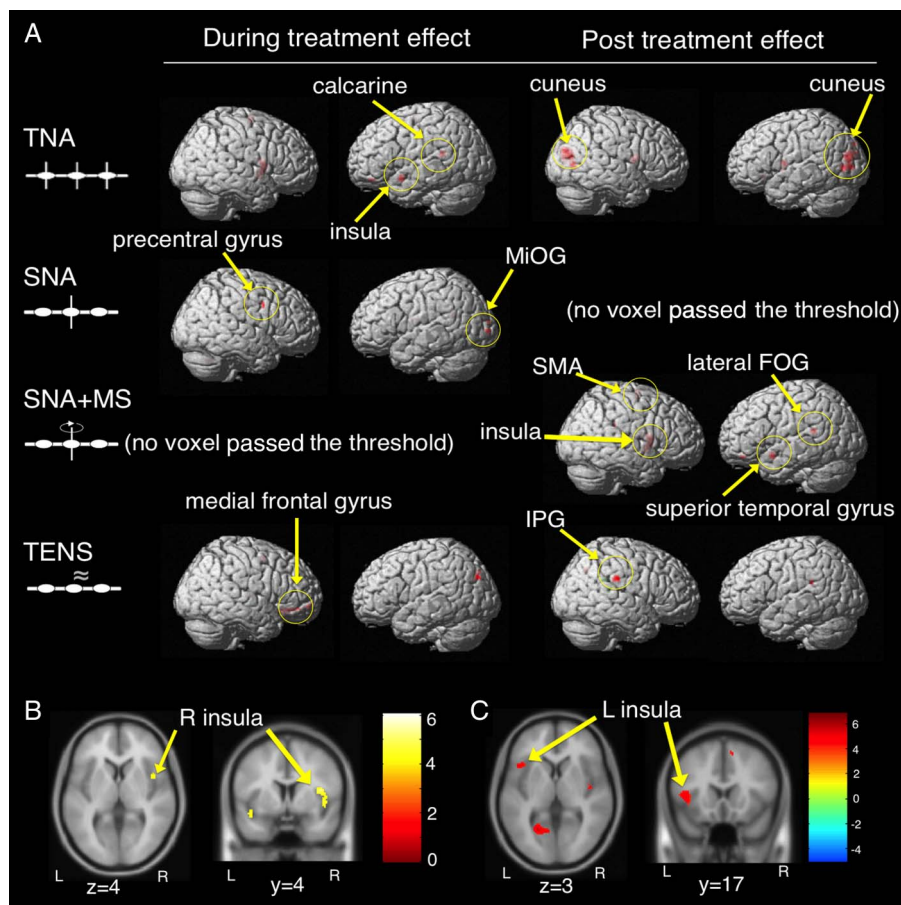
Figure 3 Comparison of default mode network (DMN)-related activity during and post-treatment with acupuncture or transcutaneous electrical nerve stimulation (TENS). (A) Images demonstrating areas of increased DMN-related activity associated with the different treatment modalities. (B) Increased right insula activity observed as post-treatment effect in SNA+MS group. (C) Increased left insula activity observed as during treatment effect in TNA group. 'During treatment' and 'post-treatment' effects were derived from the subtraction of images from session 1 and 2, and 1 and 3, respectively. Data were analysed statistically by paired t-test. The threshold was set at p=0.001 (uncorrected) with a cluster extent threshold of 10 voxels. FOG, frontal-orbital gyrus; IPG, inferior parietal gyrus; MiOG, middle occipital gyrus; SMA, supplementary motor area.

was increased in the TNA group. The precuneus is involved in a wide range of cognitive processing, including imagery, episodic memory retrieval and self-processing operations.[33] Recent studies have revealed that the precuneus contributes to inter-individual differences in pain sensitivity.[34] A functional reorganisation of the DMN has been revealed in chronic pain conditions[14] and major depressive disorder,[35] and remodulation of the DMN is associated with therapeutic effects in the treatment of chronic low back pain with acupuncture.[36]

Effects of sensations on DMN activities should also be noted. Pain, as one of the strongest sensations, is very effective at modulating DMN activity.[37] Typical *de qi* sensations (such as soreness, deep pressure and dull pain) or non-*de qi* sensations (such as sharp pain and aching) may also have DMN modulatory effects.[4] Furthermore, *de qi* sensations vary significantly between acupuncture points. For example, acupuncture at LI4 with manipulation usually produces an aching sensation rather than soreness, which is

followed by dull pain and pressure.[23] It is possible that sensations may correlate with DMN modulatory effects, although further studies are required. To minimise the potentially confounding effect of differential sensations, we used a visual scale metric and reduced the acupuncture-induced sensations in this study; that is, the amplitude of manipulation in the SNA+MS group was limited to reduce aching in order to produce a similar degree of *de qi* sensation as in the TNA and SNA groups.

Limitations

In this study, we observed short-term changes in DMN activity; however, further investigations are required to explore the role of sustained central nervous system modulation by acupuncture. Moreover, the present study characterised sequential changes in the DMN within four individual treatment groups but did not apply any between-group statistical comparisons. This is an unavoidable limitation of this particular approach, as brain activities between

individuals vary significantly. In this relatively small exploratory study, it was only feasible to compare the DMN activities of each subject before, during, and after treatment; however, a large-scale clinical trial may overcome this statistical barrier and shed light on general principles between experimental and control groups in a larger population.

CONCLUSIONS

Methods conventionally used to deliver an enhanced acupuncture dose induce different modulatory effects on DMN activities. The central effects of acupuncture can be accentuated by increasing the number of needles or by enhancing stimulation by manipulation. The TNA technique appeared to induce more extensive DMN activity compared with other methods. Conventional methods of increasing acupuncture dose in order to enhance the strength of treatment could potentially be applied as a means of modulating brain activity.

Contributors Y-JL conducted the studies, analysed the data and wrote the manuscript. J-HC supervised Y-JL and designed the study. J-CH assisted the study design while Y-YK, W-JK, DMN, C-CC, C-MC and T-CY provided facilities and helped to analyse the data.

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