Blockade of the brachial plexus abolishes activation of specific brain regions by electroacupuncture at LI4: a functional MRI study

Weidong Gu,1 Wei Jiang,2 Jingwei He,3 Songbin Liu,2 Zhaoxin Wang4

ABSTRACT

Objective Our aim was to test the hypothesis that electroacupuncture (EA) at acupuncture point LI4 activates specific brain regions by nerve stimulation that is mediated through a pathway involving the brachial plexus.

Methods Twelve acupuncture naive right-handed volunteers were allocated to receive three sessions of EA at LI4 in a random different order (crossover): (1) EA alone (EA); EA after injection of local anaesthetics into the deltoid muscle (EA+LA); and (3) EA after blockade of the brachial plexus (EA+NB). During each session, participants were imaged in a 3 T MRI scanner. Brain regions showing change in blood oxygen level-dependent (BOLD) signal (activation) were identified. Subjective acupuncture sensation was quantified after functional MRI scanning was completed. Results were compared between the three sessions for each individual, and averaged.

Results Blockade of the brachial plexus inhibited acupuncture sensation during EA. EA and EA+LA activated the bilateral thalamus, basal ganglia, cerebellum and left putamen, whilst no significant activation was observed during EA+NB. The BOLD signal of the thalamus correlated significantly with acupuncture sensation score during EA.

Conclusions Blockade of the brachial plexus completely abolishes patterns of brain activation induced by EA at LI4. The results suggest that EA activates specific brain regions through stimulation of the local nerves supplying the tissues at LI4, which transmit sensory information via the brachial plexus.

Trial registration number ChiCTR-OO-13003389.

BACKGROUND

Acupuncture, a Traditional Chinese Medicine (TCM) therapy that has been used in China for more than 2000 years, has become increasingly popular in the West as a treatment for pain and a wide variety of chronic disorders that may be difficult to manage with conventional treatments.1 An increasing body of evidence has demonstrated the clinical effectiveness of acupuncture for disorders such as knee osteoarthritis, migraine, and chronic low back pain.2 Following the endorsement of acupuncture by the National Institutes of Health, acupuncture has become more accepted in the West and an increasing number of studies have been directed towards elucidating the mechanisms of action underlying the effects of acupuncture. However, several fundamental aspects of acupuncture treatment remain poorly understood.

Historically, according to TCM theory, needling of acupuncture points has been believed to have specific therapeutic effects, either locally or at a distance via a proposed system of hypothetical acupuncture meridians.3 However, there is no structural or functional evidence to support the existence of meridians.3 Recently, brain imaging tools such as positron emission tomography and functional MRI (fMRI) have been used to investigate the neural mechanisms underlying acupuncture needle stimulation4–7 and its effects.8 9 Cho et al10 reported that acupuncture at acupuncture points historically used for the treatment of ophthalmological conditions activates the visual cortex bilaterally.11 Similar results were reported by Siedentopf et al,12 and Yan et al13 demonstrated that acupuncture at LR3 (Taichong) and LI4 (Hegu) induces specific patterns of brain activation compared with sham acupuncture at non-acupuncture points. These patterns...
may be related to the therapeutic effects of acupuncture. In addition to evoking activation in specific brain areas, recent studies have also demonstrated that acupuncture modulates the connectivity of several functional networks within the brain, including the default mode network (DMN) and the anticocceptive, affective and amygdala-associated brain networks.\(^{17} \)\(^{18} \)\(^{19}\) Taken together, these studies suggest that changes in the neural activity of brain networks are associated with acupuncture needle stimulation and its therapeutic effects; however, the precise neural mechanisms underlying acupuncture are still not well understood.\(^{20}\) In particular, the role of peripheral nerves in the modulation of these functional brain networks by acupuncture remains under investigation.

LI4, which is located in the first dorsal interosseous space of the hand, is the most frequently used acupuncture point in Chinese acupuncture, especially for analgesia and sedation.\(^{21}\) It is also widely used for diverse disorders including post-stroke paralysis, facial palsy, epilepsy, agitation, depression, and emesis.\(^{22} \)\(^{23} \)\(^{24}\) LI4 is supplied by the median nerve, which originates from the brachial plexus. In the present study, we tested the hypothesis that electroacupuncture (EA) at LI4 activates specific brain regions by nerve stimulation mediated through a pathway involving the brachial plexus.

METHODS

Participants

Twelve healthy right-handed volunteers who were naïve to acupuncture (three male, nine female, mean age 49 years, range 38–58 years) participated in the experiment. None had a history of psychiatric or neurological disorders, head trauma with loss of consciousness, or use of tranquilisers in the 3 days before the study. No participant was in pain or distress at the time of the experiment. Before the commencement of the study all participants were counselled regarding the purpose of the study, the procedures involved, and the potential discomforts and risks. All gave informed (written) consent and were free to withdraw from the experiment at any time. All research procedures were approved by the local ethical committee of Minhang Hospital, Fudan University and the study was prospectively registered with the Chinese Clinical Trial Register (http://www.chictr.org): registration number ChiCTR-OO-13003389.

Experimental protocol

Participants initially either received a session of EA alone (EA), EA after injection of local anaesthetic (LA) into the deltoid muscle (EA+LA), or EA after blockade of the brachial plexus (EA+NB). The EA+LA session was included because LA can be absorbed systematically during a brachial plexus block and may inhibit neuronal excitability in the brain.\(^{25}\) To control for this possibility, an equivalent dose of LA was injected into the deltoid muscle before acupuncture during the EA+LA session. Each subject received all three types of treatment (EA, EA+LA, and EA+NB) sequentially; however, the order of treatments was randomised and balanced. The interval between each treatment was 1 week.

EA was performed at LI4 on the left hand by an experienced acupuncturist. Since participants lost sensation at LI4 and could not move their arm after the brachial plexus block, the acupuncturist was not blind to the treatment session. Sterile, disposable and non-magnetic stainless steel needles (25 mm in length and 0.28 mm in diameter, Hwato, China) were used. The depth of needling at LI4 was 1.25 cm. An EA instrument (G9805-C, Shanghai Medical Electronic Instrument Factory, Shanghai, China) was used to administer EA with a continuous rectangular waveform (pulse width 30 ms) at a frequency of 4 Hz. The negative electrode of the EA instrument was connected to the needle at LI4, and the positive electrode (9×14 cm) was placed on the calf. One week before the experiment, the intensity of EA was tested for each participant and adjusted in order to generate a moderate de qi sensation, which was generally accompanied by mild muscle contraction in the index finger (5–12 mA). The same individual intensity as that established before the experiment was used during all three treatments for each participant.

A block design was adopted with a 10 min scanning time for each functional run. Each run started with 3 min of rest, followed by 2 min of EA stimulation, then 3 min of rest, followed by another 2 min of EA stimulation. The interval between two functional runs was approximately 3 min. A total of five functional runs were performed in each session (EA, EA+LA, and EA+NB). After the fMRI scanning was completed, participants were asked to quantify the acupuncture sensation by completing the Chinese version of the Massachusetts General Hospital Acupuncture Sensation Scale (C-MASS).\(^{26}\)

Ultrasound-guided brachial plexus block

In the EA+NB session, participants underwent an ultrasound-guided interscalene block of the brachial plexus after monitors (electrocardiogram (ECG), blood pressure, heart rate, and pulse oxygen saturation (SpO\(_2\))) were placed. The interscalene area was identified and prepared with chlorhexidine and alcohol. With the ultrasound transducer held in a coronal-oblique position, the brachial plexus was imaged at the supraclavicular level and then followed cephalad until three nerve fascicles were evident in the space between the scalene muscles. A 5 cm, 22-gauge needle was inserted under direct in-plane ultrasound guidance until its tip was clearly visualised lying in the interscalene groove. A 25 mL solution containing lidocaine 5 mg/mL and ropivacaine 5 mg/mL was injected at an approximate rate of 5 mL per
15 s with aspiration every 5 mL to monitor for intravascular injection. Before the injection, the LA solution was divided into three equal aliquots: one for the upper trunk (C5, C6), one for the middle trunk (C7), and one for the lower trunk (C8, T1) of the brachial plexus. A single puncture was used for the three injections. In brief, under ultrasound guidance, the needle was first advanced toward the upper trunk. One aliquot of LA solution was injected once the needle tip lay adjacent to the upper trunk. After injection, the needle was withdrawn a little and its direction was adjusted toward the middle trunk, around which the second aliquot was injected. Finally, the lower trunk was blocked in the same manner (figure 1). Participants rested for 1 h before EA and fMRI scanning. The duration of a brachial plexus block with ropivacaine or lidocaine is typically 337 and 137 min, respectively.28 The duration of a combined block with lidocaine and ropivacaine was 390–480 min in the present study, which was more than enough time to cover the rest period and fMRI scanning (about 60 min each).

In the EA+LA session, a 25 mL solution containing lidocaine 5 mg/mL and ropivacaine 5 mg/mL was injected into the left deltoid muscle. Participants similarly rested for 1 h before EA and fMRI scanning.

### Assessment of brachial plexus block

The intensity of the block was evaluated after 30 min. Sensory function including pain and temperature sensation was assessed in five different regions corresponding to the nerve distributions in the forearm and hand (ulnar, median and radial nerves, plus the medial and lateral cutaneous nerves of the forearm). Temperature sensation was tested using an ice cube. Pain sensation was tested by pinprick using a 23-gauge needle. A two-point scale for temperature (cold or not cold) and a three-point scale for pain (sharp, blunt or absent sensation) were used. Motor function was assessed using a modified Bromage scale (3=extension of elbow against gravity, 2=flexion of wrist against gravity, 1=finger movement, 0=no movement).29

### Functional and anatomical MRI acquisition

fMRI scanning was performed using a 3T (Tesla) Siemens Trio MRI scanner, including one anatomical run and five functional runs of 600 s each. For functional images, 35 axial slices (field of view (FOV) =240×240 mm², matrix=64×64, in-plane resolution=3.75×3.75 mm², thickness=4 mm, without gaps) covering the whole brain were obtained using a T2-weighted echo planar imaging (EPI) sequence (TR (repetition time)=2000 ms, TE (echo time)=30 ms, flip angle=90°). A high-resolution structural image for each participant was also acquired using three dimensional MRI sequences for anatomical co-registration and normalisation (TR=1900 ms, TE=3.43 ms, flip angle=7°, matrix=256×256, FOV=240×240 mm², slice thickness=1 mm).

### Data analysis

Acupuncture sensation scores were expressed as mean ±SEM. After confirming normality of distribution using the Kormogorove-Smirnov test, one-way analysis of variance followed by Student-Newman-Keuls test was performed to compare the acupuncture sensation scores between the three sessions. Differences were considered to be statistically significant at p<0.05.

The MRI technician and those performing the data analysis were blind to treatment session. SPM8 was adopted for MRI data analysis (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). For each participant, the first three volumes of each run were discarded. EPI images were realigned to the first remaining volume of the first run to correct for head motions. Then the anatomical image was co-registered with the mean EPI image, segmented and used to generate normalised parameters to Montreal Neurological Institute (MNI) spaces. Next, all EPI data were projected to an MNI template with a re-sampled voxel size of 2×2×2 mm³. Finally, the functional images were spatially smoothed with a Gaussian kernel with a full width at half maximum of 8 mm. High-pass temporal filtering with a cut-off of 128 s was carried out to remove low-frequency drifts.

**Figure 1** Ultrasound images illustrating the brachial plexus block procedure. (A) Ultrasound image of the interscalene brachial plexus (yellow arrows) before the block. (B) Ultrasound image of the brachial plexus block during injection of local anaesthetic (LA) solution, illustrating the needle (white arrow) and complete envelopment of the nerve roots in the interscalene groove by LA (outlined in blue).
For the first level individual analysis, a box-car model combined with a canonical haemodynamic response function based on the general linear model was constructed. The six estimated head movement parameters were also included in the design matrix to minimise the residual effects of head motion. The estimated parameters values were used for second level group analysis. The one-sample t test was used to identify regions engaged during each session, and the independent t test was used for comparisons between different treatments. Brain regions with a voxel-wise threshold of $p<0.005$ and at least 50 consecutive voxels were reported. A simulation was used to control type I error within a search area of $30\times30\times30$ mm$^3$, corresponding to 49 voxels. A further correlation analysis was applied to evaluate the relationship between the blood oxygen level-dependent (BOLD) signal and subjective acupuncture sensation during EA with a threshold of $p<0.05$. In this correlation analysis, activation maps of the first level analysis were used as masks with a lenient voxel-wise threshold of $p<0.01$ and at least 50 consecutive voxels.

**RESULTS**

**Intensity of brachial plexus block**

The scores for motor function, pain and temperature sensation were 0 in 11 participants after blockade of the brachial plexus. One participant had weak motor function and pain sensation after the nerve block.

**Acupuncture sensation score**

There were no significant differences in acupuncture sensation score between the EA and EA+LA sessions ($21.1\pm2.3$ vs $20.9\pm3.2$, $p=0.96$). However, blockade of the brachial plexus significantly inhibited acupuncture sensation scores during EA ($1.3\pm0.9$; $p<0.01$ compared with EA and EA+LA).

**fMRI results**

EA significantly activated the thalamus, basal ganglia and cerebellum bilaterally, as well as the left putamen. These brain regions were also activated during EA+LA. However, no significant activations were found during EA+NB, despite the application of a very lenient standard (voxel-wised threshold of $p<0.005$, 50 voxels), as shown in table 1 and figure 2.

Direct comparison between the EA and EA+NB sessions showed significant brain activations in the thalamus and cerebellum bilaterally. Similarly, direct comparison between the EA+LA and EA+NB session revealed significant brain activation in the same regions (table 2 and figure 2).

The correlation analysis revealed that the BOLD signal change of the thalamus correlated positively ($r=0.629$, $p<0.05$) with the subjective acupuncture sensation score during EA (figure 3) using a lenient voxel-wised threshold of $p<0.05$ with 50 consecutive voxels.

**DISCUSSION**

The present study has demonstrated that EA at LI4 evokes activation in the thalamus, basal ganglia and cerebellum bilaterally, and in the left putamen, and that the BOLD signal change of the thalamus correlated significantly with acupuncture sensation during EA. Notably, EA after injection of LA into the deltoid muscle evoked brain activation similar to EA alone, suggesting that LA absorbed systemically after intramuscular injection does not affect brain activation by EA. By contrast, blockade of the brachial plexus inhibited acupuncture sensation during EA and abolished brain activation by EA at LI4. Collectively, the data indicate that EA at LI4 activates brain regions by stimulating local nerves in the tissues at LI4.

In recent decades, it has become increasingly clear that the regulatory effects of acupuncture are produced through its effects on the nervous, endocrine, and immune systems. There is a growing body of evidence demonstrating the response of the central nervous system (CNS) to needling. fMRI studies have revealed specific activities in the CNS during acupuncture. Shi et al demonstrated that acupuncture at BL40 produces a broad pattern of activation in subjects with acute low back pain, including the limbic system.
and the DMN, and also activated the attentional and somatosensory systems compared with sham acupuncture. Von Deneen et al.\textsuperscript{32} showed that increased hypothalamus-putamen-insula functional connectivity after acupuncture at ST36 and SP9 was positively correlated with acupuncture-induced changes in glucose level. Consistent with previous studies,\textsuperscript{13, 33} herein we demonstrated that EA at LI4 activated the bilateral thalamus, caudate, cerebellum, and left putamen.

![Brain regions activated by electroacupuncture (EA) during three treatment sessions: EA alone (EA); EA following injection of local anaesthetic (LA) into the deltoid muscle (EA+LA); EA following a brachial plexus block (EA+NB); and functional maps showing significant clusters for comparisons between individual sessions: EA>EA+NB and EA+LA>EA+NB. EA significantly activated the bilateral thalamus, basal ganglia, cerebellum, and the left putamen. EA+LA activated the left thalamus, right anterior insula and bilateral basal ganglia, cerebellum and inferior frontal gyrus. There was no significant brain activation during EA+NB. Direct comparison between EA and EA+NB showed significant brain activations in bilateral thalamus and cerebellum (EA>EA+NB). Direct comparison between EA+LA and EA+NB revealed significant brain activations in bilateral thalamus, cerebellum, putamen, and right inferior frontal gyrus (EA+LA>EA+NB). Voxel-wised threshold was p<0.005.](http://aim.bmj.com/)

**Figure 2** Brain regions activated by electroacupuncture (EA) during three treatment sessions: EA alone (EA); EA following injection of local anaesthetic (LA) into the deltoid muscle (EA+LA); EA following a brachial plexus block (EA+NB); and functional maps showing significant clusters for comparisons between individual sessions: EA>EA+NB and EA+LA>EA+NB. EA significantly activated the bilateral thalamus, basal ganglia, cerebellum, and the left putamen. EA+LA activated the left thalamus, right anterior insula and bilateral basal ganglia, cerebellum and inferior frontal gyrus. There was no significant brain activation during EA+NB. Direct comparison between EA and EA+NB showed significant brain activations in bilateral thalamus and cerebellum (EA>EA+NB). Direct comparison between EA+LA and EA+NB revealed significant brain activations in bilateral thalamus, cerebellum, putamen, and right inferior frontal gyrus (EA+LA>EA+NB). Voxel-wised threshold was p<0.005.
present study shows, for the first time, the relationship between activation of brain regions and acupuncture sensation score, although the clinical relevance of this relationship requires further investigation.

Traditional acupuncturists emphasise the importance of acupuncture sensation (de qi) in clinical practice and this is supported by research evidence. The effect of acupuncture appears strongest when an intricate feeling occurs in patients following manipulation of the needle. This feeling is often described as a sensation of soreness, numbness, heaviness, and dis-tension in the deep tissue beneath the site of needling. It has been demonstrated that intramuscular injection of LA at acupuncture points, before needling in the laboratory, eliminates de qi and abolishes the analgesic effect of acupuncture. Asghara et al demonstrated differences in patterns of brain activation between scans associated with de qi versus pain sensation. In the present study, acupuncture sensation during EA at LI4 was inhibited following blockade of the brachial plexus. At the same time, brain activation during EA was completely abolished, which suggests that EA activates brain regions by stimulating the nerves supplying the tissues in the vicinity of the acupuncture point (rather than through hypothetical meridians).

There has been considerable research interest in the identification of the individual types of nerve fibre that mediate acupuncture sensation. It has been demonstrated that acupuncture sensation involves a multitude of fibre types, ranging from the fast-conducting myelinated Aβ fibres with higher thresholds to the slow-conducting unmyelinated C fibres with lower thresholds. Experiments by Lu have revealed that the slower conducting Aβ and C fibres are involved in the dull component of the de qi sensation, while numbness and tingling may involve Aβ fibres. Using selective blockade of conduction in Aβ- and C-type afferents by applying capsaicin to the sciatic nerves bilaterally, acupuncture-induced analgesia can be completely abolished in the rat. These studies support the observation that the mechanisms of acupuncture involve the nervous system.

We used ultrasound to guide the brachial plexus block in order to increase the success rate and improve safety. However, it was difficult to determine whether nerve conduction in the brachial plexus had been completely blocked in the present study. Lambert et al demonstrated that exposure of desheathed bullfrog sciatic nerves to 0.5% bupivacaine abolishes the compound action potential, suggesting that exposure of nerves to 0.5% bupivacaine results in total conduction block. We used a similar concentration of LA containing 0.5% lidocaine and 0.5% ropivacaine in the present study. The reason for using a mixed solution is that lidocaine shortens the time to onset of the block and ropivacaine has less toxicity than bupivacaine. Assessment of the brachial plexus block showed that motor function, pain and temperature sensation were blocked using this combination of LA.

In conclusion, our results have demonstrated that EA at LI4 evoked brain activation in specific areas including the bilateral thalamus, basal ganglia and cerebellum, and the left putamen. The activation of the thalamus significantly correlated with acupuncture sensation. Blockade of the brachial plexus abolished the fMRI changes induced by EA by LI4, which suggests that EA activates brain regions by stimulating local nerves supplying the tissues at LI4.

Author affiliations
1Department of Anesthesiology, Huadong Hospital, Fudan University, Shanghai, People’s Republic of China
2Department of Anesthesiology, Minhang Hospital, Fudan University, Shanghai, People’s Republic of China

Table 2 Coordinates and T values of brain regions modulated by experimental conditions (comparisons between acupuncture sessions)

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Volume* Location</th>
<th>MNI coordinates</th>
<th>T</th>
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<tr>
<td>EA&gt;EA+NB</td>
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<tr>
<td>L 462</td>
<td>Thalamus</td>
<td>−6 −16 8 3.77</td>
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<td>Cerebellum</td>
<td>−30 −70 −34 4.09</td>
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<td>Cerebellum</td>
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<td>EA&gt;LA&gt;EA+NB</td>
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<tr>
<td>R 62</td>
<td>Inferior frontal gyrus</td>
<td>58 20 6 3.56</td>
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*p<0.005, uncorrected, spatial extent >50 voxels. *Volumes are given as number of 2.0 × 2.0 × 2.0 mm voxels.

Figure 3 Correlation between blood-oxygen-level dependent (BOLD) signal change in the thalamus, measured by functional MRI, and acupuncture sensation score during electroacupuncture (EA) stimulation at LI4. Voxel-wised threshold was set at p<0.05.
Department of Acupuncture, Shanghai Xinzhuang Community Health Service Center, Shanghai, People’s Republic of China

Key Laboratory of Brain Functional Genomics, Ministry of Education, Institute of Cognitive Neuroscience, School of Psychology and Cognitive Science, East China Normal University, Shanghai, People’s Republic of China

Contributors W G was responsible for study design, recruitment of participants, and statistical analysis, and helped to draft and finalise the manuscript. WJ was responsible for the nerve block and recruitment of participants. JH was responsible for the acupuncture. ZW was responsible for study design, fMRI data acquisition and analysis. All authors read and approved the final manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Minhang Hospital, Fudan University.

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Correction


On page 2 in the Methods subsection entitled “Experimental protocol”, the pulse width should have been stated as 0.5 ms and not 30 ms as published.

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