Acupuncture reveals no specific effect on primary auditory cortex: a functional magnetic resonance imaging study

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Although acupuncture is effective in the treatment of several clinical conditions, its specificity has been questioned. We studied the effects of needle stimulation applied to 'ear-specific' acupuncture point GB43 on activations in primary auditory cortex using functional magnetic resonance imaging in comparison with sham acupuncture. Twenty healthy volunteers participated in this cross-over investigation. Multi-subject analysis showed no significant activations in the gyrus of Heschl during stimulation of the GB43 point or a sham point. In single-subject analysis, activation within the primary auditory cortex was seen in two out of 20 volunteers. We found no evidence for specificity of acupuncture point GB43 in relation to primary auditory activation, previously suggested by two independent research groups. *NeuroReport* 00:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Over the past decade, acupuncture has been shown to be effective in the treatment of several conditions, using the methodology of randomized controlled trials [1–3]. In the most rigorous trials in favor of acupuncture, the stimulation of specific acupuncture points (verum acupuncture) was compared with the invasive control procedure (sham acupuncture). Sham acupuncture itself produces nonspecific physiological reactions, which result in a measurable clinical effect [4]. Despite these few data from clinical research, the question whether the stimulation of specific acupuncture points elicits specific physiological effects remains unclear [5].

Several investigations, using the technique of functional magnetic resonance imaging (fMRI), reported an increase of blood oxygen level-dependent (BOLD) signal in the occipital regions of the brain under stimulation of vision-related acupuncture points [6–8] and in the temporoparietal regions, under stimulation of auditory-related acupuncture points [9,10], suggesting that specificity of acupuncture could be verified using neuroimaging techniques. Later rigorous investigations, using the sham control procedure for acupuncture (regarded as somatosensory stimulation), however, could not support the previous data on visual specificity of acupuncture points [11,12].

Two experimental investigations reported that both manual needle [9] and laser stimulation [10] of acupuncture point GB43, claimed by Traditional Chinese Medicine (TCM) to be specific for the auditory system [13], produced activation of temporoparietal auditory cortex, suggesting the specificity of acupuncture without a plausible neurophysiological background. However, the methodological part of the investigation by Cho *et al.* [9] is unclear; Siedentopf *et al.* [10] used an inactive laser device as placebo control procedure, which is not comparable with sham acupuncture.

Regarding the fact that sham acupuncture produces nonspecific physiological effects, we aimed to replicate the previous findings of GB43 stimulation (verum acupuncture), using the rigorous cross-over design with sham acupuncture as a control condition.

Materials and methods

Participants

Twenty healthy, right-handed, male volunteers (age 27 ± 4 years; mean $\pm$ SD) were enrolled in this study. Eighteen volunteers were naïve to acupuncture and all of them were blind with regard to the purpose of the investigation. Clinical examination, including standard audiometric tests (audiogram), revealed no psychological, neurological, or auditory abnormalities at the time of the experiment. None of the participants were taking medication or the recreational drugs at the time of the study. Informed consent was obtained from every individual, and the local ethics committee approved this project.

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Acupuncture

A certified acupuncturist inserted stainless steel acupuncture needles (0.25 × 25 mm; Seirin Corp, Shizuoka, Japan) perpendicularly to the skin surface at a depth of approximately 0.5 cm in each participant before the functional scanning and withdrew them after the scanning procedure. Each volunteer received acupuncture at two different sites, situated at the same dermatome on the left foot: acupuncture point GB43, located between the fourth and fifth toe and a sham point, between the third and fourth toe (Fig. 1a). Each point was stimulated during one fMRI session. To prevent the influence of residual effects of acupuncture stimulation, a recovery interval between the sessions lasted at least 3 days. The order of sessions was randomized.

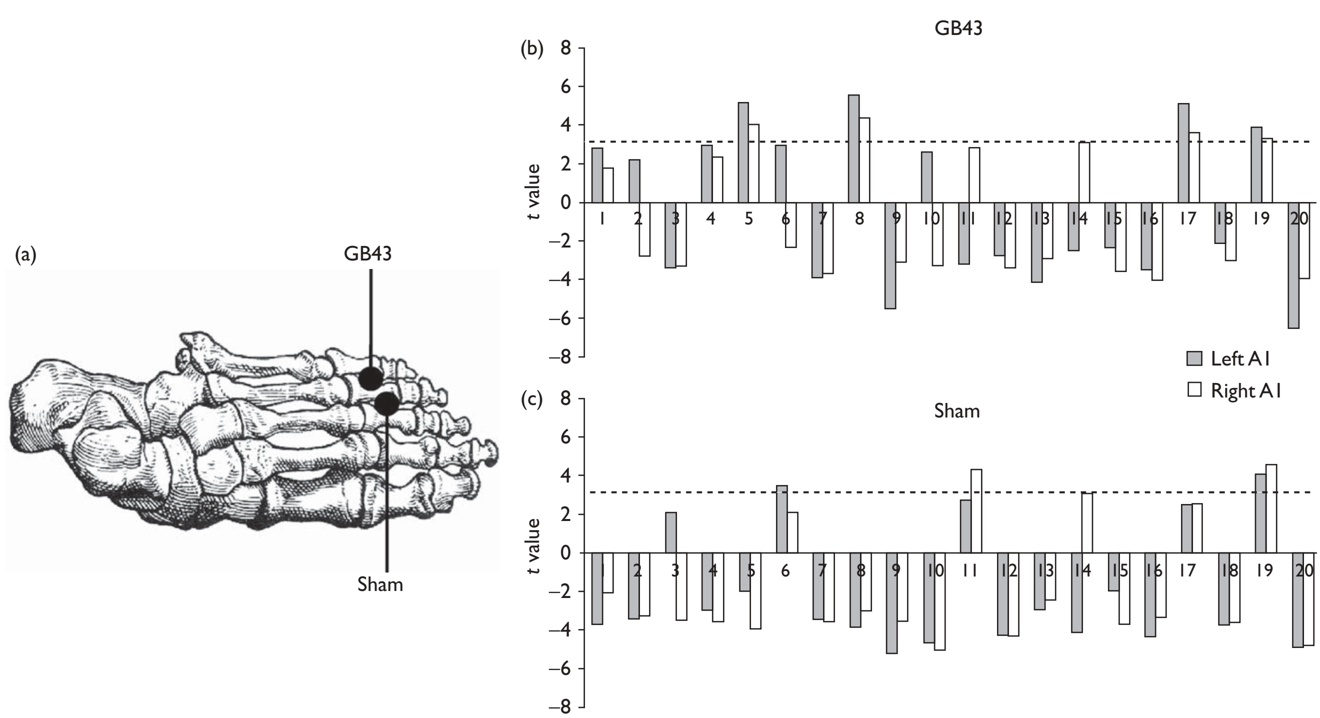
Experimental design

The volunteers were instructed to lie still, with closed eyes, in a supine position on the MRI scanner. The acupuncturist inserted the needle before fMRI scanning and before presentation of conditions was started. Lights in the scanner room were switched off, so that the volunteers, equipped with earplugs, were exposed only to muted scanner noise and acupuncture stimuli. Every session consisted of three scans, two anatomical reference scans and one functional scan in-between. The functional scan used a block design, consisting of five blocks of rest and five blocks of stimulation, with each block lasting 30 s. Each rest block was followed by a stimulation block, resulting in 300 s of total scanning time. The acupuncturist entered the MRI room just before the functional scan and stimulated the acupuncture needle by twirling with a frequency of 3 Hz only during stimulation blocks. The acupuncturist received the signal when to start and finish the stimulation by the change of colored light, which was synchronized through computer with fMRI block scans. The needle was removed after the functional scan.

Functional magnetic resonance imaging and data analysis

All sessions were carried out on a 1.5-T MR scanner (Magnetom Symphony Siemens, Erlangen, Germany), equipped with an 8-channel headcoil. T2*-weighted single-shot echo-planar image sequences were applied for the functional scan (33 slices, slice thickness = 3 mm, repetition time = 3000 ms, echo time = 50 ms, flip angle = 90°, field of view = 192 mm, matrix = 64 × 64, voxel size = 3 × 3 × 3 mm). To process and analyze the functional data with the 'BrainVoyager QX 1.9' software

Fig. 1



(a) Stimulated sites of the left foot: acupuncture point GB43, claimed to be specific for the auditory system, is located between the fourth and fifth toe proximal to the margin of the web. Sham acupuncture point is located between the third and fourth toe in the same area of peripheral innervation (spinal dermatome S1) as the acupuncture point GB43. *t* Values obtained in general linear model on single-subject analysis during: (b) verum acupuncture (stimulation of 'ear-related' acupuncture point GB43); and (c) sham acupuncture (stimulation of nonacupuncture point). The threshold was set at *P* value of less than 0.001, uncorrected; dashed line means *t* value of 3.34, set as criteria for contrasting the results.

(Brain Innovation B. V., Maastricht, The Netherlands), an additional high-resolution T1-3D flash sequence was conducted (176 slices, slice thickness = 1 mm, TR = 11 ms, TE = 5.2 ms, flip angle = 15°, FoV = 256 mm, matrix = 256 × 256, voxel size 1 × 1 × 1 mm). Raw data were processed and analyzed using the software 'BrainVoyager QX 1.9'. First, a slice scan time correction was performed. The functional images of each volunteer were realigned to the first scan of the first session to correct possible head movements. Echo-planar images were then coregistered to the high-resolution volume of the structural images. Images were normalized into Talairach space and spatially smoothed with a three-dimensional isotropic Gaussian kernel (full-width-at-half-maximum: 6 mm). Temporal filtering was done with the linear trend removal and a high-pass filter with a cutoff of 0.01 Hz.

Preprocessed data were statistically analyzed, using the general linear model on multi-subject and single-subject level. For multi-subject analysis, a design matrix was created from the functional data of all volunteers, using the canonical hemodynamic response function [14]. To make inferences at the population level, a random-effect analysis was performed. Similarly, statistical analysis was done on single-subject level. Single-subject statistics were performed to correct possible imprecise overlay of cortical activation maxima because of the Talairach normalization procedure [15]. However, as statistical conclusions were only drawn for a single participant, a fixed-effect analysis with separate study predictors and correction of serial correlations was conducted for the single-subject analysis.

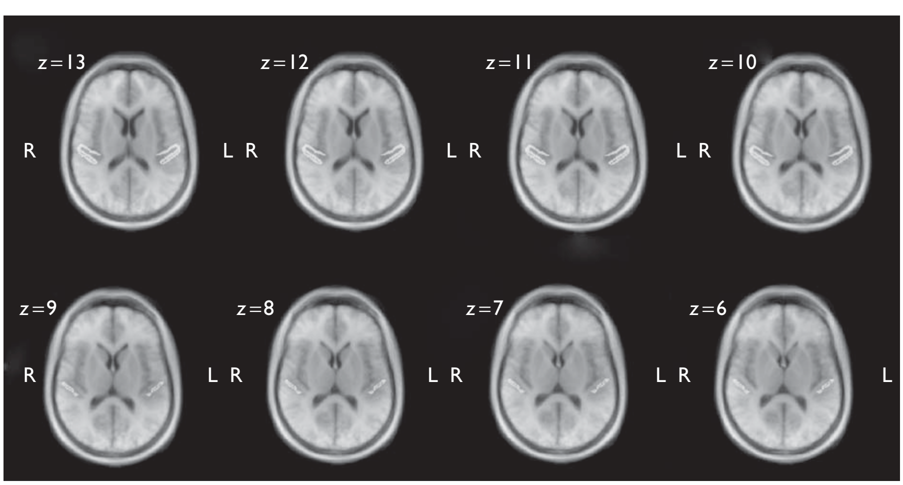
On both levels, BOLD responses were investigated for verum acupuncture (GB43), sham acupuncture and the contrast 'GB43 minus sham'. As the hypothesis was focused on whether or not the primary auditory cortex was activated, a region-of-interest analysis was performed. A standard template for the primary auditory cortex was provided with 'BrainVoyager QX' Software. Acquired statistical maps were adjusted to a threshold of P value of less than 0.001 (uncorrected), which resembled a minimum t value of 3.34 on a single-subject level and 3.88 on a multi-subject level. After each session, the volunteers where asked: (i) to report the pain intensity during acupuncture, using a visual analogue scale (VAS-100), where 0 = no pain and 100 = maximal imaginable pain; (ii) about suspected location of needling site; and (iii) any other perceptions matching the 'Qi' sensation described in TCM (heaviness, fullness, warmth, cooling, tingling, numbness, pressure). Differences in pain intensity on VAS-100 were analyzed using the Wilcoxon's signed-rank test, for dichotomous data on needle location and the incidence of 'Qi,' the McNemar test was used.

Results

Multi-subject analysis showed no significant activation (exceeding the minimum t value at $P < 0.001$ uncorrected) in the gyrus of Heschl on both hemispheres during stimulation of GB43 point (Fig. 2) and the sham point. Therefore, the contrast 'GB43 minus sham' presented no activations either.

In a single-subject analysis, four volunteers (no. 5, 8, 17, and 19) showed activations during stimulation of GB43 (Fig. 1b). During sham acupuncture (Fig. 1c), activation was registered in three volunteers (no. 6, 11, and 19). After contrasting 'GB43 minus sham', bilateral activation within the primary auditory cortex was seen in volunteer

Fig. 2



Activation maps obtained from general linear model on multi-subject analysis during verum acupuncture (stimulation of the 'ear-related' acupuncture point GB43). No significant activations were found in the primary auditory cortex (template – white curved line – adjusted to gyrus of Heschl, eight transversal slices are shown; coordinates of slice position were given in the upper left corner of each slice). L, left; R, right.

no. 5 (57 voxels activated with *t* value of maximally activated voxel 4.55) and volunteer no. 8 (398 voxels activated with *t* value 6.69).

Pain intensity after the stimulation of GB43 did not differ from that under sham condition (median 38 vs. 34; $P > 0.05$). Eight volunteers reported paresthesia ('Qi' sensation) during both GB 43 and sham procedure ($P > 0.05$).

Discussion

We investigated the effect of acupuncture stimulation of the 'ear-specific' point GB43 on fMRI activation in the primary auditory cortex in comparison with sham acupuncture. In multi-subject analysis we did not detect a difference between acupuncture and sham procedure with regard to activation of the Heschl gyrus, which approximates the cytoarchitectonic region of the primary auditory cortex [16].

Individual analysis revealed activation in the primary auditory cortex in four out of 20 volunteers during verum acupuncture. However, only two of these volunteers revealed activation in the gyrus of Heschl after contrasting with sham acupuncture. Single-subject analysis showed that the negative result, obtained in multi-subject analysis, was not caused by problems of misregistration in the Talairach space. In addition, we compared verum acupuncture with the sham procedure, which elicited a pain of comparable intensity and an equal incidence of paresthesia in volunteers. Differences in attention in those participants who showed significant activation in the Heschl gyrus to those who showed none are unlikely as the scoring of pain and paresthesia did not differ to the other participants. These effects, however, cannot completely be excluded.

In contrast to the findings of Cho *et al.* [9] and Siedentopf *et al.* [10], we did not find a significant BOLD effect in the primary auditory cortex after stimulation of 'ear-specific' acupuncture point GB43. Our data, however, suggest that on a single-subject level some participants (four out of 20 volunteers in this study) may show activation in primary auditory cortex.

This anecdotal finding may be the cause for reports on activation of primary visual and auditory cortex during stimulation of acupuncture points, claimed by TCM to be related to visual or auditory systems.

The inability to confirm specific effects of GB43 stimulation on the activation of the auditory cortex in this study may be related to several reasons. First, previous investigations did not use significance thresholds, which are now consensus [17]. In the very early days of fMRI activation sites were often described in the same way as structural images were treated. Any increase of BOLD magnitude during the activation period in comparison with the resting period was assessed as activation, without taking into account important problems, such as corrections for multiple comparisons. Second, prior publications described the subjects on the level of a single-subject analysis (four out of 20 volunteers in this study revealed the activation in the primary auditory cortex after verum acupuncture), showing the individual hemodynamic response of cortical microvasculature to neuronal activation under peripheral stimulation of differently innervated acupuncture points [18]. Third, an adequate sham stimulation has to be applied in the same participant group, which should be located in the same innervation area but not on a classical acupuncture point, which has been associated with the somatosensory input. In this study even the sham stimulation evoked significant activation in the gyrus of Heschl in three of 20 participants, but only two of 20 showed increased gyrus of Heschl activation when comparing classical versus sham acupuncture.

Cho *et al.* [6] described activations in the primary visual cortex Brodmann's area (BA) 17 during acupuncture of the BL67 point, which was similar to visual light stimulation. These effects were, however, not supported by appropriate group statistics. Another problem is the application of an adequate sham procedure: Siedentopf *et al.* [7] stimulated the BL67 using laser and compared it with a placebo (noninvasive) procedure. Using a single-subject analysis, the authors found activations of the BA 18, 19 of the visual cortex ipsilateral to laser stimulation comparable with no cortical effects during a placebo procedure [7]. Li *et al.* [8] applied acupuncture and electric current to four 'eye'-specific points on the right foot, including the BL67 and compared this with light stimulation. The authors described the bilateral activations of the BA 18 in the majority of the studied volunteers, using group multi-subject analysis of fMRI data, although the invasive control procedure (sham acupuncture) was not performed.

For the auditory domain, Cho *et al.* [9] reported that bilateral activations in the primary auditory cortex, was comparable to listening to music. This result was published as a figure of BOLD-signal changes in a book chapter lacking appropriate description of the methodological background and statistics, thus encouraging us to carry out this study. Siedentopf *et al.* [10] studied the effects of laser stimulation at GB43 versus placebo condition (inactive device) with an fMRI in 22 healthy volunteers, with 11 volunteers being stimulated on the right foot and 11 on the left. Only the laser stimulation of the right foot was associated with activations in the BA 40 and 22 ipsilateral to the stimulation site. This finding is, however, presented as a result of the group analysis, leaving unclear whether contrast conditions were applied and how many volunteers revealed activation patterns in the auditory cortex.

To reduce the individual variability in this study, we sought to standardize the physiological conditions of the volunteers (cross-over design, appropriate number of volunteers, blind in regards to the aim of the study, the same pain intensity, and incidence of paresthesia during acupuncture), randomized intervention (the same experienced acupuncturist for all volunteers), and fMRI investigation (the same time of day). As hearing impairment can cause the reorganization of the auditory cortex [19], we included only healthy volunteers with normal audiograms. We used 'state-of-the-art' statistical analysis on both multi-subject and single-subject levels, contrasting verum acupuncture with the most rigorous control – sham acupuncture [20].

The limitations of the study include: the poor temporal resolution of the fMRI technique, compared with electrophysiological methods such as electroencephalography and magnetoencephalography. What we are going to improve and include in future studies is the monitoring of volunteers' attention, which was not performed in the present investigation and might have influenced the brain perception of somatosensory stimulation [21].

Conclusion

Studying the activation of the primary auditory cortex using the fMRI technique in an experimental cross-over design with a sham control condition, we found no evidence for specificity of the acupuncture point GB43 in relation to primary auditory activation, previously suggested by two independent research groups.

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