Deconvolution Analyses with Tent Functions Reveal Delayed and Long-sustained Increases of BOLD Signals with Acupuncture Stimulation

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We used deconvolution analysis to examine temporal changes in brain activity after acupuncture stimulation and assess brain responses without expected reference functions. We also examined temporal changes in brain activity after sham acupuncture (noninsertive) and scrubbing stimulation. We divided 26 healthy right-handed adults into a group of 13 who received real acupuncture with manual manipulation and a group of 13 who received both tactical stimulations. Functional magnetic resonance imaging (fMRI) sequences consisted of four 15-s stimulation blocks (ON) interspersed between one 30-s and four 45-s rest blocks (OFF) for a total scanning time of 270 s. We analyzed data by using Statistical Parametric Mapping 8 (SPM8), MarsBaR, and Analysis of Functional NeuroImages (AFNI) software. For statistical analysis, we used 3dDeconvolve, part of the AFNI package, to extract the impulse response functions (IRFs) of the fMRI signals on a voxel-wise basis, and we tested the time courses of the extracted IRFs for the stimulations. We found stimulus-specific impulse responses of blood oxygen level-dependent (BOLD) signals in various brain regions. We observed significantly delayed and long-sustained increases of BOLD signals in several brain regions following real acupuncture compared to sham acupuncture and palm scrubbing, which we attribute to peripheral nocireceptors, flare responses, and processing of the central nervous system. Acupuncture stimulation induced continued activity that was stronger than activity after the other stimulations.

We used tent function deconvolution to process fMRI data for acupuncture stimulation and found delayed increasing and delayed decreasing changes in BOLD signal in the somatosensory areas and areas related to pain perception. Deconvolution analyses with tent functions are expected to be useful in extracting complicated and associated brain activity that is delayed and sustained for a long period after various stimulations.

Keywords: acupuncture, deconvolution, delayed, fMRI, long-sustained increase

Introduction

Acupuncture has been used worldwide to treat a variety of diseases and symptoms for more than 2,000 years, but its mechanisms and efficacy are not clear. In the past 20 years, the development of imaging techniques, such as functional magnetic resonance imaging (fMRI), has opened the door for the study of brain activity in response to acupuncture stimulation. Numerous studies have been performed with fMRI and a variety of acupuncture-related stimulations, such as electroacupuncture,1,2 manual acupuncture,1–13 sham acupuncture of the skin some distance from a known acupoint4–6 or by noninsertive acupuncture and poking of the skin over an acupuncture point,7–11 pressure to the acupoint,12 laser acupuncture,13 and electrical acupuncture stimulation.14 fMRI studies of acupuncture have typically utilized a block design that is used as a
general linear model regressor in the analysis of fMRI data based on the assumption that mechanoreceptors and nociceptor-associated afference work coincidentally with acupuncture stimulation, which is the conscious perception and evaluation of this afference. However, recent studies have questioned the equivalence of stimulations with sensation in block-design acupuncture experiments, and the persistence of the sensation introduced by acupuncture needling has been demonstrated. A temporal-shifted blood oxygen level-dependent (BOLD) model has also been reported to produce higher statistical power than an unshifted BOLD model with acupuncture stimulus. However, no reports have elaborated on delayed BOLD response after acupuncture stimulation. Deconvolution analysis was useful for extracting a BOLD impulse response induced by the stimulation. Typically, deconvolution analysis has been used to quantitate the evolution of activity during some neuronal process in related design experiments; it is also useful for detecting an unpredictable BOLD response in block design experiments. However, we could not use an event-related design because acupuncture stimulation should be continuous for a certain duration, so we instead used Analysis of Functional NeuroImages (AFNI) 3dDeconvolve software to predict the BOLD response after stimulation, extracting hemodynamic response functions (HRFs) of the fMRI signal on a voxel-wise basis. This program uses a sum of scaled and time-delayed versions of the stimulus time series.

To extract delayed and long-sustained brain activity caused by acupuncture stimulation, we performed deconvolution analysis of temporal changes in brain activity to analyze brain responses without expected reference functions.

Materials and Methods

The ethics committee of the Meiji University of Integrative Medicine (Kyoto, Japan) approved the study, and subjects provided written consent after they were instructed regarding the type of stimulation. We divided the subjects, 26 healthy right-handed adults (16 men, 10 women, aged 21 to 33 years), into 2 groups of 13; one group received real acupuncture stimulation with manual manipulation, and the other received both sham acupuncture and scrubbing stimulation.

We selected the LI-4 acupoint on the dorsum of the right hand radial to the midpoint of the second metacarpal bone as the stimulation site. Real acupuncture stimulation consisted of inserting a non-magnetic silver needle (diameter, 0.20 mm; length, 39 mm; Asahi Industry, Inc. Kawaguchi, Japan) into the LI-4 to a depth of approximately 15 mm before initiating the fMRI scan and manually twirling the needle bidirectionally approximately 180° at one Hz during the scan. Two types of tactile stimulation were used as controls—tapping with a von Frey monofilament (sham acupuncture) and scrubbing with a scrubbing sponge. Sham acupuncture consisted of gentle tapping of the skin surface on the LI-4 acupoint with a 5.88 von Frey monofilament. During the stimulation block, tapping was continuously applied at approximately 4 Hz. Another control stimulation was administered by scrubbing the subject’s right palm with a scrubbing sponge in one direction at approximately 4 Hz. A single licensed and experienced acupuncturist performed all acupuncture and tactile stimulations. In the preliminary experiment, we confirmed disappearance of subjective sensation within 45 s after cessation of acupuncture manipulation. All fMRI run sequences consisted of four 15-s stimulation blocks (ON) interspersed between one 30-s and four 45-s rest blocks (OFF) for a total scanning time of 270 s (Fig. 1).

Subjects were supine and wearing earplugs in the scanner. To minimize head motion, soft foam pads were inserted into the standard head coil. Functional MR imaging scans were conducted on a 1.5-tesla Signa MR scanner (GE Healthcare, Milwaukee, WI, USA). Anatomical images were acquired with a 2-dimensional (2D) T1-weighted conventional spin echo sequence (repetition time [TR], 350 ms; echo time [TE], 8.0 ms; flip angle [FA], 90°, field of view [FOV], 22 cm; 30 axial slices; slice thickness, 5 mm; matrix size, 256 × 192) and a 3-dimensional (3D) T1-weighted gradient echo sequence (TR, 8.9 ms; TE, 1.8 ms; inversion time, 600 ms; 270(s))

Fig. 1. All functional magnetic resonance imaging (fMRI) run sequences consisted of four 15-s stimulation blocks (ON) interspersed with one 30-s and four 45-s rest blocks (OFF) for a total scanning time of 270 s.
time courses of the extracted IRFs were tested at each time point across the stimulations. For statistical comparisons, we performed one-way analysis of variance (ANOVA) followed by Bonferroni correction for the activated areas colocalized in 3 stimulations, and we used unpaired t-test for the activated areas colocalized in 2 stimulations.

Results

The fMRI group maps of the main effects demonstrated significant brain activation in all stimulation conditions (Table). Figure 2 shows the activated areas and temporal evolution of brain responses during each stimulus. These data were consistent with the IRFs from the deconvolution analysis. In those receiving real acupuncture, we observed areas of stimulus-induced activation on both sides in the secondary somatosensory cortex (SII) and the insula in 4T and on both sides in the primary somatosensory cortex (SI), primary motor cortex (MI), anterior cingulate cortex (ACC), supplementary motor area (SMA), thalamus, and prefrontal cortex in 6-8T (Fig. 2, Red and Table, Real). The fMRI performed during sham acupuncture showed activation in the contralateral SI and SMA and on both sides in the SII and insula during simulation (3-5T) (Fig. 2, Blue and Table, Sham). During scrubbing stimulation, we observed activated areas in the contralateral SI, MI, and SMA and

<table>
<thead>
<tr>
<th>Table. Cerebral regions showing significant blood oxygen level-dependent (BOLD) increases in signal in each stimulation versus the baseline by Analysis of Functional NeuroImages (AFNI) 3dDeconvolve software (uncorrected P&lt;0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real (N=13)</td>
</tr>
<tr>
<td>BA1 – 3 (SI)</td>
</tr>
<tr>
<td>BA40, BA43 (SII)</td>
</tr>
<tr>
<td>Thalamus</td>
</tr>
<tr>
<td>BA13 (insula)</td>
</tr>
<tr>
<td>BA6 (SMA)</td>
</tr>
<tr>
<td>BA4 (MI)</td>
</tr>
<tr>
<td>BA24, BA32 (ACC)</td>
</tr>
<tr>
<td>BA9, BA10 (prefrontal cortex)</td>
</tr>
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</table>

ACC, anterior cingulate cortex; BA, Brodmann area; MI, primary motor cortex; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; SMA, supplementary motor area. The site of statistically significant activation is shown as L (left [contralateral side]), R (right [ipsilateral side]), or (–) (no significant activation).
Fig. 2. Overlapped group statistical maps for each tent function in the 3 groups. Regions exhibiting significant activation in real acupuncture (Red: N = 13), sham acupuncture (Blue: N = 13), and scrub condition (Green: N = 13). A random analysis was performed, and activations are overlapped onto an axial slice of a standard brain (P < 0.001, uncorrected). 1T−11T indicate the temporal axis, and each interval of T (tent function) represents 6 s (= 2 TR) (i.e., 1T represents 0 s, 2T represents 6 s).

Fig. 3. Time course of beta amplitude changes (means ± standard error [SE]) for real acupuncture (red: N = 13), sham acupuncture (blue: N = 13), and scrub (green: N = 13) condition in the (a) primary somatosensory cortex (SI), (b) supplementary motor area (SMA), (c) secondary somatosensory cortex (SII), (d) insula, (e) primary motor cortex (MI), (f) prefrontal cortex, (g) anterior cingulate cortex (ACC), and (h) thalamus. The areas identified as exhibiting activation in the 3 stimulations are the (a) SI, (b) SMA, (c) SII, and (d) insula. We performed one-way analysis of variance (ANOVA) followed by Bonferroni post hoc comparison at each time point among the 3 groups. The area identified as exhibiting activation in 2 of the stimulations was the (e) MI. We performed unpaired t-test at each time point to reveal the time range of the significant impulse response function (IRF) adaptations during the trial. Activation was also observed in the (f) ACC, (g) prefrontal cortex, and (h) thalamus by real acupuncture.
on both sides in the SII and insula (3–5T) (Fig. 2, Green and Table, Scrub). These results demonstrated many similarities in activation between sham acupuncture and scrubbing stimulation. Real acupuncture induced more widespread brain activity and more delayed and long-sustained brain activity than the tactile stimulations.

Figure 3 shows the extracted IRFs in the various brain regions. In the SI and SMA, significantly larger increases in signal during stimulation were induced by palm scrubbing than by real and sham acupuncture. However, real and sham acupuncture induced delayed increases in the signals after stimulation. In the SII and insula, only real acupuncture showed delayed and sustained signal increases after stimulation. In contrast, responses evoked by sham acupuncture and scrubbing decreased more rapidly after the cessation of stimuli than responses to real acupuncture. MI activation was only observed in real acupuncture and scrubbing, and the increases in signal during stimulation induced by scrubbing were significantly larger than the signal induced by real acupuncture. In contrast, real acupuncture induced delayed and long-sustained increases in signal after stimulation. Only real acupuncture induced activation in the ACC, prefrontal cortex, and thalamus, and delayed and long-sustained increases of signal were observed after stimulation in these areas.

Discussion

In this study, we investigated the temporal dynamics of BOLD signal responses during various stimulations, including real acupuncture, sham acupuncture, and palm scrubbing. We utilized deconvolution analyses to analyze brain responses without expected reference functions. We found stimulus-specific impulse responses of the BOLD signals in various brain regions. We observed significantly delayed and long-sustained increased signals in several brain regions during real acupuncture compared to sham acupuncture and palm scrubbing.

In this study, we used deconvolution analysis with tent basis function to analyze brain responses without using expected reference functions. Independent component analysis (ICA) is a known method for blind signal separation in fMRI research. However, typical ICA in whole brain fMRI study are applied to the spatial domain; ICA is not suited to separate components that represent a partially overlapped time course at one voxel, such as original and prolonged response. Furthermore, in a repeated experimental design, such as a block design paradigm, deconvolution analysis could utilize every time course of signals relating to stimulus to strengthen the extraction of BOLD impulse response. Therefore, we considered deconvolution using tent functions suitable in this study.

We found more prominent activation of the sensorimotor regions of the brain (SI, SII, SMA, and MI) during scrub stimulation than real and sham acupuncture. We also observed a tight correspondence of the IRF of palm scrubbing in these areas to the period of stimulation, with a rapid increase and immediate decrease to baseline. However, real and sham acupuncture invoked IRFs that showed delayed increase over the period of stimulation, and these increased signals persisted long after cessation of the stimulus, especially real acupuncture. The scrubbing task activated the brain only during stimulation, a finding reported in a previous study.

It has been reported that SI processing is performed with input provided by mechanoreceptors, but the activation of much of the SI has not been confirmed by acupuncture stimulation. The peripheral receptors involved in acupuncture stimulation have been suggested to be the polymodal receptors that are nociceptive receptors of the C-fibers. It is also thought that the input is the transmission through C-fiber in response to production of a chemical substance following deep tissue injury by manual acupuncture stimulation. One previous study reported that pain stimuli (C-fiber input) evoked weak activity in the SI. In fact, substantially fewer areas in the SI were activated by real and sham acupuncture than palm scrubbing. This is likely due to the hardly activated mechanoreceptors by the acupuncture needle and von Frey filament stimulation of the pinpoint region on the skin. A magnetoencephalogram study reported that C fibers that mediate pain induce late and sustained cortical activity. Therefore, we suggest that polymodal receptors and C-fibers were involved in the delayed and long-sustained increased signals invoked by acupuncture stimulation.

Real and sham acupuncture elicited temporal changes that exhibited similar trends. In general, most subjects felt the de-qi sensation during acupuncture stimulation at the LI-4 acupoint more frequently than at other acupoints of the crus. A previous study reported that the de-qi sensation is related to unmyelinated nerve fibers (C-fibers). C-fibers produce a flare response (e.g., in neurogenic inflammation) in response to acupuncture stimulation. Flare responses induced by polymodal receptors on the skin are also observed with sham acupuncture. Neurogenic inflammation induces
local hyperalgesia. In addition, repeated C-fiber input following C-fiber stimulation results in a progressive, windup, increase in response of nociceptive spinal neurons.\textsuperscript{34} The delayed and long-sustained increases in signal change occur in response to the flare response.

The apparent relevance of neural responses evoked by acupuncture stimuli to most or all functions associated with pain has been reported. These include areas of the brain involved in pain affect (ACC, insula, prefrontal cortex, and SMA) and pain modulation (ACC).\textsuperscript{35} A previous study has reported that input from the C-fibers arrives at the SII and insula 500 ms after stimulation,\textsuperscript{28} but processing is believed to be long because pain perception is a higher brain function that involves complicated and associated brain activity. Therefore, we considered that delayed and long-sustained increases in signal affect brain activity with real acupuncture stimulation.

**Conclusion**

Real acupuncture stimulation induced delayed and long-sustained increases in brain activity, which we attribute to peripheral nocireceptors, flare responses, and processing in the central nervous system. Using tent function deconvolutions to process the fMRI data of acupuncture stimulation, we found delayed increasing and delayed decreasing changes in BOLD signal in the somatosensory region and in areas related to pain perception. Deconvolution analysis with tent functions is expected to be useful for extracting more complicated and associated brain activity that is delayed and long-sustained after various stimulations.

**References**

17. Ho TJ, Duann JR, Chen CM, et al. Temporally shifted hemodynamic response model helps to extract acupuncture-induced functional magnetic res-


34. Li J, Simone DA, Larson AA. Windup leads to characteristics of central sensitization. Pain 1999; 79:75–82.