Consecutive Acupuncture Stimulations Lead to Significantly Decreased Neural Responses

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Abstract

Objective: Functional magnetic resonance imaging (fMRI), in combination with block design paradigms with consecutive acupuncture stimulations, has often been used to investigate the neural responses to acupuncture. In this study, we investigated whether previous acupuncture stimulations can affect brain activations of later acupuncture stimulations.

Design: All subjects were measured twice in the same scanning session and a block design was used.

Setting: The study was conducted at Kyung Hee University, Seoul, Republic of Korea.

Subjects: Fifteen (15) healthy right-handed male volunteers participated in the study.

Intervention: The subjects received two acupuncture stimulations on BL62 (*Shenmai*) on the right foot with a time interval of 5 minutes. In addition, sham stimulations were tested with the same paradigm.

Outcome measures: One-sample *t* tests were conducted in order to map the brain activations induced by the acupuncture and sham stimulations. Moreover, paired-sample *t* tests were conducted to investigate the signal changes between the first and second acupuncture stimulation.

Results: During the first acupuncture stimulation, in the left hemisphere, significant foci of activation were found in the hypothalamus, thalamus, claustrum, cerebellum, inferior frontal gyrus, and the superior temporal gyrus. In the right hemisphere, a significant focus of activation was found in the middle frontal gyrus. In addition, in both hemispheres, a significant focus of activation was found in the inferior parietal lobule. Interestingly, however, during the second acupuncture stimulation, the only areas that were also significantly activated were the cerebellum in the left hemisphere and the inferior parietal lobule in the right hemisphere.

Conclusions: We found that consecutive acupuncture stimulations on BL62 affected the neural responses in a significant way, resulting in decreased activations during the second acupuncture stimulation. This is an important finding, suggesting that in future fMRI studies on acupuncture, researchers should take this methodological issue more seriously.

Introduction

ACUPUNCTURE IS AN IMPORTANT therapeutic technique in alternative and complementary medicine. Despite its relatively recent arrival in Western countries, acupuncture has been increasingly accepted as an alternative or complementary method,^{1–3} and research into its effects is gaining popularity.^{4,5}

The development of neuroimaging techniques, such as positron emission tomography and functional magnetic resonance imaging (fMRI), made it easier to investigate the neural responses to acupuncture stimulations in humans.⁶ fMRI in particular is popular since it is a noninvasive neuroimaging technique that can demonstrate the neural changes induced by acupuncture. As a result, fMRI has been one of the most important tools to investigate acupuncture

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efficacy in the last 20 years.⁷⁻⁹ In most of these fMRI studies on acupuncture, a block design is used^{10,11} in which consecutive acupuncture stimulations are given in order to detect neural responses.

So far, previous research in which the same consecutive stimulations (not acupuncture) were used showed that the neural responses to the later stimulations are different. In a visual stimulation study with electroencephalography-fMRI, for instance, it was found that both signals contained stimulation-specific linear decreases.¹² Moreover, a significant decrease in strength of activation occurred from the first scanning session to the several following sessions in the anterior cingulate cortex, primary somatosensory cortex, and supplementary motor cortex, which may be explained by an analogous decrease in pain ratings.¹³ It was suggested that during the serial brain imaging studies, a decrease in subjective perception of pain and brain activity from the first to the final scan might be affected by habituation.^{12,13}

Until now, several studies on the so-called "carryover effect" of acupuncture stimulations have been conducted,^{14,15} showing that consecutive acupuncture stimulations induced significantly different results when they were analyzed with different portions of acupuncture fMRI data.¹⁴

In this study, in line with previous neuroimaging research on consecutive stimulations,¹²⁻¹⁴ we formulated the following two hypotheses: First, we expected that the first acupuncture stimulation would affect brain activations of the second acupuncture stimulation. Moreover, we hypothesized that consecutive acupuncture stimulations would lead to significantly decreased activations.

Materials and Methods

Target population

Fifteen (15) healthy right-handed male volunteers participated in this study following written informed consent according to the institutional guidelines of the Human Research Committee of Kyung Hee University. All subjects were between 20 and 35 years old (the mean age was 24.5 ± 3.0 years) and had previously experienced acupuncture treatments, since such treatments are very common in Korea.

Acupuncture

Disposable needles were used (diameter, 0.25 mm; length, 30 mm; stainless steel, Dongbang Acupuncture Inc., Seoul, Korea). At BL62 (*Shenmai*), a needle was inserted into the skin to a depth of 10 mm and rotated bidirectionally at two times a second (2 Hz). For the sham acupuncture stimulation, the skin at right BL62 was stimulated by poking it with a blunt-type needle at 2 Hz.¹⁶

Acupoint-BL62 (right). BL62 is located on the lateral aspect of the foot, directly inferior to the prominence of the lateral malleolus, in the depression between the inferior border of the lateral malleolus and the calcaneus. According to the World Health Organization (WHO) Standard Acupuncture Point Locations (Fig. 1), BL62 is the starting point of the Yang Heel Vessel, which is related to movement, sleep, and arousal.

FIG. 1. Acupuncture and sham, during functional magnetic resonance imaging scanning, was accomplished at BL62.

Design

Two (2) separated blocks were used, with each paradigm lasting 4 minutes. As can be seen in Figure 2, after the patient rested for 60 seconds, the acupuncture needle was inserted into the skin and rotated bidirectionally at 2 Hz for 30 seconds, after which it was removed immediately. After a rest period of 60 seconds, the acupuncture manipulation was repeated in the same manner followed by another 60-second rest period. During the fMRI experiment, all acupuncture manipulations on each participant were performed by 2 licensed oriental medical doctors who had 5 years of clinical experience.¹⁶ There was a 5-minute intermission between each session, and the order of doctors was randomized. After this first part of the study, sham stimulations were performed and the same paradigm as for the acupuncture stimulations was used. Participants did not know which doctor was performing acupuncture/sham and they were told that they would get acupuncture stimulations 4 times during fMRI scanning. To reduce variances between physicians, an acupuncture tube was used for defining the depth of insertion and the number of rotations was unified at 2 Hz. Following Traditional Chinese Medicine practice, insertion

FIG. 2. The scanning paradigm consisted of three rest conditions and the first and second acupuncture stimulation conditions.

of acupuncture needles was performed in a manner to elicit a characteristic somatosensory reaction in participants, which is called *de qi*. *De qi* is a unique sensation of numbness, tingling, fullness, and/or dull ache that is induced at the site of acupuncture and may spread some distance from the acupuncture point during needle manipulation. After acupuncture, the psychophysical response of every participant was obtained.

Data acquisition

Images were acquired with a 3-Tesla MRI scanner (ISOL Tech, Kyunggido, Korea). Blood oxygen level-dependent (BOLD) functional imaging was obtained using a gradient-echo T2*-weighted pulse sequence (echo time [TE] = 25 ms, repetition time [TR] = 3000 ms, matrix = 64 × 64, field of view = 220 mm, flip angle = 90 degrees). The volume number was 30 and each slice was 5 mm thick without a gap. The three-dimensional-T1 (TE = 5.7 ms, TR = 10 ms, flip angle = 10 degrees)-weighted images were collected with a high resolution.

Data analysis

SPM5 (www.fil.ion.ucl.ac.uk/spm) was used for the data preprocessing and statistical analysis. The functional echo planar imaging-BOLD images were realigned and the subject-mean functional magnetic resonance images were coregistered with the corresponding structural images. All images were normalized to the standard SPM5 template and were spatially smoothed with 8 mm with full width at half maximum, using a Gaussian filter.

One-sample *t* tests were conducted in order to map the brain activations induced by the acupuncture and sham stimulations. Moreover, paired-sample *t* tests were conducted to investigate the brain areas, which showed a significant signal change between the first and second acupuncture stimulation ("1st < 2nd acupuncture stimulation" and "1st > 2nd acupuncture stimulation"). In all analyses, a threshold of uncorrected *p* < 0.005 was used and only those results were reported with a spatial extent > 50 voxels. Finally, all local maxima are reported as Talairach coordinates.

Results

After scanning, subjects rated the type and intensity of sensations they felt during the acupuncture stimulations. As can be seen in Table 1, 10 of the 15 subjects experienced distinct sensations of *de qi* during acupuncture. Among them, 4 subjects experienced *de qi* by both doctors, whereas 6 subjects experienced *de qi* by only by 1 of them. There was no statistical difference between the first and second sensations.

As can be seen in Table 2, during both the first and second acupuncture stimulation, the left cerebellum and the right inferior parietal lobule (BA 40) were significantly activated. However, the right middle frontal gyrus (BA 9), left inferior frontal gyrus (BA 44), left superior temporal gyrus (BA 13), left inferior parietal lobule (BA 40), left hypothalamus, left thalamus, and left claustrum were only significantly activated during the first acupuncture stimulation and the right thalamus only during the second acupuncture stimulation. During sham, no significant brain activations were found with our threshold (Fig. 3).

TABLE 1. CHARACTERISTICS OF THE SUBJECTS SPECIFIED FOR SUBJECT NUMBER, GENDER, AND AGE AND THE EXPERIENCED ACUPUNCTURE SENSATIONS (*De Qi*)

Subject	Gender	Age	<i>De qi</i> ^a	
			<i>P</i> ^b 1	<i>P</i> 2
1	Male	23	Yes	Yes
2	Male	29	No	No
3	Male	24	No	No
4	Male	23	No	Yes
5	Male	24	Yes	Yes
6	Male	32	No	No
7	Male	23	No	Yes
8	Male	25	No	No
9	Male	25	Yes	No
10	Male	26	Yes	Yes
11	Male	23	No	Yes
12	Male	22	Yes	No
13	Male	26	Yes	Yes
14	Male	20	No	No
15	Male	22	Yes	No

^aThe table shows whether or not the subjects experienced distinct sensations of *de qi*.

^bP, physician.

As can be seen in Table 3, the results of the paired-sample *t* test between the first and second acupuncture stimulation showed that the left paracentral lobule (BA 6), left cingulate gyrus (BA 24), left medial globus pallidus, left and right insula (BA 13), left claustrum, left and right thalamus, and left cerebellum were only significantly activated in the first > second acupuncture stimulation comparison and not in the first < second acupuncture stimulation comparison (Fig. 4).

Discussion

We investigated the neural responses to consecutive acupuncture stimulations in a group of healthy subjects. Our hypothesis was that the first acupuncture stimulation would affect the brain activations of the second acupuncture stimulation. More specifically, in line with previous stimulation studies (on other stimulations than acupuncture), which showed that consecutive stimulations lead to decreased neural activations,^{12,13,17} we also expected that the neural responses to the second acupuncture stimulation would be decreased compared to the first acupuncture stimulation.

The results of the survey on *de qi* showed that there was no statistical difference between the first and second sensations. This finding supports that the subjects' neural responses were not because of the feeling, but because of the acupuncture stimulations.

In this study, the right inferior parietal lobule and the left cerebellum were activated during both the first and second acupuncture stimulation. However, the right middle frontal gyrus, left inferior frontal gyrus, left superior temporal gyrus, left inferior parietal lobule, left hypothalamus, left thalamus, and left claustrum were only activated during the first acupuncture stimulation and the right thalamus only during the second acupuncture stimulation. These results confirm our hypothesis that the first acupuncture stimulation

would affect brain activations of the second acupuncture stimulation.

According to the results of the paired-sample *t* test between the first and second acupuncture stimulation, there were brain activations in the left paracentral lobule, left cingulate gyrus, left medial globus pallidus, left and right insula, left claustrum, left and right thalamus, and left cerebellum only for the first > second acupuncture stimulation comparison. For the first < second acupuncture stimulation comparison, no significant areas were found. As can be seen in Figures 3 and 4, our results showed decreased brain activations during the second acupuncture stimulation, thereby confirming our hypothesis that consecutive acupuncture stimulations would lead to significantly decreased activations. Moreover, the fact that no significant brain activations were found within our threshold during sham stimulation is also in line with this idea. Sham stimulations may have been depressed by the acupuncture stimulations.

Our neuroimaging results are in line with those in previous studies, in which significant decreases in strength of activation were found between the first scanning session and the following sessions.^{12,13} Moreover, analyses of a series of single-epoch data from a single individual revealed a stimulus-by-stimulus decrease in the activation in the anterior cingulate cortex that paralleled the decrease in the subject's psychophysical responses.¹⁷ These and our results suggest that during consecutive stimulations, decreases in subjective perception of pain and brain activations from the first to the final scan might be caused by habituation.^{12,13}

During the alterations of on- and off-stimulations, the effects of the stimulation administered in the experiment may persist for such a long time that its effects are still present even when the subject is receiving new stimulations after previous ones. This is called the "carryover effect" and is a

very important and often under-stressed problem in experimental design and data analysis.^{14,17,18}

So far, several studies have been conducted on the "carry-over effect" of acupuncture stimulations,^{14,15} showing that consecutive acupuncture stimulations caused significantly different results when they were analyzed with different portions of acupuncture fMRI data.¹⁴

In our study, we found significantly different results between the first and the second stimulation. More decreased brain activations were found during the second acupuncture stimulation. Importantly, this was not only the case within a block, but also between two separated blocks.

Our study gives further evidence that neuroimaging results obtained with acupuncture studies in which a block design with consecutive acupuncture stimulations was used should be interpreted very carefully. These results are not the "exact" brain activations resulting from acupuncture, but "affected" activations decreased by previous stimulations. Therefore, it is very important that in future neuroimaging studies on acupuncture, researchers take this methodological issue seriously.

Limitations of our study are that only BL62 was used and that we only investigated a time interval of 5 minutes. More studies with other acupoints and various time intervals will be needed before the neural effects of consecutive acupuncture stimulations are completely understood.

Conclusions

We found that consecutive acupuncture stimulations on BL62 affect the neural responses in a significant way, resulting in decreased activations during the second acupuncture stimulation. This is an important finding that shows that in future fMRI studies on acupuncture, researchers should take this methodological issue into account when developing their scanning design.

TABLE 2. RESULTS OF THE ONE-SAMPLE *t*-TEST, SPECIFIED FOR THE FIRST AND SECOND ACUPUNCTURE STIMULATION^{a,b}

Regions	First acupuncture stimulation					Second acupuncture stimulation				
	Talairach					Talairach				
	x	y	z	t value	BA ^c	x	y	z	t value	BA
Frontal cortex										
Middle frontal gyrus R	43	8	36	3.76	9					
Inferior frontal gyrus L	-51	5	16	4.62	44					
Temporal cortex										
Superior temporal gyrus L	-44	-21	9	4.13	13					
Parietal cortex										
Inferior parietal lobule L	-61	-24	26	6.24	40					
Inferior parietal lobule R	54	-28	24	4.72	40	47	-50	35	3.25	40
Subcortical										
Hypothalamus L	-10	-6	-6	6.58	-					
Thalamus L	-7	-19	10	6.44	-					
Thalamus R						6	-25	7	3.77	-
Claustrum L	-38	-7	-2	5.64	-					
Cerebellum L	-3	-47	-33	5.4	-	-32	-59	-47	4.41	-

^aThe table describes the location of the peak voxel and the corresponding brain regions and Brodmann areas. Results are reported if uncorrected *p* < 0.005. The cluster-level is at least 50 voxels per cluster and the voxel size is 3.43×3.43×4 mm.

^bBlank areas in table indicate no significant change.

^cBA, Brodmann area.

R, right; L, left.

FIG. 3. Brain areas with a significant positive signal change, during the first and second acupuncture (ACUP) and sham (SHAM) stimulation, obtained with a one-sample t -test (uncorrected $p < 0.005$). The bar in the figure shows the t -value. R, right hemisphere; L, left hemisphere.

TABLE 3. RESULTS OF THE PAIRED-SAMPLE t TEST, SPECIFIED FOR THE COMPARISON: "1ST > 2ND ACUPUNCTURE STIMULATION" AND "1ST < 2ND ACUPUNCTURE STIMULATION"^{a,b}

Regions	1st > 2nd Acupuncture stimulation ^c					1st < 2nd Acupuncture stimulation ^d				
	Talairach					Talairach				
	x	y	z	t-value	BA ^e	x	y	z	t-value	BA
Frontal cortex										
Paracentral lobule L	0	-30	63	5.21	6	- ^f	-	-	-	-
Limbic cortex										
Cingulate gyrus L	-5	-1	33	4.82	24	-	-	-	-	-
Subcortical										
Medial globus pallidus L	-12	-6	-6	5.6	NA	-	-	-	-	-
Insula L	-38	3	9	4.45	13	-	-	-	-	-
Insula R	40	-10	15	4.17	13	-	-	-	-	-
Caudate L	-33	-15	9	3.78	NA	-	-	-	-	-
Thalamus L	-12	-12	17	5	NA	-	-	-	-	-
Thalamus R	19	-26	17	4.21	NA	-	-	-	-	-
Cerebellum L	-4	-42	-30	5.22	NA	-	-	-	-	-

^aThe table describes the location of the peak voxel and the corresponding brain regions and Brodmann areas. Results are reported if uncorrected $p < 0.005$. The cluster-level is at least 50 voxels per cluster and the voxel size is $3.43 \times 3.43 \times 4$ mm.

^bBlank areas in table indicate no significant change.

^c"1st > 2nd" means the brain areas that were more activated during the first acupuncture stimulation than during the second acupuncture stimulation.

^d"1st < 2nd" means the brain areas that were more activated during the second acupuncture stimulation than during the first acupuncture stimulation.

^eBA, Brodmann area.

^fIndicates not significantly activated.

NA, not applicable; R, right; L, left.

FIG. 4. Brain areas with a significant signal change between the first and second acupuncture stimulation obtained with a paired-sample t -test (uncorrected $p < 0.005$). The bar in the figure shows the t -value. R, right hemisphere; L, left hemisphere.

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Disclosure Statement

No competing financial interests exist.

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