fMRI study of acupuncture-induced periaqueductal gray activity in humans

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BOLD fMRI was used to study acupuncture-induced activation (increase in the BOLD signal from undetectable) of the periaqueductal gray (PAG) and two somatosensory cortical areas in seven healthy human subjects. Mechanical stimulation (push-pull) was given to the LI4 (Hoku) acupoint or to a non-acupoint. The stimulation paradigm consisted of 5 runs, each consisting of four 30 s On/30 s OFF periods over 30 min. The scan for each ON period was analyzed individually. The PAG and cortical areas showed different activity patterns. PAG activity was episodic and reliably demonstrated after 20–25 min of stimulation; both cortical areas, however, were active >90% of the time. Stimulation of a non-acupoint (leg) resulted in reduced levels of PAG and cortical activity. *NeuroReport* 15:1937–1940 © 2004 Lippincott Williams & Wilkins.

Key words: Acupuncture; fMRI; Periaqueductal gray (PAG); Somatosensory cortex

INTRODUCTION

Acupuncture is an old form of treatment with widespread applications in the induction of analgesia and relief of painful and noxious stimuli. Acupuncture analgesia often works through prolonged stimulation of a number of sites, known as acupoints. Following acupuncture stimulation, a significant pain threshold increase has been reported in animals [1–4] and humans [3–5].

The periaqueductal gray (PAG) is a brain stem area associated with noxious stimuli and their modulation [6]. The anatomy and biochemistry underlying the role of the PAG in analgesia is quite complex, involving multiple neurotransmitter systems [1,3,4,7–12] and extensive connections with several sites known to be involved in the interpretation of sensory data [3,4,13].

Functional imaging of the CNS during acupuncture has shown the activation (increase in the BOLD signal from undetectable) of a variety of sites, including somatosensory cortical areas, insula, hippocampus and amygdala [14,15]. To date, there have been no demonstrations of PAG activity during acupuncture using BOLD fMRI. In this paper we report that mechanical stimulation of the Hoku acupoint results in PAG activity. Because the PAG activity was infrequent and sporadic, we also analyzed the activity patterns in two somatosensory cortical areas (Brodmann areas 3 and 40). The Hoku point (LI#4; large intestine #4), located at the dorsal surface of the web between the thumb and the index finger, is a recognized acupoint for the induction of the analgesic effects of acupuncture [3,4]. We conclude that these areas respond to acupuncture with two distinct patterns of activity and that stimulation of a non-acupoint reduces the level of PAG activity [16].

MATERIALS AND METHODS

Participants: Seven healthy human subjects participated in the study (one female, six males; ages 27–52 years); six had never experienced acupuncture. The study was approved by the NJMS Institutional Review Board and all subjects gave informed consent. The patients had no neurological problems, chronic disease, or head injury; one subject was taking medication for hypertension.

Acupuncture: Acupuncture was performed by an experienced acupuncturist. Acupuncture stimulation of the LI4 point and a non-acupoint (right leg, 8–10 cm posterior to the ST-37 acupoint) consisted of strong mechanical stimulation, as described below. A specially designed needle (0.18 mm stainless steel, sterile, disposable; SEIRIN, OMS Medical Supplies Inc., Braintree, MA, USA) was inserted into the Hoku point or into the leg; the needle was pushed, pulled and twisted manually, once per second (1 Hz) for 30 s (the ON phase). The needle was then withdrawn for 30 s (the OFF phase). The timing of the stimulus was cued to a metronome; the acupuncturist wore a headset through which he could hear the metronome. Functional images were recorded during the entire time.

One subject (#3) was restudied using electroacupuncture. Low-intensity electrical stimulation (2 Hz) was applied to the Hoku point with a time-course identical to that for the mechanical acupuncture. Three subjects (2,4 and 7) were rescanned for the control study using the same paradigm.

The testing period was 30 min, 40 s, and was subdivided into five runs each lasting 4 min and 30 s (Fig. 1); each 4 min run had four cycles (A-D) of 30 s ON/30 s OFF, preceded by a 32 s rest period and followed by a 120 s rest period. Immediately following the session the subjects were asked about the sensation of 'De-Qi' – a feeling of numbness, soreness and fullness that is associated with successful acupuncture.

Neuroimaging: For scanning, subjects were fitted with a headset, designed to reduce noise (about 30 dB) and head motion, and their forehead was taped to the frame of the head cradle.

Conventional and fMRI was performed on a GE 1.5 T Echospeed Horizon scanner. Initially, an anatomical high-resolution T1 image was taken for subsequent image overlay. The parameters were: spin-echo sequence, TR/TE: 450/30, FOV: 24 cm, matrix size: 256 256, slice thickness: 5 mm with no gap, 28 slices. Functional data were acquired with a gradient echo EPI sequence TR/TE: 4000/60, and matrix size: 64 × 64; other parameters were the same as for the T1 image.

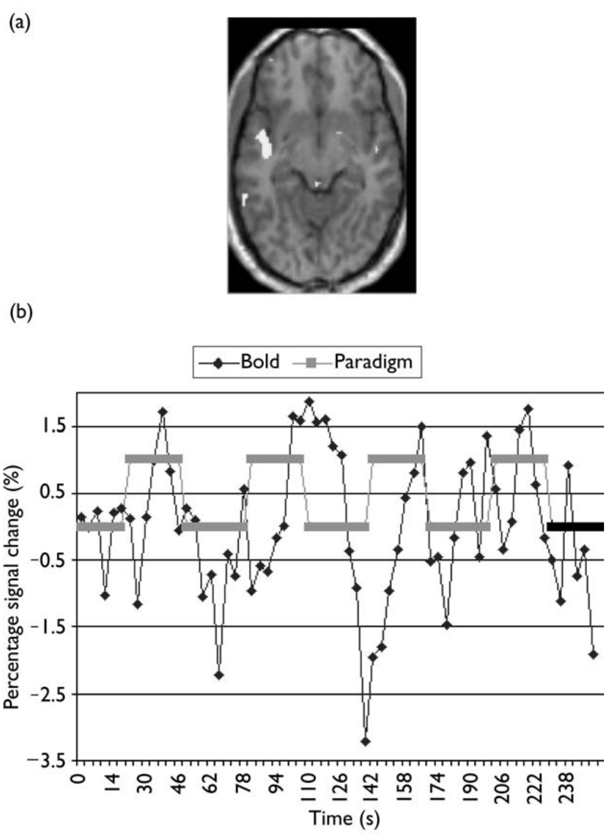

Fig. 1. Representative fMRI image (**a**) and associated hemodynamic curve (**b**) for Subject 1 Run 4b. In this run PAG activity is above threshold ($p < 0.05$) in (b) and (d) and below threshold in (a) and (c).

The raw data was transferred to a remote Sun SPARC 60 workstation and reconstructed off-line using IDL software (Interactive Data Language, Research Systems, Inc., Boulder, Co, USA). The results were analyzed using SPM99 software [17]. Images were realigned for head motion, normalized, and smoothed temporally and spatially.

The activation maps were co-registered onto the high-resolution T1 images [18], and resampled to a voxel unit of 2 mm × 2 mm × 2 mm (x, y, and z directions). The activation sites and volumes were generated using a digital brain atlas [19] and confirmed by the investigators.

The scan for each 30 s ON period was analyzed separately. The activated areas were identified visually (the threshold used was $p < 0.05$). We were careful in delineating the borders of the PAG since the brain stem is reported to be difficult to analyze functionally [20]. For each area of interest we then calculated the activation volume and the peak significance value (Table 1).

RESULTS

PAG activation was observed in all subjects (Table 1). In most subjects the active site was in the inferior part of the nucleus. Within the PAG, the activated areas ranged from left-ventral to left dorsal-lateral to dorsal midline; occasionally the active site switched sides

Figure 1 is a representative fMRI image and associated hemodynamic curve for Subject 1, Run 4b. In this run PAG activity is above threshold in B and D and below threshold in A and C.

Stimulation of the LI-4 acupoint: The overall response pattern among the subjects was similar (Table 1, Fig. 2). No subject showed PAG activity in all five runs; in six of seven subjects the PAG was usually active twice over the 30 min test session. Within each 4 min run, the PAG was usually active for one 30 s period. Activation was accompanied by either an increase or decrease in the BOLD signal.

There was a tendency for the frequency and levels of PAG activity to increase with length of stimulation (Table 1; Fig. 2); by the end of the session, i.e., run 5, only one subject showed activity. The degree of PAG activity ranged from 3 to 50 voxels and plateaued between runs 2-4 (range 40–28).

When the subjects were queried about the feeling of ''De Qi'', four subjects (2,3,4,5) reported the acupuncture was 'painful'.

To determine if the PAG could be activated under a less painful paradigm, we restudied subject 3 using a milder electrical stimulation paradigm (Table 1). Low-level electrical stimulation of the Hoku point resulted in similar patterns of PAG activation in four of the five runs.

Stimulation of a non-acupoint: Control stimulation was performed on 3 subjects (Table 1). Subject 4, showed no PAG activity; while in subjects 2 and 7 the activity in the PAG was reduced.

Somatosensory cortex, Brodmann Areas 3, 40: Stimulation of the LI-4 acupoint resulted in activity in somatosensory-related areas 3 and 40 (Fig. 2). In contrast to the PAG, these areas were active >90% of the time. Stimulation of the non-acupoint resulted in similar, but reduced frequency levels and lower activation volumes (data not shown).

Table I. PAG activity as a function of time.

	RUN1	RUN2	RUN3	RUN4	RUN5
1		●D: 35↓, (0.05)**	B: 49↑, (0.03)	B: 50↑, (0.0003) D: 50↑, (0.00003)	
2			B: 50↓, (0.002)	B: 7↓, (0.04) C: 18↓, (0.02)	
3	A: 42↑, (0.009)			A: 29↑, (0.0002)	
4	D: 18↑, (0.003)			C: 6 ↓, (0.03)	
5	B: 24↓, (0.0006)		D: 3↑, (0.04)		
6		C: 40↑, (0.0001) D: 35↓, (0.004)	D: 33↓, (0.0001)	D; 7↓, (0.006)	
7		B: 50↓, (0.00008)			D:35↑, (0.001)
3*	B: 15↑, (0.003)	B: 17 ↓, (0.001)		D:19↑, (0.0005)	C:24↓, (0.0005)
2†	C: 6↓, (0.02)		A: 9↓, (0.01)		
4†	No PAG activity				
7†		D: 18↑, (0.05)			

Runs 1-5 are subdivided into 4 30-second stimulation periods: A-D.
●: run subdivision with # of activated voxels at threshold ($p < 0.05$), e.g., D:35 (35 voxels in this scan) means there are 35 voxels observed in session D;
↑↓: direction of BOLD signal, ↑: increased, ↓: decreased;
**: (p value of peak);
*: electroacupuncture;
†: control subject; subject 4†: no PAG activity.

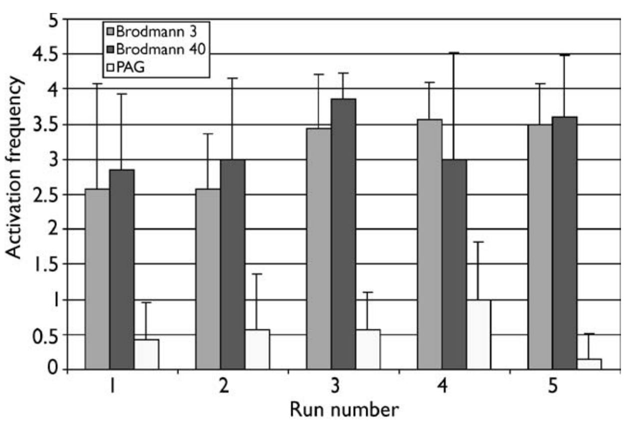

Fig. 2. Frequency of activation (mean ± s.d.) of PAG and cortex (Brodmann areas 3,40) following stimulation of the Hoku acupoint for all subjects. The mean frequency was calculated by averaging the total number of activations for all subjects in each run (observed activity of each run). Each run is composed of four 30 s on periods. For PAG we used both increased (↑) and decreased (↓) activation; for cortex the data is only for increased activation.

DISCUSSION

In this study we report acupuncture-induced activation of the PAG. To date, this is the first demonstration of acupuncture-induced PAG activity in humans using fMRI. PAG activity has been demonstrated with fMRI using noxious stimuli [13,15]. We also report that somatosensory cortex (Brodmann areas 3 and 40) and PAG show distinct activity patterns in response to acupuncture.

In our paradigm, strong acupuncture was given over a relatively long time-period, i.e., 30 min, to either a recognized acupoint (LI4) or to a non-acupoint. All subjects showed a basically similar episodic pattern of PAG activation. Most subjects had extended times, sometimes 10–15 min, during which the PAG BOLD signal was not above threshold. When we applied the same stimulation to a non-acupoint, PAG activity was further reduced. In our study activity in the PAG was often seen in the ventral and ventral-lateral areas; areas that are associated with analgesia-related activity [6,8].

In recent years there have been several fMRI studies of CNS activity during acupuncture [14,15,21]. In these studies the authors used a variety of stimulation paradigms involving both mechanical and electrical stimulation; the acupuncture sessions were usually short (i.e. <10 min) and often mild. None of the authors reported PAG activity. In our paradigm, strong stimulation was given mechanically over a relatively long time, i.e. 30 min, and PAG activation was reliably demonstrated after 20 min. However, the difference in results cannot be solely linked to the intensity of the stimulus since we saw a similar pattern of PAG activity with milder electro-acupuncture and also with stimulation of a non-acupoint.

In our studies we noted that stimulation of the acupoint resulted in stronger activity in PAG and cortex. Our findings in PAG are similar to those reported for rats [8].

One striking finding of our study was the identification of two distinct temporal patterns of activity in response to acupuncture stimulation. While the PAG was active only episodically, the two areas of somatosensory cortex were active throughout the session. These temporal response properties of the PAG and cortex have not, to our knowledge, been previously reported. Both patterns were strongly similar in all seven subjects (eight if we include the electroacupuncture data). The time-course of activity in the PAG is very similar to that seen in analgesia in humans [3–5,12] and animals [1,2,8,9].

We noted both positive and negative BOLD signals in the PAG and cortex. The interpretation of the direction of the BOLD signal is a complex and controversial topic but appears to be a characteristic feature of the somatosensory system [14,15]. In a recent study [21] that measured blood flow, oxygen consumption and the BOLD signal, the authors concluded that the direction of the BOLD signal (BOLD-positive, BOLD-negative and BOLD-silent) probably reflects a complex interaction between neuronal activity and the hemodynamics of the particular area. The differences in time-scale of fMRI measurements and neuronal activity (seconds *vs* milliseconds) make it difficult to do more than speculate as to the mechanisms underlying our observations.

CONCLUSION

PAG and somatosensory cortex respond to acupuncture with two distinct activity patterns. Stimulation of the acupoint, as opposed to the non-acupoint, increases the levels of activity in both areas without altering their basic activity patterns.

REFERENCES

1. Mayer DJ, Wolfle TL, Akil H, Carder B and Liebeskind JC. Analgesia from electrical stimulation in the brainstem of the rat. *Science* 1971; **174**: 1351–1354.
2. Chapman CR, Colpitts YM, Benedetti C, Kitaeff R and Gehrig JD. Evoked potential assessment of acupunctural analgesia: attempted reversal with naloxone. *Pain* 1980; **9**:183–197.
3. Han J-S. Physiologic and neurochemical basis of acupuncture analgesia. In: Cheng TO (ed.). *The International Textbook of Cardiology*. Oxford: Pergamon; 1986, pp. 1124–1132.
4. Han J-S. Physiology of acupuncture: review of thirty years of research. *J Alt Compl Med* 1997; **3**: S101–S108.
5. Andersson SA and Holmgren E. On acupuncture analgesia and the mechanism of pain. *Am J Chin Med* 1975; **3**:311–334.
6. Fields HL and Basbaum AI. Central nervous system mechanisms of pain modulation. In: Wall PD and Melzack R (eds). *Textbook of Pain*. London: Churchill Livingstone; 1999, pp. 309–329.
7. Da Costa Gomez TM and Behbehani MM. An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. *Brain Res* 1995; **689**:21–31.
8. de Medeiros MA, Canteras N, Suchecki D, Eugenio L and Mello AM. Analgesia and c-Fos expression in the periaqueductal gray induced by electroacupuncture at Zusanli point in rats. *Brain Res* 2003; **973**:196–204.
9. Tang N-M, Dong H-W, Wang X-M, Tsui Z-C and Han J-S. Cholecystokinin antisense RNA increases the analgesic effect induced by electroacupuncture or low dose morphine: conversion of low responder rats into high responders. *Pain* 1997; **71**:71–80.
10. Commons KG, van Bockstaele EJ and Pfaff DW. Frequent colocalization of mu opioid and NMDA-type glutamate receptors at postsynaptic sites in periaqueductal gray neurons. *J Comp Neurol* 1999; **408**:549–559.
11. Helmstetter FJ, Tershner SA, Poore LH and Bellgowan PS. Antinocioception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventral medulla. *Brain Res* 1998; **779**:104–118.
12. Mayer DJ, Price DD and Rafii A. Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. *Brain Res* 1977; **121**:368–372.
13. Tracey I, Ploghaus A, Gati JS, Clare S, Smith S *et al.* Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci* 2002; **22**:2748–2752.
14. Hui KK, Liu J, Makris N, Gollub RL, Chen AJW, Moore CI *et al.* Acupuncture modulates the limbic system and subcortical gray structures of the human brain: evidence from fMRI studies in normal subjects. *Hum Brain Mapp* 2000; **9**:13–25.
15. Peyron R, Laurent B and Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis. *Neurophysiol Clin* 2000; **30**:263–288.
16. Liu WC, Cook D, Hong D-L, Kalnin A, Feldman S and Komisaruk B. Responses in periaqueductal gray matter by acupuncture stimulation: a preliminary fMRI study. *Soc Acupuncture Res* 2003 (abstract).
17. Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD and Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp* 1995; **2**:189–210.
18. Talairach J and Tournoux P. *Co-Planar Stereotaxic Atlas of the Human Brain*. Stuttgart: Thieme; 1998.
19. Kikinis R, Shenton M, Losifescu D, McCarley R, Saiviroopom P, Hokama H *et al.* A digital brain atlas for surgical planning, model driven segmentation and teaching. *IEEE Trans Visualization Computer Graphics* 1996; **2**:2323–2341.
20. Gati JS, Menon RS, Ugurbil K and Rutt BK. Experimental determination of the BOLD field strength dependence in vessels and tissue. *Magn Reson Med* 1997; **38**:296–302.
21. Seiyama A, Seki J, Tanabe HC, Sase I, Takatsuki A, Miyauci S *et al.* Circulatory basis of fMRI signals: relationship between changes in the hemodynamic parameters and BOLD signal intensity. *Neuroimage* 2004; **21**:1204–1214.

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