Electroacupuncture-induced neural activation detected by use of manganese-enhanced functional magnetic resonance imaging in rabbits

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Objective—To investigate the effects of acupuncture on neural activity detected by use of manganese-enhanced functional magnetic resonance imaging (fMRI) and elucidate the relationship between somatic acupoint stimulation and brain activation.

Animals—40 New Zealand White rabbits.

Procedure—Manganese-enhanced fMRI was performed in anesthetized rabbits manipulated with electroacupuncture (EA) on Zusanli (ST-36) and Yanglingquan (GB-34) acupoints. Image acquisition was performed on a 1.5T superconductive clinical scanner with a circular polarized extremity coil. T1-weighted images were acquired sequentially as follows: baseline, after mannitol injection, after manganese infusion, and 5 and 20 minutes after initiation of EA.

Results—Changes in focal neural activity were detected by use of manganese-enhanced fMRI. Stimulation on Zusanli (ST-36) for 5 minutes resulted in activation of the hippocampus, whereas stimulation on Yanglingquan (GB-34) resulted in activation of the hypothalamus, insula, and motor cortex. Activation became less specific after 20 minutes of EA. Furthermore, stimulation on ipsilateral acupoints led to bilateral brain activation.

Conclusions and Clinical Relevance—Each acupoint has corresponding cerebral linkage, and stimulation on these points resulted in time-dependent neural activation. Understanding the linkage between peripheral acupoint stimulation and central neural pathways may provide a useful guide for clinical applications of acupuncture. (Am J Vet Res 2001;62: 178–182)

In the past decade, the advance of image technology has made magnetic resonance imaging (MRI) a strong tool for evaluation of many disorders in humans and other animals. Recently, functional MRI (fMRI) has proven to be a useful method with high temporal and spatial resolution for noninvasive mapping of brain function. Alternations in neuron activity are inferred from MRI characterizations of cerebral hemodynamics. Presently, the most widely used fMRI technique for noninvasive mapping of human brain activity is gradient-echo planar imaging, using blood oxygen level-dependent (BOLD) contrast. By using the endogenous contrast agent deoxyhemoglobin, it is possible to continuously measure the change of blood oxygenation during resting and activation states. A small but significant local increase in signal is observed in activated cortical regions, using susceptibility sensitive gradient-echo pulse sequences. However, the signal-to-noise ratio of BOLD contrast is low, approximately 2 to 3% at 1.5T and 4 to 7% at 3T.

Manganese-enhanced fMRI can provide an approach to direct imaging of brain function. Manganese is paramagnetic and enters cells through voltage-gated calcium channels. When manganese accumulates in brain tissue, it provides higher spatial resolution for fMRI in static states, even in awake animals, compared with fMRI performed without manganese contrast. For more than 2,000 years, acupuncture has been practiced in Asian countries to maintain a harmonious balance of energies within an individual. In 1980, the World Health Organization recommended acupuncture as an effective treatment for 43 health problems in humans, including respiratory and gastrointestinal tract disorders, ocular disorders, and neuromuscular disorders. Several lines of evidence suggest that acupuncture is not only effective for treatment of human diseases but also for disorders in other animals. Advances in knowledge of spinal gate theory and the discovery of opioid receptors have allowed for characterization of mechanisms of acupuncture-mediated analgesia. Recently, MRI has been used for the diagnosis of many disorders in companion animals, such as elbow dysplasia, cerebellar degeneration, and musculoskeletal diseases. Although fMRI has been used to study the central pathway of acupuncture stimulation in humans, informative data about time-dependent acupuncture effects on brain activity measured by use of manganese-enhanced fMRI remain to be elucidated. The purposes of the study reported here were to determine the effects of acupuncture on neural activity in the brain of rabbits by use of manganese-enhanced fMRI and elucidate the relationship between somatic acupoint stimulation and brain activation.

Materials and Methods

Animals and experimental protocol—Forty New Zealand White rabbits of either sex and weighing 2.5 to 3.5
kg were used in this study. They were treated according to the principles outlined by the National Institutes of Health. After withholding food for 12 hours but allowing free access to water, rabbits were anesthetized with ketamine hydrochloride (35 mg/kg of body weight, IM). Anesthesia was maintained with additional low doses of ketamine (5 to 10 mg/kg) as needed. Anesthetic depth was maintained at a steady level without affecting heart rate or arterial blood pressure and without detection of signs of pain in response to skin traction. Catheters were placed in a femoral artery and vein for monitoring heart rate and blood pressure. A catheter was also placed in a carotid artery for administration of drugs. In brief, a polyethylene tube was positioned in the external carotid artery (ECA), and the artery was ligated distal to the entrance of the tube. The tip of the catheter was located at the junction between the ECA and internal carotid artery (ICA) so that blood flowed to the brain through the ICA during periods when drugs or saline (0.9% NaCl) solution were not infused. Hypertonic (20%) D-mannitol solution (5 ml/kg) was administered via the ICA to break down the blood brain barrier, and MnCl₂ (120 mM in isotonic saline solution) was administered for manganese-enhanced fMRI.

Functional magnetic resonance imaging—Multislice TIWI conventional spin-echo images (TR, 400 milliseconds; TE, 12 milliseconds) were acquired, using a circular polarized extremity coil in a 1.5T superconductive magnet. A 116 X 128 matrix with a field of view of 5 cm was used. Slice thickness was 4 mm.

To separate nonspecific and specific signal enhancement, we obtained 5 series of images. A baseline fMRI was performed at 0 minutes. Five minutes later, mannitol was infused and a second series of images was obtained. At 10 minutes, MnCl₂ was infused and a third series was obtained. Electroacupuncture (EA) on the Yanglingquan (GB-34) acupoint was initiated at 15 minutes. The fourth series of images was obtained 5 minutes after initiation of EA (ie, minute 20 of the experiment), and 10 minutes later (ie, minute 30 of the experiment), MnCl₂ was again administered. The final series of images was obtained 20 minutes after initiation of EA (ie, minute 35 of the experiment).

To detect specificity and time-dependent effects of EA stimulation, mannitol and MnCl₂ were infused as described in the first experiment, but images were acquired only after EA on Zusanli (ST-36) and Yanglingquan (GB-34) acupoints. For better resolution and registration of anatomic structures, the 3D fast low angle shot (TR, 50 milliseconds; TE, 10 milliseconds; flip angle, 50°; matrix, 128 X 128; field of view, 64 mm; slice thickness, 0.5 mm) was acquired after completion of EA.

Electroacupuncture—Electroacupuncture was performed by applying an electric current to 2 No. 32 needles positioned near 1 acupoint. Needles were positioned 2 to 3 mm apart to prevent a short electrical current. Zusanli (ST-36) and Yanglingquan (GB-34) acupoints (Fig 1), which have been standardized, were chosen to elucidate the relationship between somatic acupoint stimulation and brain activation. Electroacupuncture was performed with an electrical nerve stimulator. Stimulation was pulse-waved with alternating frequencies of 2 and 15 Hz. Wave width was 300 microseconds, and stimulation duration was 20 minutes. Intensity of stimulation was adjusted between 1 and 2 mA. Location of EA stimulation was contra- or ipsilateral to the carotid artery used for drug administration.

Figure 1—Diagram indicating the 2 acupoints stimulated in vivo for detection of electroacupuncture (EA)-induced brain activation by use of manganese-enhanced functional magnetic resonance imaging (fMRI) in rabbits. Acupoint Yanglingquan (GB-34) and Zusanli (ST-36) were located by palpating anatomic landmarks such as the tibial tuberosity and fibular head.

Figure 2—Transverse images obtained by use of manganese-enhanced fMRI of the brain of rabbits at baseline (A), after infusion of mannitol into the internal carotid artery (B), after infusion of MnCl₂ into the internal carotid artery (C), and 5 minutes after EA stimulation of acupoint the Yanglingquan (GB-34; D). Images were obtained at the level of hypothalamus and thalamus. In the image obtained 5 minutes after EA stimulation, increases in signal intensity are evident in the insula (long arrow), motor cortex (arrowhead), and hypothalamus (short arrow).

Figure 3—Transverse images obtained by use of manganese-enhanced fMRI of the brain of rabbits 5 minutes after initiation of EA stimulation on Yanglingquan (GB-34) or Zusanli (ST-36). Stimulation on Yanglingquan resulted in activation of the insula (long arrow), motor cortex (arrowhead), and hypothalamus (short arrow).
Results
To distinguish specific enhancement from nonspecific enhancement, we acquired images before and after infusion of mannitol, after infusion of MnCl₂, and after EA in 11 rabbits (Fig 2). Brain activation was not detected immediately after infusion of mannitol or MnCl₂, whereas the insula, hypothalamus, and motor cortex were activated after 5 minutes of EA on Yanglingquan (GB-34).

Specific changes in focal brain activity were detected after stimulation of the 2 acupoints. When Yanglingquan (GB-34) was stimulated for 5 minutes (n = 13 rabbits), changes in brain activity were detected in the hypothalamus, insula, and motor cortex, whereas after stimulation on Zusanli (ST-36; n = 9 rabbits), brain activity changes were primarily detected in the hippocampal region (Fig 3).

To study time-dependent neural activation after stimulation of somatic acupoints, EA was performed on acupoints Zusanli (ST-36) and Yanglingquan (GB-34) in 4 rabbits. Brain activation detected on images acquired 5 minutes after EA were more specific, compared with images obtained after 20 minutes (Fig 4).

Normally, EA stimulation on the left Yanglingquan (GB-34) acupoint caused activation of the insula and motor cortex on the right side of the brain. However, stimulation on the left Yanglingquan (GB-34) acupoint of 3 rabbits also resulted in activation of the left hemisphere (Fig 5), suggesting that stimulation on ipsilateral acupoints leads to bilateral neural activation.

Discussion
In this study, we were clearly able to demonstrate time-dependent effects of EA by use of manganese-enhanced fMRI. Moreover, EA stimulation on different acupoints resulted in obvious and specific changes in brain activity.

Because depth of anesthesia may affect brain activity (eg, nonspecific activation with minimal anesthesia and hypoactivity with deep anesthesia), a steady depth of anesthesia is mandatory to allow measurement of specific brain activity. Because there are no signs of pain in response to skin traction in animals at a surgical plane of anesthesia (ie, the depth of anesthesia necessary for fMRI studies), it is hard to assess how anesthesia and analgesia affect fMRI results. Nevertheless, several lines of evidence suggest that acupuncture effects can be detected in anesthetized animals.²⁻⁵ We have maintained rabbits at a surgical plane of anesthesia without affecting heart rate or arterial blood pressure and without detecting signs of pain in response to skin traction.²⁶ Furthermore, results of this and a previous² study indicated that fMRI of the brain could be performed in rabbits anesthetized with ketamine.

Intravenous administration of mannitol followed by manganese is a useful fMRI technique to separate stimulation-specific signal enhancement from nonspecific enhancement.²²⁻²³ Manganese is a good contrast agent, and in many biological systems, it is handled similarly to calcium. Manganese is known to enter cells through voltage-gated calcium channels. In the present study, we used manganese, a paramagnetic calcium analog,²⁴ to monitor calcium influx in the brain. Our results indicated that manganese can be used as a contrast agent to monitor brain activation by use of fMRI. Moreover, manganese-induced activity-specific contrast was independent of cerebral blood flow.

Recently, fMRI has been used to study brain linkages during acupuncture stimulation in humans.¹⁶⁻²⁰ For example, manual acupuncture on Zusanli (ST-36) induces activation of the hypothalamus and deactiva-
tion of the limbic system. Activation of central pathways, including descending antinoceptive systems (eg, hypothalamus, raphe nuclei,periaqueductal gray matter), and deactivation of the limbic system leading to attenuation of affective responses to pain have been postulated as a mechanism to explain acupuncture-induced analgesia. However, it is generally accepted that clinically relevant acupuncture-induced analgesic effects usually require prolonged and multiple stimulation sessions. Because of ethical considerations, it is difficult to assess analgesic effects of acupuncture in humans by use of fMRI.

Our study was designed to investigate acupuncture effects in a time-dependent manner. Results indicated that 5 minutes of EA stimulation on Zusanli (ST-36) induced activation in the hippocampal region, whereas 5 minutes of EA stimulation on Yanglingquan (GB-34) resulted in activity changes in the motor cortex and insula. Results of previous studies suggest that prolonged acupuncture stimulation (10 to 20 minutes) is required to induce an antinociceptive effect.\textsuperscript{11,25,26} Our results indicated that brain activation became less specific after 20 minutes of stimulation on acupoints Zusanli (ST-36) and Yanglingquan (GB-34). This phenomenon may explain the clinical observation that analgesia can be induced by EA stimulation on several acupoints.\textsuperscript{11} Changes in brain activity detected by use of manganese-enhanced MRI indicate mobilization of cellular ions as a result of activation or deactivation of central neurons. Therefore, it remains unclear whether the actual change in brain activity (activation or deactivation) after EA stimulation on Zusanli (ST-36) for 20 minutes was similar to that after EA stimulation on Yanglingquan (GB-34). Furthermore, activity changes detected 20 minutes after EA stimulation may have been attributable to the neurotoxic effects of manganese. However, to minimize this possibility, we designed our study so that MnCl$_2$ was injected 5 minutes before obtaining images 20 minutes after initiation of EA.

The advantages of using fMRI for these types of studies are 2-fold. First, the linkage of acupoints and corresponding cerebral function can be demonstrated in vivo. Second, less time is required for processing manganese-enhanced fMRI images were obtained from at least 3 rabbits for each of 3 manganese-enhanced fMRI approaches. Many fMRI studies of normal subjects have reported that manganese-enhanced fMRI allow only qualitative and not quantitative evaluation of brain images. Although images were obtained from at least 3 rabbits for each phase of the present study, we could not statistically compare changes before and after manganese infusion and EA. Nonetheless, fMRI can be used to determine linkages between acupuncture stimulation and changes in brain activity, which, in turn, may provide a useful guide for clinical applications of acupuncture.

References
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