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Exploring Nociceptive Response by BOLD fMRI in α-chloralose Anesthetized Rats

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Abstract— **The technique of blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) was used to provide a spatial-temporal mapping of nociceptive activation in brain. We contrived to obtain an illustration of the pain related regions by injecting formalin at the hindpaw using a 4.7 T MR system in α-chloralose anesthetized rats. In order to obtain the pain response, we avoided any invasive surgery on animals to purify the signal of nociception. The dynamic data were analyzed by mapping correlation coefficient and the time activity curves were calculated by atlas-based region of interest selection. The BOLD signals showed obvious difference in anterior cingulated cortex, somatosensory cortex, medial thalamus, and striatum after stimulation. The results not only show the global somatotopic organization of noxious stimulation on hindpaw in rats, but also provided invaluable information for neuroscience research.**

I. Introduction

The mechanism and circuitries of nociception in brain is extremely complex, including not only the response about noxious environmental stimuli, but also about cognitive and emotional factors. Recent evidences in small animal image experiments indicate that the processing of pain in brain include anterior cingulated cortex, somatosensory cortex, motor cortex, insula, prefrontal cortex, claustrum, interpeduncular nucleus, periaqueductal gray, hippocampus, thalamus, hypothalamus, piriform cortex, etc. Moreover, there are obviously bilateral activation including anterior cingulate cortex, frontal cortex, thalamus and sensory-motor cortex [1-15]. In the previous study, we have known that neuronal activity of nociception consists of different brain areas. In neuroscience research, scientists often apply single-unit recording and field potential recording to map the neuronal activity. These traditional electrophysiological methods have a better temporal resolution but do not have the capability to record the whole brain signals in a limited time period. Functional images, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), can provide intact functional signals by using brain mapping techniques. In 1990, Ogawa proposed the blood oxygenation level dependent (BOLD) fMRI method based on hemodynamic response [16;17]. And then in 1992, Kwong applied this theory to depict the dynamic brain activity of visual and motor cortex [18]. Several groups also used the BOLD theory to report these significant findings [19-21]. Since then it comes a brand-new era of neuroscience in fMRI. The principle of BOLD is based on the existence of paramagnetic substance, such as deoxy-Hemoglobin (Hb), which could interfere with magnetic field and further reduce the intensity of signal. In general, the local cellular activity is supported by blood flow, and the consumption of glucose is directly related to synaptic activity [22-25]. Therefore, these mechanisms could roughly infer that the blood flow increase is related to the activation of neurons [26;27]. The activation of neurons can stimulate vasodilatation indirectly and over-compensate the regional cerebral blood flow (rCBF) to bring more oxygen and glucose. Hence, it causes the ratio of paramagnetic deoxy-Hb in blood to be reduced when the neural activity increases in brain, so more intensive signal can be obtained in activated brain areas [28-30]. In this study we intended to determine the formalin induced nociceptive maps in α-chloralose anesthetic rats by using the BOLD fMRI technique.

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II. Materials and Methods

A. Animal Experiments

Eight adult male Wistar rats (weight 250~300g) were anesthetized with α-chloralose. The α-chloralose (70 mg/kg) is given intraperitoneally, and then the animal was positioned on stereotaxic holder. The body temperature of

the animal was maintained by a warm-water circulated system. A 30 G needle was fitted with a PE-50 catheter and inserted subcutaneously in the left hindpaw for the subsequent formalin administration. All efforts were used to alleviate animal suffering and to use a minimal number of animals.

B. Image Experiments

For MR experiments, the images were performed on Bruker Biospec BMT 47/40 4.7 T system equipped with an actively shielded gradient system (0-5.9 G/cm in 500 μs). A 20 cm volume coil was used as RF transmitter and a 2 cm surface coil placed on the head was used as receiver. A T_2-weighted scout image was scanned in mid-sagittal plan to localize the anatomical position by identifying the anterior commisure (bregma -0.8 mm). The four slices T_2-weighted template images (bregma -0.8 mm, -2.8 mm, -4.8 mm and -6.8 mm) were acquired using spin echo sequence (TR = 4000 ms, TE = 80 ms, FOV = 4 cm, SLTH = 2 mm , NEX = 2, and acquisition matrix was 256×128 with a matrix of 256×256 after zero-filling). A 40-repetitive four slices gradient echo images (TR = 215 ms, TE = 20 ms, flip angle = 22.5°, FOV = 4 cm, SLTH = 2 mm, NEX = 2 and acquisition matrix was 256×64 with a matrix of 256×256 after zero-filling) were acquired at the same position and each time frame took 27 s. The first 20 consecutive frames were collected as base line and the remnant 20 were collected after 5% formalin (0.05 ml) was injecting into the hindpaw.

C. Data Analysis

Functional images were analyzed using Matlab 7. The cross-correlation coefficients were calculated with a model of OFF-ON paradigm pixel by pixel after the slices were realigned to the time series. The coefficients provide the relativity of brain neuronal activation and noxious stimulation of hindpaw. In order to improve the accuracy of the anatomical localization, we registered the high resolution T_2-weighted images with the atlas (Paxinos and Watson, 1998). Thus, complete information about the activated areas can be clearly mapped (Fig.1).

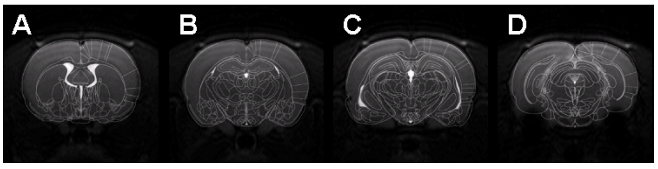

Fig. 1. T_2 weighted images were registered and fused with the rat atlas. These templates provide accurate anatomical information and precise ROI selection. The locations of the four images were indicated as (A) Bregma -0.8 mm. (B) Bregma -2.8 mm. (C) Bregma -4.8 mm. (D) Bregma -6.8 mm.

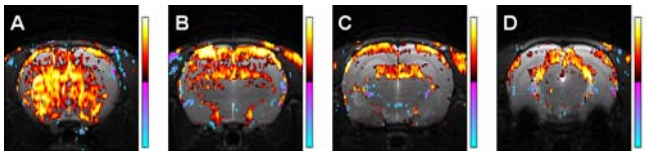

Fig. 2. Formalin stimulated functional images. The event related activation were labeled by hot and cold colors when the correlation coefficients were over 0.6 and under -0.6 respectively. (A) Bregma -0.8 mm. (B) Bregma -2.8 mm. (C) Bregma -4.8 mm. (D) Bregma -6.8 mm.

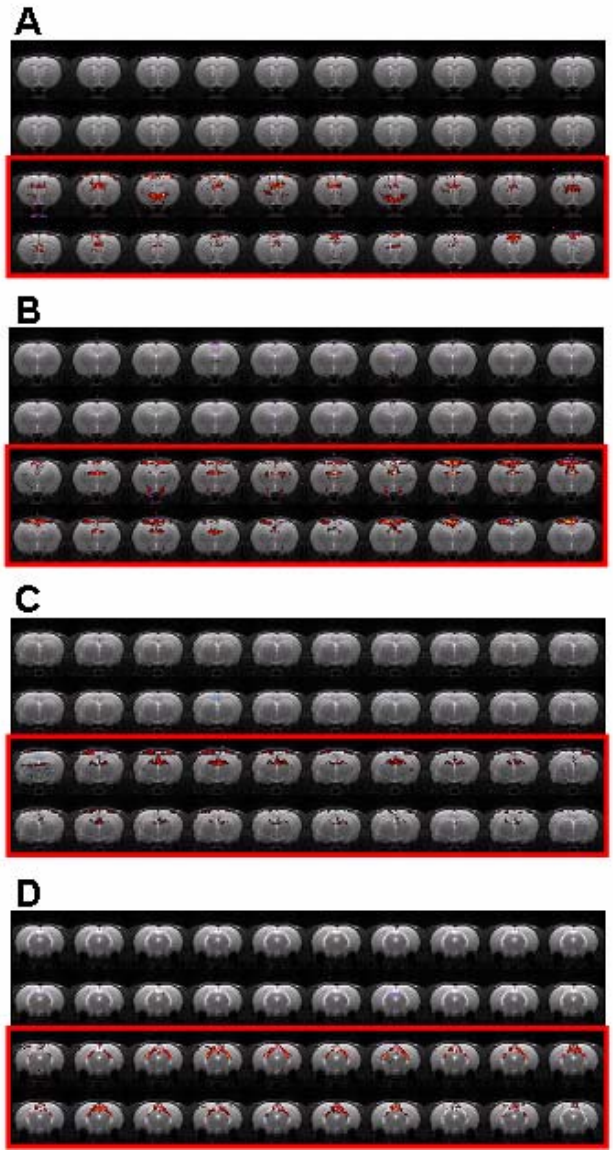

Fig. 3. Dynamic cine images display 4 sets of MR images. The time courses of each set of images are rising from left to right, up to down. Images acquired during painful stimulation were marked in red. The colored-pixel values of each sub-image are formed by subtracting the mean-map of the baseline data. (A) Bregma -0.8 mm. (B) Bregma -2.8 mm. (C) Bregma -4.8 mm. (D) Bregma -6.8 mm.

III. Results

In our formalin studies, we found that the BOLD signals showed more increases in the anterior cingulated cortex, medial thalamus and somatosensory cortex than the other brain areas. In addition, striatum had also shown significant response. The correlation map could illustrate the pain related areas in brain and demonstrated that almost all the trends of BOLD signals were positive (Fig. 2). Furthermore, we observed similar phenomena using the dynamic cine image method and time activity curve analysis (Fig. 3 and Fig. 4); the results showed more clearly that the activation change in time and space during the noxious stimulation. Based upon the event-related response on these images, we could conclude that the previously mentioned brain area have high relation with nociception. All the activated areas showed bilateral activation.

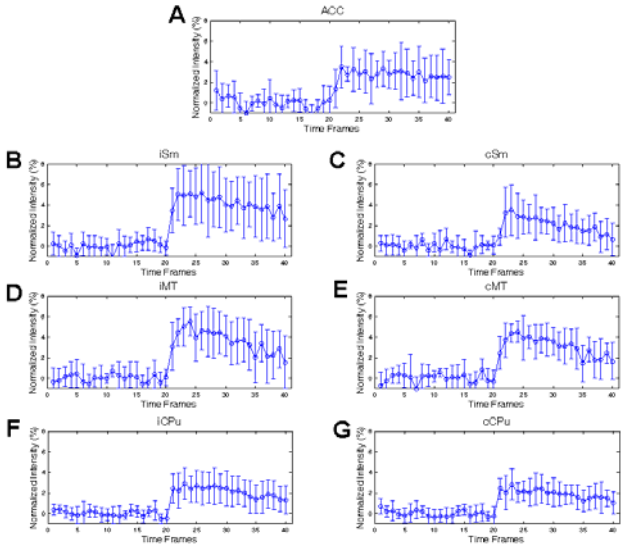

Fig. 3. Time Activity Curves in different brain areas (n=8). The signal intensities were normalized to percentage signal difference with each baseline data. The formalin was injected at frame 21. (A) anterior cingulated cortex, (B) ipsilateral somatosensory cortex, (C) contralateral somatosensory cortex, (D) ipsilateral medial thalamus, (E) contralateral medial thalamus, (F) ipsilateral striatum and (G) contralateral striatum.

IV. Discussions and Conclusions

The BOLD technique, unlike electrophysiological recording, provides a global view to analyze a constellation of neurons in multiple brain areas at the same time. Many fMRI experiments in rats used invasive catheter to control anesthesia and to administer other pharmacological compounds. However, the wound of surgery may influence specific brain activity during dynamic image acquisition, and confuse the signal of pain. In this study, we avoided any invasive surgery to eliminate its influence on the response. Thus, the formalin-induced nociceptive response from the hindpaw can be more clearly identified. Since potential factors which could affect the pain sensation were excluded, we inferred that the signals of the BOLD images were directly related to formalin–induced neuronal activity in brain. The temporal patterns of BOLD signal were observed to rise immediately after formalin injection. Maximal BOLD intensity was generally observed within 3 min. This time delay of the peak intensity could be resulted from the time required to maximally increase the rCBF due to neuronal activation [22;31]. Our study, covering a volume from bregma +1.2 mm to bregma -7.8 mm of the rat brain, nearly includes the whole brain areas. This extends the previous studies focusing in the frontal areas of the rat brain [32;33]. In addition, we found that in cortical regions, the activated areas showed more increase than halothane anesthetized study and the formalin-induced response occurred earlier [34]. The activated positions in this study elucidated the functional localization and somatotopic projection of a pure pain sensation. These results highlight the advantages of functional images, that is, to tell which brain region or level control the persisted pain and reaction within time.

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