BOLD fMRI mapping of brain responses to nociceptive stimuli in rats under ketamine anesthesia

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Received 31 May 2007; received in revised form 5 December 2007; accepted 12 December 2007

Abstract

Ketamine is one of the most commonly used anesthetics, but its effects on nociceptive responses are not clearly defined. This study used blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) to hemodynamically map responses to formalin stimuli under ketamine anesthesia. All imaging was performed on a 4.7-T fMRI system. During dynamic image acquisition, formalin was injected into the rat hindpaw as a painful stimulant. Correlation coefficients were calculated, and each image was registered and fused with the corresponding rat brain atlas so as to avoid inaccuracies arising from manual definition of the brain area and to achieve atlas-based normalization among subjects. Formalin injections were found to increase BOLD signals in the cingulate cortex, sensory-motor cortices, insular cortex, striatum, nucleus accumbens, medial thalamus, ventrolateral thalamic group, and hippocampus. Moreover, in contrast to previous pain investigations, the frontal subcortical regions were strongly activated in ketamine-anesthetized rats.

Keywords: fMRI; Ketamine; Pain; Rat; Formalin

1. Introduction

Nociception in small animals has been widely studied over the past decade with the aim of understanding pain mechanisms and the associated neuronal interactions. New pain-relief drugs and technologies have been developed in parallel to alleviate human suffering of pain. The dependence of these advancements on animal testing reinforces the importance of studying pain mechanisms in small animals. Improvements in the resolution and signal-to-noise ratio of medical imaging techniques have allowed them to be applied to small animals. Meanwhile, the ability to record noninvasive, in vivo hemodynamic responses using blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has sparked great interest amongst pain researchers in recent years [1–3].

Anesthesia is usually essential for immobilizing animals during imaging procedures. Ketamine, a noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonist [4,5], is one of the most commonly used anesthetics for animal sedation. However, its effect on pain signals has not been well defined. Previous pharmacological MRI studies have shown that an acute ketamine challenge increases the BOLD contrast in frontal cortex, hippocampus (HIP), limbic structures, and olfactory lobe [6,7]. Other studies showed that injecting animals with a sufficient dose of ketamine markedly increased glucose metabolism in these same brain regions [8,9]. In addition, it has been reported that ketamine acts on opioid receptors, indirectly suggesting that ketamine can modulate pain signals in the brain [10,11].

Formalin is widely used in small-animal studies of nociception since it evokes pure chemically induced pain without the influence of touch, pressure, temperature, or other stimuli. Formalin also provides a continuous rather than a transient noxious stimulus, thus creating a model...
for persistent pain. Formalin-induced behavioral states have been investigated previously [12,13], and the corresponding BOLD signal changes in the rat spinal cord have also been characterized [14,15]. For studying pain in the brain, injecting formalin into the α-chloralose-anesthetized rat paw has been shown to cause significant signal alterations in forebrain regions [16,17]. However, pain processing mediated by ketamine is less well understood. Therefore, the present study used BOLD fMRI and several data analysis techniques to determine the formalin-stimulated brain nociceptive maps under ketamine anesthesia.

2. Materials and methods

2.1. Animal experiments

Seven adult male Wistar rats weighing 250–300 g (National Laboratory Animal Center, Taiwan) were anesthetized by intraperitoneal administration of 90 mg/kg ketamine. Anesthetized rats were positioned on a stereotaxic holder, and the body temperature was maintained using a warm-water circulating system. Two ear bars and an incisor fixer were used to position the rat head, with tapes used to restrain the body. The CO₂ concentration was monitored continuously (Datex-Ohmeda Capnomac Ultima respiration–ventilation monitor). Images were acquired only when the rat was in a stable condition with a ventilation rate of 55–60 breaths/min and a CO₂ concentration of 3–3.5%. Pain was induced by a single injection of 50 µL of 5% formalin into the hindpaw using a 250-µL microsyringe and a 30-G needle fitted with a PE-50 catheter. The catheter was filled with formalin, with the formalin on the needle being wiped off with cotton prior to insertion into the left hindpaw. The injected volume was carefully calibrated by repeatedly measuring the ejected formalin liquid. Surgical tape was affixed to the hindpaw to secure the syringe during injection. Experiments were designed to minimize animal suffering, and were approved by the Institutional Animal Care and Use Committee, National Taiwan University, College of Medicine.

2.2. Imaging experiments

fMRI images were captured using a 4.7-T spectrometer (BioSpec 47/40). A 20-cm volume coil was used as the RF transmitter and a 2-cm surface coil placed on the head was used as the receiver. A T₂-weighted scout image was taken in the mid-sagittal plane to localize the anatomical position by identifying the anterior commissure (bregma −0.8 mm). T₂-weighted template images (bregma +1.2 mm, −0.8 mm, and −2.8 mm) were acquired using spin echo sequences with a repetition time (TR) of 4000 ms, echo time (TE) of 80 ms, field of view (FOV) of 4 cm, slice thickness (SLTH) of 2 mm, number of excitations (NEX) of 2, and an acquisition matrix of 256 × 128 (zero-filled to 256 × 256). The stereotaxic frame in the magnetic bore was adjusted to the correct angle until the scanned image showed no rotation. When motion artifacts were visible, the animal was remounted on the stereotaxic head holder. A set of 40-repeats, 3-slice gradient echo images was then acquired at the same location with a TR of 215 ms, TE of 20 ms, flip angle of 22.5°, FOV of 4 cm, SLTH of 2 mm, NEX of 2, an acquisition matrix of 256 × 64 (zero-filled to 256 × 256), and a temporal resolution of 27 s. For dynamic image acquisition, the first 20 consecutive frames were categorized as baseline, and the remaining 20 were collected after formalin was injected into the hindpaw. The animal was excluded from the analysis if motion occurred after stimulus onset.

2.3. Data analysis

BOLD images were analyzed using the custom-built ISP-MER data processing system [18]. Correlation coefficients were calculated on a pixel-by-pixel basis to allow correlation between neuronal activation and noxious stimuli. Each temporal profile was calculated with an OFF-ON paradigm, and a correlation coefficient of \( r = \pm 0.6 \) was used as a threshold value for generating time–activity curves (TACs). In order to improve the accuracy of anatomical localizations, the BOLD images were registered with a digital atlas of the rat brain [19]. The digital atlas was captured from the atlas PDF file and transformed into a 2D binary matrix using a MATLAB program. The edges of fMRI and atlas images were then

Fig. 1. T₂-weighted images were registered and fused with the rat atlas using scaling and shifting operations. These templates provided anatomical alignment and corresponding ROI selection. Image locations: (A) bregma +1.2 mm, (B) bregma −0.8 mm, and (C) bregma −2.8 mm.
registered using manual shifting (translating all the contents of the images by the assigned shift) and scaling (involving bicubic transformation in the \(x\) and \(y\) directions to address the difference in resolutions between fMRI and the atlas). This resulted in the activated signals having a clear spatial reference (Fig. 1).

An atlas-based region of interest (ROI) was selected by predefining the coordinates of each brain area in the digital atlas. These coordinates comprised the edge of each brain structure, and generated a corresponding ROI (Fig. 1). Since the coordinates were simultaneously transformed throughout the registration process, the data within each ROI could be calculated automatically. The TACs were created by averaging the time courses of activated pixels in the ROIs, and only activation of more than two pixels was considered to be a response. Since the brain images of the seven rats were transformed into a rat atlas reference domain after the registration process, the images could be normalized to the atlas by intersubject averaging, and then displayed as incidence images representing the averaged correlation coefficient. Overall responses to formalin stimulation under ketamine anesthesia could thus be displayed on this map.

3. Results

3.1. Incidence image

Incidence images generated by averaging the nociceptive maps from the seven rats are shown in Fig. 2. The averaged formalin-evoked images illustrate that the responsive areas were correlated with formalin stimulation under ketamine anesthesia. The activated regions include the cingulate cortex (Cg), motor cortex (M), primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insular cortex (IC), striatum (CPu), nucleus accumbens (Acb), medial thalamus (MT), ventrolateral thalamic group (VLT), and HIP. The major responses to stimulation were observed in the frontal subcortical areas, especially in the CPu and Acb. The activation was weaker in the thalamus, which may due to weaker activation or less-reactive animals. Most of the activated areas were bilateral, with clear lateralization only observed in the sensory cortices.

![Fig. 2. Incidence images calculated by averaging the correlation coefficient maps of seven animals during formalin stimulation. Each correlation map was normalized to the rat brain atlas before averaging. Strong activations were observed in the CPu and Acb. The minimal value of each incidence image was set at 0.375 of the maximal value. Negative activation was omitted from the analysis. Image locations: (A) bregma +1.2 mm, (B) bregma −0.8 mm, and (C) bregma −2.8 mm.](image)

![Fig. 3. Averaged TACs in different brain areas. Formalin was injected at frame 21. Each brain area was separated into ipsilateral (blue) and contralateral (red) regions according to the mid-sagittal plane. The signal intensities were normalized to the matching baseline data. Data represent mean and standard deviation values (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).](image)
BOLD signals appeared immediately after formalin stimulus (Fig. 3), with the signal generally peaking within 3 min and remaining elevated for at least 9 min. Formalin injection resulted in a 5–10% increase in the BOLD signal in the above-mentioned areas. This large signal increase may be attributable to threshold setting, since only the temporal pattern that was highly correlated with the paradigm was used to generate the TACs. The activated intensities differed significantly ($p < 0.05$) between the two hemispheres only observed in S1, S2, and IC.

4. Discussion

Mapping the activated pixels into different brain areas is important to determining the hemodynamic pattern therein. One key aspect of the present study was that all images were registered and fused with the rat atlas. Echo planar imaging was not selected since it would cause less-manageable artifacts and geometry distortion; while the fast gradient echo was used to reduce spatial distortion and increase the accuracy of anatomical alignment. The availability of information about the spatial distribution allowed data to be more precisely determined by applying atlas-based ROI selection. Unlike manual ROI methods, data processing in the present study did not introduce artificial bias.

Small-animal fMRI studies have mapped the somatotopic projection from the forepaw, hindpaw, and tail of rats using electrical stimuli [20–23]. These studies determined brain locations that were responsive to intense electrical stimulation, but this cannot be considered to be a pure pain stimulus since coactivation of Aβ, Aδ, and C fibers may cause confusion of both innocuous and noxious responses. In addition, noxious electrical stimulation can result in less-consistent activation than pain induced by chemical stimulants [16].

Significant blood pressure changes have been reported to increase BOLD signals due to increases in cerebral blood flow [24]. Previous studies demonstrated that injecting 50 μL of 5% formalin into the left hindpaw does not significantly elevate the blood pressure, with no nonspecific BOLD changes being observed [16,17,24,25]. This suggests that formalin does not result in nonspecific activation.

Ketamine has been shown to have a high affinity to dopamine D₂ and serotonin 5-HT₂ receptors [26]. This contributes to the activation of dopaminergic neurons in frontal subcortical regions since the extracellular turnover of dopamine in Acb is sensitive to a ketamine challenge [7]. Recent electrophysiological recordings showed that painful stimuli would increase the firing rate of rat dopaminergic neurons in the ventral tegmental area, which is the origin of the mesolimbic dopaminergic system [27]. While the present study found that responses induced by formalin appeared mainly in the CPu and Acb (Fig. 2), these areas were considered to be involved in pain circuits and rich in dopaminergic receptors [28]. This indicates that although a high level of dopamine is induced by initial ketamine anesthesia [29], it is highly probable that the formalin may further influence the dopamine-related vascular response in the CPu and Acb.

For the slight activation in HIP, an immunohistochemical study showed that unilateral injection of formalin induced bilateral c-fos expression in the HIP, implicating reciprocal neural connections from the HIP in the perception of chronic pain [30]. Besides, other regions showing lower activation are consistent with the spinothalamic tract and its related projections. These areas include S1, S2, MT, VLT, and Cg, which are closely associated with pain processing. The signal increases in these regions are also consistent with the previous report involving noxious electrical stimulation of the sciatic nerve [31]. The spinothalamic pathway is mainly considered to be a contralateral projection. Figs. 2 and 3 show that the S1 cortex is the best candidate for detecting the laterality of a painful stimulus [32], with it being difficult to observe clear lateralization in other brain regions. Previous BOLD formalin experiments showed that activation often occurred bilaterally in the both cortical and subcortical regions under α-chloralose anesthesia [16,17], while Shah et al. also reported bilateral activation in most brain structures under halothane anesthesia [25]. The reasons for this are still unclear, but it might reflect the interhemispheric transfer of nociceptive information by either commissural connections or the thalamus [33–36].

Difference of activation patterns between the present study and the previous BOLD formalin experiments may be influenced by the effects of anesthetics, since anesthesia would mediate the representation of specific functions of the brain [37]. α-Chloralose is a widely used analgesic in BOLD studies due to its minimal suppression of neuronal activity [38]. A 2-deoxyglucose autoradiography study found that α-chloralose-anesthetized rats is the best model for showing sensory responses and neurovascular coupling in cortical regions [39]. Some studies using ketamine have shown that neuropathic and chronic pain is mediated via the NMDA receptors [40–41], and that systematic administration of NMDA receptor antagonists will greatly reduce pain perception in both humans and animals [42–44]. A recent study found that ketamine blocks sodium- and voltage-gated potassium channels of the NMDA receptor in superficial dorsal horn neurons, which modulates the firing rate of neuronal signal transmission [45]. These observations may result from strong nociceptive responses in cortical regions during α-chloralose anesthesia [16,17], whereas only slight activation was evident in the present results.

5. Conclusion

A great challenge in neuroscience is understanding pain. Pain signals are complex physiological events with diverse underlying mechanisms compared with other sensory modalities, and hence understanding these neuronal
interactions requires simultaneously measurements of whole-brain spatiotemporal activity. The present findings highlight the advantages of using BOLD fMRI, which elucidated the ketamine-mediated nociceptive map and found strong activations in frontal subcortical regions.

Acknowledgments

This work was supported by grant NSC-94-2213-E-002-001 from the National Science Council, Taiwan, ROC. The authors acknowledge technical support from the Functional and Micro-Magnetic Resonance Imaging Center supported by the National Research Program for Genomic Medicine, National Science Council, Taiwan, ROC (NSC95-3112-B-001-009).

Conflict of interest statement

There are no conflicts of interest in the present study.

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