Comparison of neural activity in the rat spinal cord using fMRI and field potentials during noxious electrical stimulation of the hind paw

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Introduction: Functional MRI indirectly detects areas of neuronal activity by observing local changes in blood oxygen. Until recently fMRI has been used only for brain imaging, however, it has since been applied to the spinal cord (spinal fMRI) (1,2). The relationship between areas of fMRI activity and neuronal activity must be verified by comparison with gold standards in order for spinal fMRI to become more widely accepted. A previous study investigated the relationship between areas of functional activity and the areas of expression of c-fos, a known marker of neuronal activity (3). This technique was limited to marking only some areas of activity in the spinal cord detected by fMRI. In the present study we compare areas of fMRI activity with local field potentials during noxious electrical stimulation of the hind paw in rats. In an area of neuronal activity there is an influx of Na^{2+} into the cells. This current results in a negative field potential extracellularly. By observing the pattern of field potentials we can observe generally, the loci of neuronal activity. Correspondence between the two methods confirms that spinal fMRI can be used to non-invasively evaluate sites of neuronal activity.

Materials and Methods: The lumbar spinal cords of nine Sprague-Dawley rats were imaged at 7T (Magnex, U.K with 205/120 gradient insert, Bruker, Germany) during halothane anesthesia (3-4% induction, oxygen/med. Air, 1-2% maintenance). The animal was intubated, placed supine on a quadrature surface coil tuned and matched to 300 MHz and ventilated using a small animal ventilator at a constant rate (60/min) (Columbus Instruments, Ohio, USA). Sequential images were acquired using a multi-slice fast spin echo imaging sequence (2 shot, TE_{eff}= 49.0 ms, 128 X 64 matrix, FOV=3cm). Acquisition of MR image data was gated to the respiration.

Six, 2 mm thick slices were centered on the vertebra and intervertebral discs between T12 and L1. Functional imaging experiments were performed using electrical stimulation (~7 mA) delivered by two silver needle electrodes inserted subcutaneously in the dorsal surface of the right hind paw; experiments were repeated three times for each animal. During fMRI studies the expired O2 and CO2 were monitored with a pulse oximeter. Following imaging, animals were recovered and rested for at least 48 hours before electrophysiology experiments were performed.

During electrophysiology, animals were anesthetized with halothane. A laminectomy was performed from T12 to L1 in order to measure extracellular potentials in the imaged region of the spinal cord. Recordings were taken using a 2-M tungsten electrode. Extracellular recordings were taken during rest and during electrical stimulation of the right hind paw (~7 mA), every 150 µm to a depth of 1800 µm from the dorsal surface of the cord, at several sites spanning these segments.

Data Analysis: Functional MRI data were analyzed using a direct correlation to the stimulation paradigm (p=0.05) using custom made software written in MatLab. Extracellular potentials were analyzed using X-win. A minimum of 50 traces had been collected and these were averaged off-line to obtain a mean response. For each individual site the delay between stimulation and response, peak response (mV), time to peak, and area of the potential were measured.

Results: Figure 1 shows 3 functional maps and measured local field potentials from one subject. The greatest number of active pixels is observed at the level of the T13 vertebra and T13/L1 disc. At this level, the largest negative field potentials are also observed. Some variation between individual activity maps is observed, as anticipated. Active pixels are observed in the right ventral horn at the T13 vertebra in 2 of 3 maps. Ventral horn activity is observed on the contralateral side in all 3 maps. At the level of the T13/L1 intervertebral disc, active pixels are observed in 2 out of 3 maps in both the right and left dorsal horn. Active pixels are observed in the left ventral horn in 2 of the 3 maps at both the L1 vertebra and the L1/L2 disc. The local field potentials measured at the same approximate location are shown adjacent to the images. Measurements taken on the right side of the animal are shown on the right side of the image. The largest field potentials are observed at the T13 vertebrae. At a slightly more caudal location (T13/L1), negative field potentials are observed as deep as 1200 µm. Smaller negative field potentials are observed on the left side indicating that neuronal activity is present in the dorsal horn on the contralateral side.

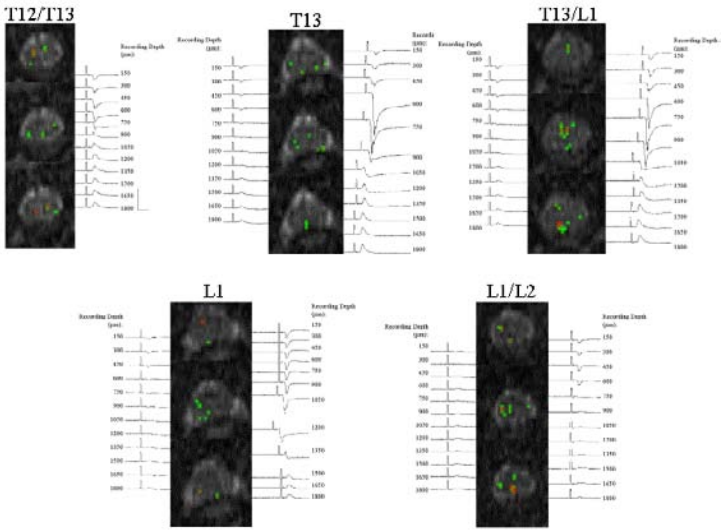

Figure 1. Three individual functional maps from one subject. Labels indicate the vertebral level of slices. Images are shown with the dorsal side of the animal at the top of the page and the right side of the animal on the right side of the image. Recorded average field potentials measured at the approximate same location are shown beside the images with recordings taken on the right side of the cord shown on the right side of the image.

Discussion: Most animals displayed the greatest local field potentials at the level of the T13 vertebra, the same level in which most of the functional activity was observed. While some variation was expected between the three functional scans in each of the animals, there were some areas that were consistently active between scans as well as across animals. A Previous study has confirmed neuronal activity observed in the superficial lamina of the ipsilateral dorsal horn (3). Spinal fMRI studies have observed functional activity on both sides of the cord and in both the dorsal and ventral horns. In the present study, negative local field potentials were observed from the most shallow recording site (150µm) to 1200 µm at some levels of the spinal cord demonstrating greater areas of neuronal activity than previously described.

The positioning of the imaged slices and recordings were taken carefully in order to best match the locations. During functional imaging, a sagittal image was used in order to center axial slices on the vertebral bodies and intervertebral discs. Therefore the position in the spinal cord can only be approximated in relation to the spine. Recently, a new technique using sagittal slices in the human spinal cord (1) provides more anatomical information including the location of the spinal nerves yielding a more precise method of placing slices in relation to the spinal cord segments.

Future studies will allow further characterization of the functional response observed by spinal fMRI in order to identify false positive activity. This study demonstrates neuronal activity is present in the areas of the spinal cord that are identified functionally active by fMRI. Spinal fMRI provides a noninvasive method of monitoring neuronal activity in the spinal cord.

Conclusion: Combined activation maps of the fMRI data are important in determining areas of functional activity that are consistent. In general, the pattern of functional activity and extracellular potentials are in agreement.

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3. Lawrence J, et al., (2004) Neuroimage, 22(4): 1802-7.