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Cortical Brain Mapping of Peripheral Nerves Using Functional Magnetic Resonance Imaging in a Rodent Model

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Abstract

The regions of the body have cortical and subcortical representation in proportion to their degree of innervation. The rat forepaw has been studied extensively in recent years using functional magnetic resonance imaging (fMRI)—typically by stimulation using electrodes directly inserted into the skin of the forepaw. Here, we stimulate using surgically implanted electrodes. A major distinction is that stimulation of the skin of the forepaw is mostly sensory, whereas direct nerve stimulation reveals not only the sensory system but also deep brain structures associated with motor activity. In this paper, we seek to define both the motor and sensory cortical and subcortical representations associated with the four major nerves of the rodent upper extremity. We electrically stimulated each nerve (median, ulnar, radial, and musculocutaneous) during fMRI acquisition using a 9.4T Bruker scanner. A current level of 0.5–1.0 mA and a frequency of 5 Hz were used while keeping the duration constant. A distinct pattern of cortical activation was found for each nerve that can be correlated with known sensorimotor afferent and efferent pathways to the rat forepaw. This direct nerve stimulation rat model can provide insight into peripheral nerve injury.

Keywords

brachial plexus; fMRI; nerve stimulation; rat imaging; sensorimotor cortical representation

INTRODUCTION

Brachial plexus and peripheral nerve injuries are extremely debilitating. These injuries result in varying loss of sensory and motor function in the affected extremity. The most serious injuries may require surgical neurotization, nerve grafting, or nerve transfer to restore essential function such as elbow and digit flexion. The procedures we use today were previously studied using animal models—in particular, the rat.^{1–4}

The early studies on brain mapping relied on electroencephalogram (EEG) stimulation to determine the spatial layout of sensory and motor regions. The development of functional magnetic resonance imaging (fMRI) provides a noninvasive tool to image the functioning human brain. This tool expands our ability to study neuroscience and has been used to study peripheral nerve injuries. A study by Beaulieu et al. used fMRI to study cortical activation in brachial plexus patients who had a crossed C7 nerve graft to neurotize the biceps muscle. Normal cortical activation for unilateral elbow flexion occurs in the contralateral cortex, whereas bilateral activation in neurotized patients was found.⁵ Hsieh et al. used fMRI to show there is no longer inhibitory influence from the ipsilateral hemisphere after unilateral brachial plexus injury.⁶ In separate studies, cortical reorganization was shown via fMRI after toe to thumb transfer and double-hand transplantation.^{7,8}

The fMRI signal relies on the nature of oxygenated versus deoxygenated hemoglobin. Oxygenated hemoglobin is diamagnetic but paramagnetic when deoxygenated. The magnetic resonance signal of water molecules in the vicinity of capillaries in the brain differs slightly depending on the level of oxygenation extraction during neural activity. Using an appropriate magnetic resonance pulse sequence, blood-oxygen-level dependent (BOLD) contrast signals can be detected. Principles of BOLD contrast in fMRI have been discussed by Chen and Ogawa,⁹ while a convenient text on fMRI intended for a physician audience is *Functional Magnetic Resonance Imaging* by Huettel et al.¹⁰

Following neural activation, there is a local transient increase in blood flow to meet the metabolic demands of the activated neurons. The concomitant increase in available oxygen, surprisingly, exceeds the metabolic needs of the neurons. This results in an increase of oxygenated hemoglobin on the venous side of the microcirculation in brain tissue. The higher BOLD signal intensities (red/orange in our study) come from increases in the concentration of oxygenated hemoglobin—a sign of increased neuronal activity. A “negative” BOLD signal (shade of blue in our study) is less straightforward and may represent an area of neuronal inhibition or an area of the brain not requiring an increase of oxygenated blood over other areas due to inactivity, and therefore following a “vascular steal” principle.¹¹

The fMRI signal intensity can be measured by the number of activated units or voxels. The term “voxel” comes from the words “volume” and “pixel,” and a voxel is a volume of tissue. In a two-dimensional image, the signal in each pixel arises from a corresponding voxel of tissue in the brain.

In this experiment, we focused on several regions of motor and sensory neural pathways (Fig. 1). These include the S1Tr/S1Fl, S2, Motor 1/Motor 2 (M1/M2), sensory motor thalamus (SMT), and caudate putamen regions. S1Tr is the somatosensory representation of the trunk, and S1Fl is the sensory representation of the forepaw. We will be referring to the S1Fl region throughout the paper. The S2 region is the secondary somatosensory cortex and is primarily responsible for the relay of signals to the opposite side of the brain. M1/M2 corresponds to the motor region for the forepaw. Approximately 10-15% of the signals to and from the somatosensory and motor cortex pass through the SMT region. The contributions of this region to the somatosensory system and motor system are subjects of ongoing research. The caudate putamen is part of the basal ganglia system; this area relays signals to other portions of the basal ganglia including the globus pallidus and substantia nigra.

The cortical representation of the rat forepaw has been studied using fMRI.¹²⁻¹⁵ Forepaw stimulation produces a signal in a particular region in the somatosensory cortex (S1Fl).¹⁶ A literature review did not reveal any studies beyond forepaw stimulation that focused on defining the motor and sensory regions of the rodent upper extremity.

This study describes a surgical approach to nerves of the rat brachial plexus to allow for direct nerve stimulation using implanted electrodes. Nerves carry both sensory and motor nerve fibers in the proximal portion of the upper extremity. Stimulating the mixed nerves provides an opportunity to study both motor and sensory components of the rat upper extremity and examine cortical regions beyond the sensory forepaw region. Specifically, we examine direct nerve stimulation of the ulnar, median, radial, and musculocutaneous nerves of the rodent upper extremity. By refining the motor and sensory cortical representation of each nerve of the rat upper extremity, we hope to develop an animal model to study and characterize peripheral nerve injuries and treatments. We further hope to expand these studies to humans to supplement our diagnostic capabilities and refine our surgical intervention and postoperative rehabilitation protocols.

MATERIALS AND METHODS

Twenty-one Sprague Dawley rats were used to study the musculocutaneous, ulnar, median, and radial nerves (N = 5 for each nerve except ulnar where N = 6). Under isoflurane anesthesia, the right femoral artery and vein were cannulated for invasive blood pressure monitoring and for continuous intravenous drug administration of pancuronium bromide (2 mg/kg/hr) and medetomidine hydrochloride (Domitor, 0.1 mg/kg/hr, Pfizer Animal Health, New York, NY) during the fMRI portion of the protocol. A tracheotomy allowed mechanical ventilation during the fMRI acquisition.

Exposure of median, ulnar, radial, and musculocutaneous nerves

An incision was made along the medial aspect of the right upper extremity and extended into the axilla and a portion of the flank. The subcutaneous tissues were retracted with suture placement. The pectoralis muscle was identified and retracted medially to expose the brachial plexus and brachial artery. The largest and most visible nerve is the median nerve. The ulnar nerve is medial and runs parallel to the median nerve while the radial nerve runs deep to the brachial artery. The long and short heads of the biceps brachii were retracted to expose the musculocutaneous nerve.

Direct nerve stimulation protocol

A 150 micrometer diameter stainless steel bipolar electrode (AISI 304, Plastics1, Roanoke, VA) was implanted around the nerve of interest. A piece of silicone plastic was attached to each proximal end of the electrode to ensure that the two ends of the electrode did not touch and to provide another point of fixation. A bipolar beryllium copper electrode was placed in the left forepaw to serve as a control. Every study began with a conventional fMRI experiment using forepaw stimulation.

We stimulated the nerves with a current of 0.5-1.0 mA and a frequency of 5 Hz provided by a Grass stimulator (88 Square Pulse Stimulator, Grass Telefactor, West Warwick, RI). One ms pulse duration was used for each nerve stimulation protocol. Each nerve stimulation sequence began with an OFF period of 40 s followed by three repetitions of ON for 20 s and OFF for 40 s (total sequence time = 3 min 40 s). The stimulation sequence was computer controlled with Lab-VIEW software (National Instruments, Austin, TX) and started by a trigger pulse delivered by the scanner.

The total surgical times varied from 1.5-2.0 hours, and the fMRI protocol was approximately two hours. The rat was euthanized using an overdose of sodium pentobarbital upon completion of the study. These studies were performed in compliance with federal regulations and the guidelines of our institution's animal care and use committee (IACUC).

fMRI image acquisition

An fMRI acquisition requires not only that the subject be motionless but also that the anesthetic conditions be optimal. The level of anesthesia cannot be too deep or it will suppress brain activity. Once the surgery was complete, the isoflurane was gradually tapered off and continuous infusions of medetomidine hydrochloride (Domitor, 0.1 mg/kg/hr, Pfizer Animal Health, New York, NY) for the maintenance of anesthesia and pancuronium bromide (2 mg/kg/hr) for muscle paralysis were started. The rat was placed on a mechanical ventilator (MRI-1 Ventilator, CWE, Ardmore, PA) using 30-70% O₂/N₂ and loaded onto a custom-built cradle fabricated using G-10 fiberglass material, suitable for fMRI experiments. The cradle was built with a warming surface controlled by a water-pump driven temperature regulator (Medi-therm III, Gaymar Industries, Orchard Park, NY).

A rapid acquisition with relaxation enhancement (RARE) anatomical image was acquired with a 256 × 256 matrix, TE = 12.5 ms, TR = 2.5 sec, and the same slice geometry as the echo planar imaging (EPI) sequence. Gradient echo scans (single-shot EPI, TE = 18.76 ms, TR = 2 sec, matrix size 96 × 96, FOV = 4 cm, number of repetitions = 110, 10 contiguous interleaved 1-mm slices, acquisition time = 3 min 40 sec) were acquired on a 9.4T Bruker AVANCE MRI scanner (Bruker BioSpin, Billerica, MA) with a 30-cm bore. Figure 2 shows a sagittal view of the locations of slices. Two sets of gradient echo images were acquired for each stimulation protocol. Images were acquired using a Bruker receiving surface coil (T9208) and a linear transmit coil (T10325).

Data acquisition

The EPI scans were registered to an ideal RARE anatomy with a 12 degree of freedom affine transformation using the Linear Image Registration Tool (FLIRT) software from the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain.¹⁷ The images for each nerve and stimulation protocol were averaged (3dcalc) using Analysis of Functional Neuroimages (AFNI) software.¹⁸ The averaged data for each nerve and stimulation level were then masked (3dAutomask), and activation was determined by an F test (3dDeconvolve) with a P-value threshold of 0.0005 using AFNI. The fMRI signal was modeled using a boxcar function as a regressor. Pixels were classified as active if the F statistic was above threshold. The color-coding of the pixels was determined by the amplitude of the fit coefficient, with blues signifying negative values and red/orange signifying positive values. The Paxinos and Watson atlas of the rat brain¹⁶ was consulted to draw the regions of interest (ROIs).

Figure 1 shows an enlarged BOLD activation map for slice 4 when the ulnar nerve is stimulated at 0.5 mA and 5 Hz. Ovals have been drawn around ROIs in the functional activation map to delineate the functional anatomy. Clearly shown are S1Tr (somatosensory S1 upper arm/trunk region), M1/M2 (motor regions), S2 (sensory region), and the thalamus. This figure should be used as a reference for the Results and Discussion sections.

RESULTS

Extensive physiological studies to investigate possible stress or pain were carried out in five animals on the bench using the same surgical preparations and stimulation protocols that we used for fMRI studies. We recorded the physiological parameters of heart rate (HR), blood oxygen saturation (SpO₂), and mean arterial pressure (MAP). There were no statistically significant differences between these parameters measured during the ON and OFF periods of our stimulation protocol. Because there was no significant difference before, during, or after our stimulation protocol, parameters were averaged together. The averages are: MAP = 136 ± 17 mmHg; HR = 289 ± 6 beats/min; and SpO₂ = 98 ± 1%.

We included a forepaw stimulation experiment in our study to verify the presence of cortical activation. This served as a control for normal animal physiological function and suitable depth of anesthesia. Figure 3 shows three contiguous representative slices, which can be correlated with the sagittal view of the rat brain in Fig. 2. The area of activation corresponds with the S1FI region.

Figure 4 shows the representative BOLD time courses from a single voxel in a region of interest, S1FI, in response to the direct nerve stimulation protocol. This is the sensory region of the forepaw. Differences in S1FI activation after direct stimulation of the four separate nerves are clearly shown.

Slices 0 through 4 with ulnar nerve activation are shown in Fig. 5. The stimulation indicates BOLD responses in many regions of the brain. Slice 0 shows the most activation in the M1/M2 region while Slice 4 shows the most activation in the S1Tr region. Comparing Fig. 3, we see that a portion of the activated region can be localized to the stereotaxic S1FI region and overlaps with the region activated by forepaw stimulation. Note that the forepaw signal in Fig. 3 is found contralateral to the sensory region activated by the ulnar nerve stimulation because the left forepaw and right ulnar nerve were stimulated. Note also the small activation of the S2 region and the negative activation of the caudate putamen with ulnar nerve stimulation.

Median nerve stimulation (Fig. 5) produces a similar pattern to ulnar nerve stimulation. Activation of the motor cortex is seen best in slices 0 and 1. The activation seen in the sensory region of median nerve stimulation (slices 2-4) corresponds to S1FI and overlaps with the

forepaw sensory region (Fig. 3). There is some activation in the secondary somatosensory region (S2) and the caudate putamen, as was found for ulnar nerve stimulation.

Stimulation of the radial nerve produces the cortical activation pattern displayed in Fig. 5. As with ulnar and median nerve stimulation, radial nerve stimulation produces activation in the mediofrontal cortical regions. Activation is seen in the somatosensory region (S1Tr). Unlike the median and ulnar nerve, the S2 region is not activated; however, the activation of the caudate putamen is similar to the median and ulnar nerve.

Musculocutaneous nerve stimulation (Fig. 5) shows a different pattern of activation compared with the other three nerves studied. Activation in the mediofrontal cortical regions is present but significantly less than the other three nerves. The area of activation appears to be in the M1/M2 region with no apparent activation of the S1Tr area. Activation in the thalamic region is present at the protocol frequency and current, as is the activation of the caudate putamen. The S2 region is not activated.

DISCUSSION

Many of the studies relating to defining the sensory or motor regions of the rat brain have used electrophysiological mapping methods.^{19,20} To our knowledge, the use of direct nerve stimulation in the upper extremity of the rat has not previously been reported.

Each stimulated nerve shows a unique cortical activation pattern. Although these are distinct, there are some overlapping regions of activation. For example, the sensory component for each nerve overlaps to varying degrees with the forepaw region (S1Fl) of the somatosensory cortex. This coincides with the anatomical distribution of the nerves since each nerve provides sensory innervation to various areas of the forepaw. There is also overlap of the motor regions of the brain—again, concurring with the known innervations of the four major nerves. For example, both the median and ulnar nerves contribute to wrist flexion and also innervate the intrinsic muscles of the forepaw.

Our method of direct stimulation of mixed motor and sensory nerves provides the brain with combined motor-sensory inputs in contrast to pure forepaw sensory stimulation. The orthodromic stimulation of the sensory fibers and antidromic stimulation of the motor nerve fibers can give rise to the observed pattern of a strong somatosensory signal and bilateral M1/M2 signals.

We detect bilateral thalamic and motor activation with mixed nerve stimulation. This activation is transmitted through the spinothalamic tract to the contralateral thalamus. The activation is also transmitted through the spinoreticular cells and then relayed to both thalami and the motor areas. The functional neuroconnectivity within and across hemispheres in the motor and sensory networks appears extensive.

The median and ulnar nerves show activation of both the sensory and motor areas concomitantly. The radial nerve has the same areas of activation but to a lesser degree. We know that these nerves have both motor and sensory significance in the upper extremity. The activation of the S2 region with the median and ulnar nerve stimulation is less understood and requires future investigation.

Figure 4 is another way to look at the data. In this figure, a single voxel is isolated and the percent signal change is shown with respect to time. We may be able quantitatively to extrapolate the various contributions of each nerve to the S1Tr region of the rat cerebral cortex. The percent signal changes for excitation of the median and ulnar nerves are relatively higher

than for the radial and musculocutaneous nerves. This is consistent with the hand receiving the majority of the sensory innervation by the median and ulnar nerves.

An interesting finding is the activation seen with musculocutaneous nerve stimulation. When one compares the activation with that of the other three nerves, one sees that while the motor region is activated, the sensory region is not activated at our stimulation level. This finding may be explained by the nature of the nerve since it serves primarily as a motor nerve with relatively little sensory contribution to the upper extremity. Unreported data show that the activation of the sensory region becomes apparent at double the current (1.0 mA) and double the frequency (10 Hz).

The negative BOLD response seen in the caudate putamen is a unique finding. Interestingly, a literature search turned up no electric stimulation studies in rats with negative or positive BOLD signals in the caudate putamen. However, pharmacological fMRI studies in rats did reveal negative BOLD responses.^{21,22} The caudate putamen is involved in both the somatosensory and motor neural network of the rat.¹⁶ This region has direct connections to the motor cortex as well as to the intralaminar thalamic nuclei, and can be activated or inhibited by nociceptive impulses. Perhaps antidromic stimulation of motor nerve fibers gives rise to this response. An analogous cerebral blood volume (CBV) experiment using monocrystalline iron oxide nanoparticle (MION)²³ and direct stimulation of the musculocutaneous nerve reversed the sign of the fMRI response in both the caudate putamen (positive) and somatosensory (negative) regions (unreported data). This may indicate that a decrease in CBV occurs in the caudate putamen region with a concomitant increase in CBV in the somatosensory system during direct nerve stimulation.

The rat model affords us the opportunity to perform various manipulations that could not be done in a human model. One limitation of this model is that under the anesthesia required to sedate the rat, brain function can differ from that of the fully awake brain. Another limitation is that direct electrical stimulation of the nerve is not physiologic.

The fMRI method is a powerful tool to probe brain function and the associated networks. By understanding these networks, we may be able to understand and treat the pathologies associated with peripheral nerve injury. This goal of developing a rodent fMRI model for brachial plexus and peripheral nerve injuries appears within reach.

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ABBREVIATIONS

fMRI, functional magnetic resonance imaging
 EEG, electroencephalogram
 BOLD, blood-oxygen-level dependent
 M1/M2, Motor 1/Motor 2
 SMT, sensory motor thalamus
 S1Tr, somatosensory representation of the trunk
 S1Fl, sensory representation of the forepaw
 S2, secondary somatosensory cortex
 RARE, rapid acquisition with relaxation enhancement
 EPI, echo planar imaging
 ROIs, regions of interest

HR, heart rate
 SpO₂, blood oxygen saturation
 MAP, mean arterial pressure
 CBV, cerebral blood volume
 MION, monocrySTALLine iron oxide nanoparticle

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Figure 1.

Cortical activation in slice 4 after direct ulnar nerve stimulation (0.5 mA, 5 Hz). Two sensory regions are delineated: the S1 trunk region (S1Tr) and the secondary somatosensory region (S2). The primary and secondary motor regions (M1/M2) are active, as is the thalamus. The negative BOLD response overlies the caudate putamen region of the brain.

Figure 2.
Midline sagittal view showing the five acquired slices (0-4) with each acquisition.

Figure 3.

A representative voxel time course when the forepaw is stimulated (2 mA, 10 Hz, 3 ms). This activated region is S1FL.

Figure 4.
Representative single voxel time course response in S1FL to excitation of four different nerves.

Figure 5.
Cortical response to direct nerve stimulation (0.5 mA, 5 Hz).