Video Article

Simultaneous fMRI and Electrophysiology in the Rodent Brain

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Abstract

To examine the neural basis of the blood oxygenation level dependent (BOLD) magnetic resonance imaging (MRI) signal, we have developed a rodent model in which functional MRI data and *in vivo* intracortical recording can be performed simultaneously. The combination of MRI and electrical recording is technically challenging because the electrodes used for recording distort the MRI images and the MRI acquisition induces noise in the electrical recording. To minimize the mutual interference of the two modalities, glass microelectrodes were used rather than metal and a noise removal algorithm was implemented for the electrophysiology data. In our studies, two microelectrodes were separately implanted in bilateral primary somatosensory cortices (SI) of the rat and fixed in place. One coronal slice covering the electrode tips was selected for functional MRI. Electrode shafts and fixation positions were not included in the image slice to avoid imaging artifacts. The removed scalp was replaced with toothpaste to reduce susceptibility mismatch and prevent Gibbs ringing artifacts in the images. The artifact structure induced in the electrical recordings by the rapidly-switching magnetic fields during image acquisition was characterized by averaging all cycles of scans for each run. The noise structure during imaging was then subtracted from original recordings. The denoised time courses were then used for further analysis in combination with the fMRI data. As an example, the simultaneous acquisition was used to determine the relationship between spontaneous fMRI BOLD signals and band-limited intracortical electrical activity. Simultaneous fMRI and electrophysiological recording in the rodent will provide a platform for many exciting applications in neuroscience in addition to elucidating the relationship between the fMRI BOLD signal and neuronal activity.

Video Link

The video component of this article can be found at http://www.jove.com/video/1901/

Protocol

1. This is a non-survival surgery. The first step is implantation of the electrodes. In this example, electrodes will be implanted in the forepaw regions of primary somatosensory cortex of both hemispheres.

1. Anesthetize the rat (male SD rat, 200-300 g) with 2% isoflurane and fix into place on a stereotactic surgical system. Before beginning surgery, ensure that the animal is well anesthetized and exhibits no response to a toe pinch. Remove the fur before opening the scalp. Separate the muscle and other tissue above the skull and block any bleeding on the bone surface using a cauterizer.
2. Prepare a pier on the skull surface (near the midline anterior V-shaped junction) as a fixation point for the shaft of the implanted electrode, using dental cement. Setting a small nylon screw set into the bone before applying dental cement may increase stability. The size of the pier shaped with cement should be approximately 5 mm high and 3 x 5 mm^2 in area at the base (see Figure 1).
3. Using a fine tipped electrical drill, carefully open the skull and expose the dura over the forepaw representation in primary somatosensory cortex of each hemisphere. The diameter of each hole should be around 1 mm, positioned 1 mm anterior to and 4 mm lateral from bregma. Under a microscope, cut a tiny opening in the dura using a syringe needle tip, being careful to avoid any vessel damage.
4. Before inserting each electrode, make sure that no bleeding or exudation is present near the incisions. The glass microelectrodes should be prepared before surgery with approximately 3~4 cm shaft-length and impedance of 1~5 Mω. Fill the capillary of the electrode with artificial CSF (ACSF) and insert each electrode obliquely (~45°, from posterior to anterior) into the brain ~0.4 mm from the opened dura using the stereotactic arm. Before fixing into place, check the electrical signal. One end of a chloridized silver wire should be dipped into the ACSF and the other end connected to the input leads to the amplifier. A silver wire, attached subcutaneously at the back of the opened skin, serves as reference electrode.
5. Before electrode fixation, double check the surgical area and make sure that no bleeding or exudation occurs, and then apply toothpaste to replace the removed skin and muscle on the skull. The use of the toothpaste improves the MR image quality by reducing the susceptibility mismatch at the skull/air interface. Attach the electrode shaft to the prepared pier with dental cement (see Figure 1).
6. After the dental cement cures, transfer the animal to the MRI cradle and fix in place. Monitor the rat s physiological condition for the remainder of the study, including body temperature, respiration rate, SPO_2 and cardiac rate.

7. Position a surface coil (transmit/receive) over the head, with the electrodes protruding from the center of the coil. An additional arch-shaped hard cover that sits atop the cradle serves as a support for fixation of the electrode leads in order to avoid motion caused by the animal breathing. The leads used for simultaneous imaging and recording extend to ~5 m (the amplifier is located just outside the magnet room) and are covered with conductive plastic which serves as a passive shield.
8. Anesthesia can be switched from isoflurane to medetomidine to reduce suppression of neural activity if desired. Examine the electrical signal a final time before transferring the animal into the magnet. In our studies, the recording parameters were as follows: x 1000 amplified, 0.1Hz~5 K Hz bandpass-filtered, 60 Hz notch-filtered, 12 kHz sample rate for analog to digital conversion.

2. At this point, the animal is inserted into the MRI scanner for simultaneous imaging and recording. The animals are anesthetized throughout the imaging procedure.

1. A 9.4 T small animal MRI system (Bruker, Germany) was used in our studies. Prior to recording, imaging parameters must be established. A three plane scout image is used to position the fMRI scans. To improve the homogeneity of the magnetic field, the volume of interest is shimmed using FASTMAP[1]. For fMRI studies, a coronal imaging slice was selected, which included bilateral forepaw primary somatosensory areas, in which the electrodes were implanted. The EPI imaging parameters were FOV, 1.92 x 1.92 cm^2; matrix size, 64 x 64; in-plane resolution, 0.3 x 0.3 mm^2; slice thickness, 2 mm; TR/TE, 500/15 ms.
2. After the imaging setup is complete, simultaneous recording and fMRI can begin. Figure 2 shows a representative EPI image and raw recordings during imaging. The rapid switching of the gradients during image acquisition results in saturated recordings, which persist for only a small portion of each scan cycle (22/500 ms). After image acquisition, the electrical signal returns to baseline with a form of non-saturated oscillation (see Figure 3). Combined fMRI and recording may be conducted during the resting state (as demonstrated in this study) or during stimulation. For stimulus studies, the imaging parameters are the same as for the resting state study, with electrical stimulation of the forepaw supplied using 9 Hz, 1~4 mA current. The rat is euthanized after the final scan.

3. After simultaneous imaging and recording, the data must be pre-processed prior to final analysis.

1. We begin with the removal of the gradient artifacts from the electrophysiological recordings (see Figure 3).
    1. The noise structure due to scanning can be extracted by averaging all ~500 ms (TR) sections, each of which corresponds to the interval between two consecutive fMRI images.
    2. Subtract the average noise structure from the original recordings. This method corrects only the unsaturated recording segments.
    3. Each saturated segment corresponding to gradient alternation during image acquisition is replaced by a line, which passes between the time point before and the time point after gradient-induced saturation.

2. The denoised recordings of local field potentials (LFPs) are then converted to power time courses, which will have the same temporal resolution as the fMRI time course. The average power within a 2-second bin is used for calculating the lowest frequencies (delta band, 1~4 Hz), with a 1 second bin for theta band (4~8 Hz) frequencies, and a bin of ~0.5 s between neighboring saturated signals for higher frequencies (> 8 Hz, alpha to gamma bands). The sliding window for all frequency bands was moved in 0.5 s increments, matching the TR of the fMRI data.
3. For image data, standard fMRI preprocessing is performed, including head motion correction, image smoothing with 0.5 mm FWHM, and linear drift removal.
4. Cross correlation analysis is conducted between the LFP power time courses and the time course from each voxel of imaging data. Varying time lags allow the examination of time-dependent correlation (see Figure 4).

Representative Results:

As an example, this technique may be used to investigate the relationship between spontaneous neural activity and BOLD fluctuations. Figure 4 shows the correlation maps between LFP power and the BOLD signal at time lags between -2.5 and 9.5 s from one rat. The low frequency BOLD fluctuations (< 0.1 Hz) from cortical areas near the electrode tip are correlated with LFP power changes (< 0.1 Hz) at a delay of 2~6 s.

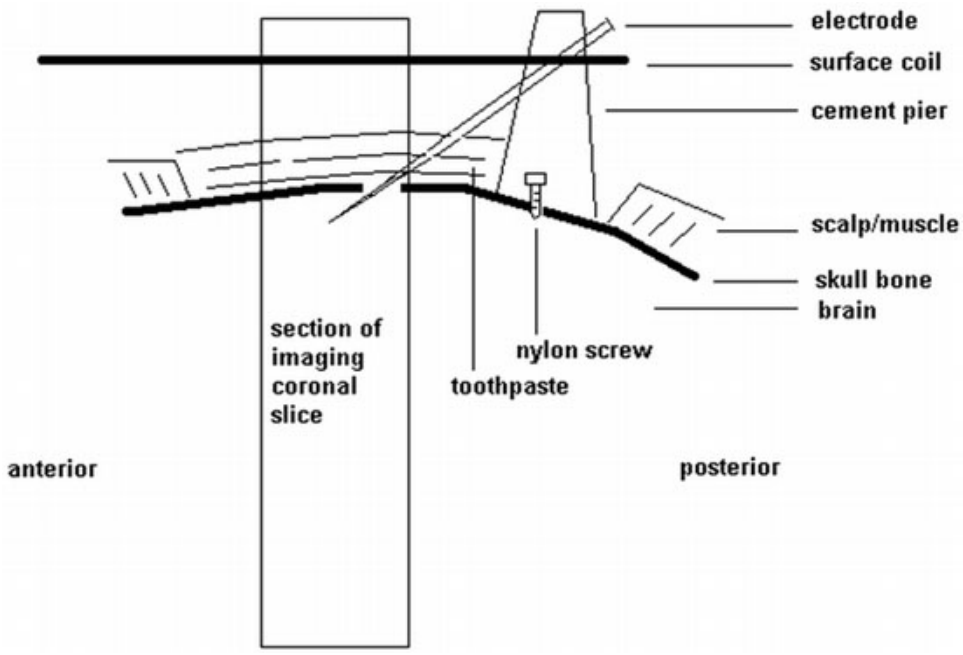

Figure 1. Schematic configuration of electrode implantation and imaging region with a surface coil.

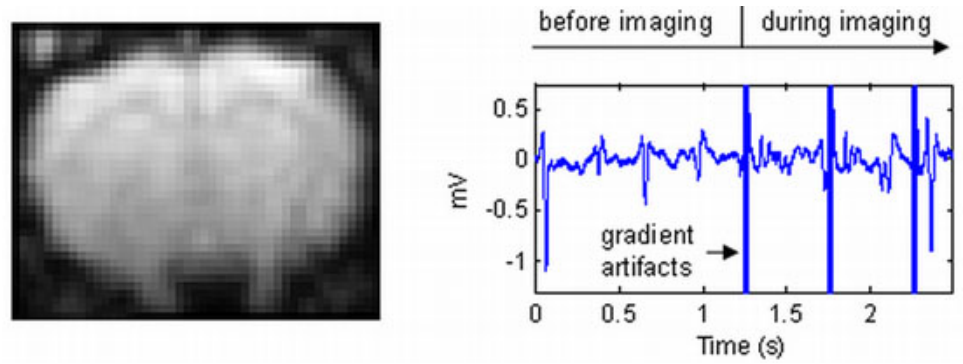

Figure 2. A representative coronal EPI image, including the electrode tips, is shown in the left panel. The right panel shows raw electrophysiological recordings before and during imaging.

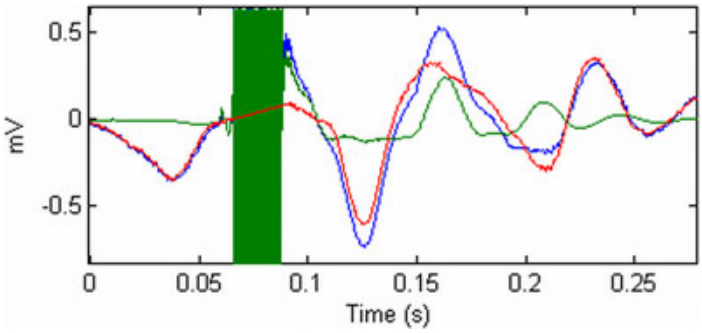

Figure 3. By zooming in on one scan cycle, it can be seen that the artifact (green) during imaging may be removed from original recordings (blue). The denoised time courses (red) were used for further analysis.

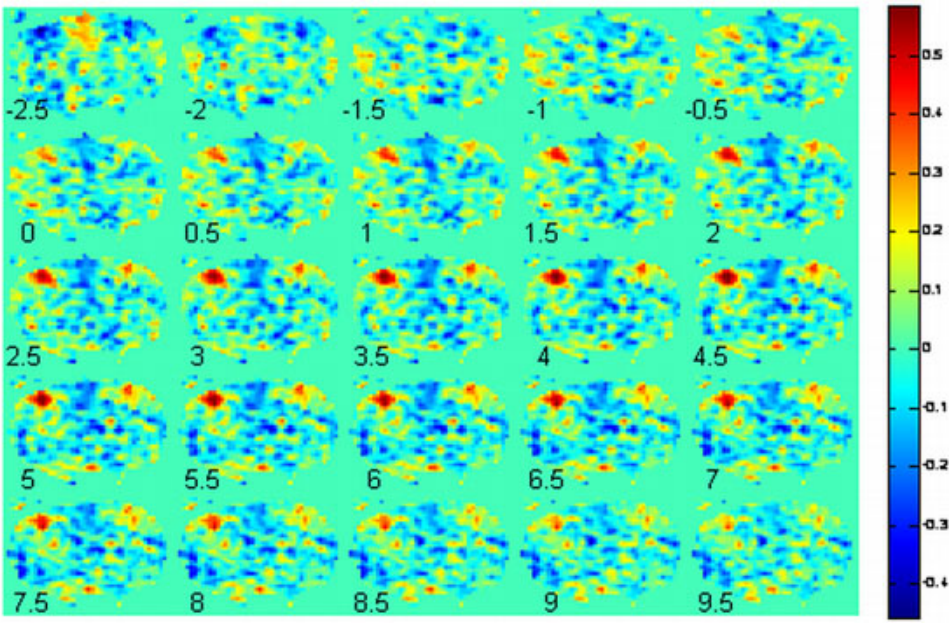

Figure 4. Coronal maps (from one typical rat) of correlation between the power of spontaneous delta band activity from one electrode and the resting-state BOLD signal at time lags from -2.5 to 9.5 s. Maximum correlation is observed in bilateral SI at approximately 4~5 s in the isoflurane-anesthetized rat. Color bar represents Pearson r.

Discussion

Both electrophysiological recording and BOLD fMRI are separately well-developed techniques. However, simultaneously recording and imaging is challenging due to the mutual interference[2] of the two modalities. Here we provide a possible solution for combined experiments in the rodent. The modified method of electrode implantation minimizes influence on the image quality, and the artifact removal for the electrical recordings is necessary to remove the noise induced by image acquisition. Simultaneous imaging and recording in the rodent will provide a powerful platform for further investigations of the coupling between spontaneous neural activity and the BOLD signal, in addition to other applications in neuroscience that take advantage of the combined strengths of electrophysiology and functional brain imaging[3].

Acknowledgements

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References

1. Gruetter, R. Automatic, localized *in vivo*adjustment of all first- and second-order shim coils. Magn Reson Med 29 (6), 804 (1993).
2. Logothetis, N.K., Pauls, J., Augath, M., *et al.*, Neurophysiological investigation of the basis of the fMRI signal. Nature 412 (6843), 150 (2001).
3. Nir, Y., Fisch, L., Mukamel, R., *et al.* Coupling between neuronal firing rate, gamma LFP, and BOLD fMRI is related to interneuronal correlations. Curr Biol 17 (15), 1275 (2007).

