A novel test bed for non-BOLD functional MRI

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Synopsis

Several fMRI contrast mechanisms have been proposed to measure neuronal activity more directly and accurately than BOLD fMRI. Conclusive findings supporting these non-BOLD fMRI methods have been difficult to obtain, mainly because of the dearth of a reliable and robust test system to vet and validate them. Here we describe the development and testing of a test bed for non-BOLD fMRI, in which calcium fluorescence imaging and MR acquisition can be performed simultaneously on the same organotypic cortical cultures. This experimental design makes it possible to directly correlate any candidate fMRI signal to a robust optical indicator of neuronal activity.

Purpose

Several functional magnetic resonance imaging (fMRI) contrast mechanisms including diffusion,[1] phase imaging,[2] proton density,[3] etc. have been proposed to measure neuronal activity more directly and accurately than BOLD fMRI. However, these approaches have proven difficult to vet and validate because of the dearth of reliable and robust test systems. At a minimum, such an fMRI test bed should include (a) a well-characterized biological model of neuronal activity free of hemodynamic and related confounds and artifacts, and (b) an independent well-established and direct neurophysiological method to detect neuronal activity simultaneously with fMR/fMRI acquisition. Here we describe the development and testing of such a test bed for non-BOLD fMRI.[4]

Methods

To achieve goal (a), perfused organotypic rat cortex cultures that do not have a vascular system were used. Organotypic cortical cultures largely maintain the in vivo cortical cytoarchitecture including cortical layers and cortical cell types[4,5], and they display bursts of spontaneous neuronal activity that is similar to *in-vivo* nervous tissue.[6] To achieve goal (b), intracellular calcium fluorescence imaging is performed simultaneously with MR measurements. As shown in Fig. 1, organotypic cultures were kept in a custom-machined environmental chamber to maintain the culture's vitality. The chamber was mounted on the top of a one-sided MR system with permanent magnets (0.32 T);[4,7] this MR system provides open access to the tissue culture unlike conventional MRI scanners. An RF surface coil was attached directly below the coverslips to transmit and receive MR signals. An optical fluorescence microscope was mounted above the MR stage, thus enabling calcium imaging from above the organotypic culture. One advantage of this optical system is its long working distance objective (87mm with the 0.63× lens), which allows for a gap between the permanent magnets and the fluorescence microscope.

Results

(1) MR acquisition and analysis. The cultured tissue is first located by 1D profiling with a spatial resolution of 40 μm (Fig. 2c). Diffusion MR (Fig. 2b and d) was performed prior to the simultaneous fluorescence and MR recording; the diffusion-weighted signal decay was well fitted by a bi-compartment model with the slow diffusion component fraction ~10.3 ± 3.1%. To test the performance of the simultaneous fluorescence and MR

experiments, a CPMG pulse sequence was used for fast recording: TR = 1 s, 1200 echoes with τ = 30μs (Fig. 2a). The decay curves in all the samples (n =14) were fit well by a single-exponential function with T_{2eff} = 59.0 ± 2.7ms, except for the faster decaying part at echo time (TE) < 5ms with a fraction 2.5 ± 0.8% and relaxation time < 10ms (Fig. 2e).

(2) Calcium imaging. Fluorescence imaging was acquired with 8.8 mm × 6.6 mm FOV and 10 frames per second. Three ROIs were selected in each cortical region (ROI 1–6), whose calcium traces showed highly spontaneous neuronal activity across the two cortical tissues. The background ROI 7 shows low fluorescence intensity without neuronal activity information. The calcium signal from the entire tissue was used for further correlation test with MR signals.

(3) Time-series analysis pipelines. Two MR CPMG parameters were defined: signal intensity I_0, the average of the first 5th-100th echoes (mean TE 3.2ms); decay ratio R, the weighting ratio between the average of the 301rd-1200th and 5th-300th echoes. The potential effects of neuronal activity on the MR signal were tested by binning the MR signal itself into two categories: active and resting states, based on its relative temporal location to each neuronal activity event (Fig. 4). For Type 1, we hypothesized that each neuronal activity event only affects the MR signal recorded after each neuronal activity event in a time window T (0.1 - 1.0s). For Type 2, we hypothesized that each neuronal activity event only affects the MR signal recorded within 2s before and following each neuronal event. Each active MR was further binned into different groups on the basis of how distant it was in time (Δ) from the neuronal activity event. Paired students t-Tests did not reveal any significant correlation between neuronal activity and two MR CPMG parameters.

Conclusion and Discussion

Here we describe the development and testing of a test bed for non-BOLD fMRI, in which simultaneous fluorescence imaging and MR acquisition can be performed on live organotypic cortical cultures. The reliability and performance of the proposed test bed was demonstrated by a conventional CPMG MR sequence acquired simultaneously with calcium imaging, which is a well-characterized means to measure neuronal activity. This experiment design will make it possible to directly correlate other candidate functional MR signals to the optical indicators of neuronal activity in future.

Acknowledgements

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Figures

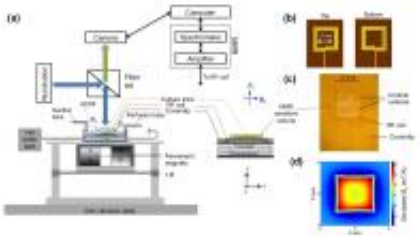

Figure 1. (a) Schematic diagram of the setup and an enlargement of the components near the specimen. (b) Layers of the two-layer multi-turn RF surface coil. (c) An image of the coil with the cortical cultures mounted. (d) A simulated 2D B_1 field distribution in the plane of the specimen.

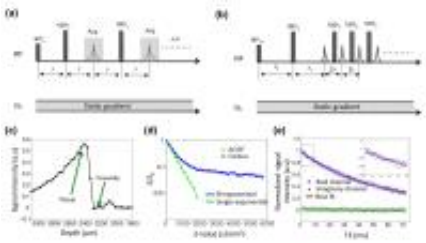

Figure 2. Diagrams of the two pulse sequences: (a) CPMG and (b) diffusion editing SE with CPMG detection. (c) MR spatial localization of the culture sample. (d) The diffusion-weighted MR decay signals. (e) The CPMG signal of the culture, which is fitted with a single-exponential function (red continuous curve).

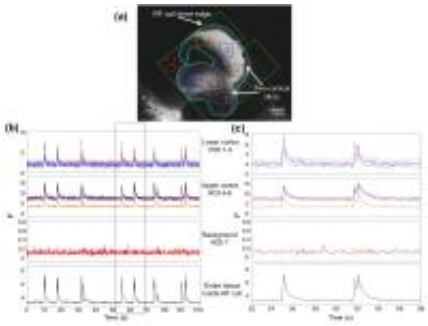

Figure 3. (a) Fluorescence image of the organotypic cortical culture (2 coronal slices co-cultured to reduce partial-volume effect in MR) and the position of seven different ROIs. (b,c) The raw calcium traces *F* of each ROI on the cortical cultures showed highly spontaneous neuronal activity.

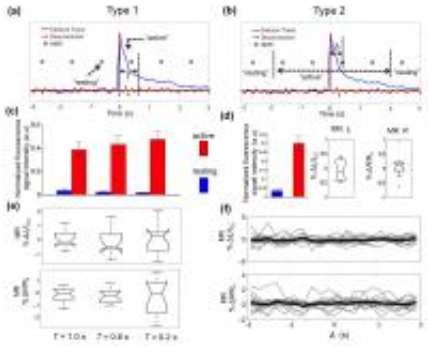

Figure 4. (a, b) Diagrams for two hypothesis tests. (c, e, d) Statistical results of the changes in fluorescence signal (c–Type 1; d–Type 2) and MR parameters (e–Type 1; d–Type 2) in the active states. (f) The time profiles of changes in the active states for Type 2 analysis.