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Mapping cortical columnar structures using fMRI

Seong-Gi Kim[a,b,*], Timothy Q. Duong[c]

[a]Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261, USA
[b]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN 55455, USA
[c]Department of Psychiatry, University of Massachusetts Medical Center, Worcester, MA 01655, USA

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Abstract

Mapping cortical columnar structures is important to understand cortical information processing. To map submillimeter columnar structures noninvasively, we have evaluated various functional magnetic resonance imaging (fMRI) techniques using a well-established feline orientation column model. The conventional positive blood oxygenation level-dependent (BOLD) signal is widespread and diffuse due to large venous vessel contributions, resulting in its poor specificity to columns. However, the early-negative BOLD signal is induced by the early oxygen consumption increase without significant change in blood flow. This negative signal has been successfully applied for columnar mapping. Tissue-specific cerebral blood flow (CBF) response is also specific to individual cortical columns, suggesting that parenchyma-specific fMRI techniques are capable to map individual single-condition functional cortical columns in animals as well as humans.

Keywords: Columns; Maps; Functional MRI; Brain mapping; Perfusion; Cerebral blood flow; Dip

1. Introduction

Mountcastle [1] and Hubel and Wiesel [2] demonstrated that cortical neurons with common functional properties are clustered into columns, spanning the entire cortical plate from the pia to the white matter. Individual functional columns in mammals are ~0.2–1-mm wide and isofunctional columns often repeat. Elucidation of the temporal and spatial properties of the brain's columnar and laminar architectures may provide the "neural code" of the brain's information processing. Visualization of such high-resolution functional localization has been achieved by 2-deoxyglucose (2-DG) autoradiography and intrinsic optical imaging. However, each of these techniques has its limitations. The 2-DG method is not viable for in vivo mapping [3], while the optical imaging of intrinsic signals requires the cortical surface to be exposed for penetration of optical light [4]. Since the penetration of light into the cortical tissue is limited, optical imaging does not contain any depth (laminar) information and can not obtain images from subcortical structures. To further understand cortical information processing, it is important to develop methods that can visualize the three-dimenstional functional architecture of the living brain repeatedly and noninvasively. Since newly developed functional magnetic resonance imaging (fMRI) techniques [5] have all the necessary requirements for columnar and laminar brain mapping, we explored various fMRI techniques for high-resolution functional mapping.

The most commonly used fMRI technique relies on changes in deoxyhemoglobin (dHb), which acts as an endogenous paramagnetic contrast agent [5]. Therefore, changes in the local dHb concentration in the brain lead to alterations in the signal intensity of magnetic resonance images (MRI) [5]. It is thought that neural activation leads to an increase in oxygen delivery without a commensurate elevation in cerebral oxygen consumption [6], which in turn causes a *decrease* in the capillary and venous dHb concentrations and thus an increase in the MRI signal. This imaging contrast is dubbed the "blood oxygenation level-dependent" (BOLD) signal [5]. Less common, alternative fMRI is based on the change of cerebral blood flow (CBF) [7]. Typically, CBF-weighted MRI employs arterial blood water as an endogenous flow tracer. Arterial blood water can be labeled by using radiofrequency pulses. Then, labeled

* Corresponding author. Department of Neurobiology, University of Pittsburgh, 3025 East Carson Street, Pittsburgh, PA 15203, USA.
E-mail address: kimsg@pitt.edu (S.-G. Kim).

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water moves into capillaries in the imaging slice and exchanges with tissue water. For fMRI experiments, two images are acquired repeatedly, one with arterial blood labeling and the other without labeling. The difference between the two images is directly related to CBF, and relative CBF changes due to physiological perturbations can be measured.

To investigate the feasibility of columnar resolution fMRI, we used a well-established cat orientation column model where the periodicity of cortical columns in area 18 is 1.1–1.4 mm and the average width of the column is ~500 μm, as demonstrated by the 2-DG technique and intrinsic optical imaging [4,8]. BOLD and CBF responses were obtained by using conventional gradient-echo BOLD and CBF-based flow-sensitive alternating inversion recovery (FAIR) techniques [7], respectively.

2. Methods

Detailed experimental procedures were described elsewhere [9–12]. In brief, female adolescent cats (0.5–1.1 kg) were treated with atropine sulfate (0.05 mg/kg im) and anesthetized with a ketamine (10–25 mg/kg im) and xylazine (2.5 mg/kg im) cocktail. Following oral intubation, the animal was mechanically ventilated using a Harvard ventilator (~25–35 stroke/min, 15–30 ml/stroke) under isoflurane anesthesia (1.0–1.3% v/v) in a 7:3 N_2O:O_2 mixture. End tidal CO_2 was kept at a physiological level (3.4–4.0%). The animal's temperature was maintained at 38 ± 1 °C.

Binocular visual stimuli consisted of high-contrast *moving* square-wave gratings (0.15 cycle/deg, 2 cycle/s) of four different orientations (0°, 45°, 90°, 135°). *Stationary* gratings of identical spatial frequency and orientation were presented during the control period. This stimulus was optimized to activate orientation-selective neurons in area 18 [13].

MR experiments were performed using a 4.7-T/40-cm horizontal magnet (Oxford Magnet, Oxford, UK), equipped with a homebuilt 15-G/cm gradient and an INOVA console (Varian, Palo Alto, CA). After placing the animal in a cradle, a small surface coil of 1.6-cm diameter was placed on top of the cat brain. A single oblique slice, ~500 μm below the cortical surface, was chosen to target the columnar structure in area 18 with minimal superficial vessel contamination as shown previously [9–11].

fMRI studies were performed using a single-shot, gradient-echo EPI technique [9,10]. The parameters were echo time = 32 ms, data matrix = 64 × 64, field of view = 2.0 × 2.0 cm^2 and slice thickness = 2 mm. For each BOLD fMRI measurement with repetition time (TR) = 0.5 s and flip angle = 40°, a total of 160 images was acquired, with 60 images prestimulation, 20 images during stimulation and 80 images poststimulation. CBF measurements were performed using the FAIR technique [11]. Paired images were acquired, one with slice-selective inversion and the other with nonslice selective inversion. Single-shot, gradient-echo echo-planar images were acquired with the following parameters: TR = 3.0 s, inversion delay = 1.5 s and flip angle = 90°. The slice-selective inversion slab was 5-mm thick. Seventy pairs of images were acquired during a three-epoch stimulus paradigm. Each epoch consisted of 10 control (60 s) and 10 stimulated images. At the end of three epochs, 10 control images were additionally acquired. To understand the source of the early-negative BOLD signal, dynamic CBF changes were measured by shifting onset of visual stimulation relative to the image collection at 0.5-s increments. Temporal resolution of 0.5 s was achieved. For this, a single-epoch paradigm of 10 control, 4 stimulation and 13 control FAIR images was used.

Repeated BOLD and CBF measurements of the same orientation stimulus were averaged before further analysis. CBF images were obtained by pair-wise pixel-by-pixel subtraction of the non-slice selective images from the slice selective images [7]. BOLD and CBF activation maps were computed on a pixel-by-pixel basis using a cross-correlation method. Details have been described elsewhere [9–12].

3. Results and discussion

3.1. BOLD-based fMRI

Fig. 1 shows a conventional BOLD functional map obtained during stimulation with single orientation. Activation predominantly lies within cortical area 18. However, the highest signal change was located at the sagittal sinus [9,10]. The conventional BOLD signal change is not specific to active orientation columns. In fact, all four

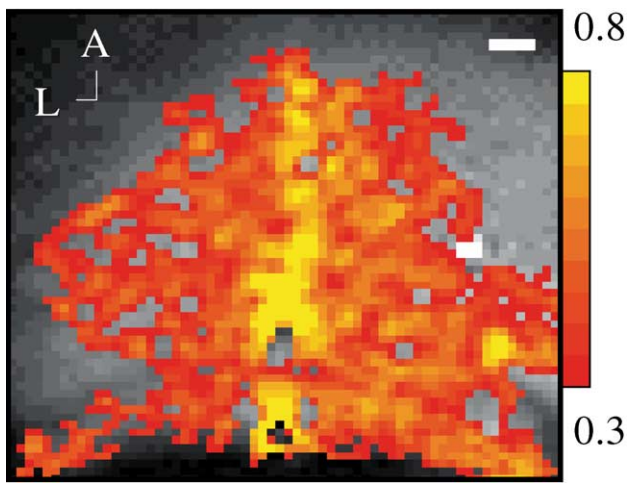

Fig. 1. A representative conventional positive BOLD fMRI map obtained during stimulation with moving gratings of a single orientation. The highest signal change is located at the sagittal sinus in the middle of the brain. A color bar indicates a cross-correlation value from 0.3 to 0.8. Very bright pixels are due to image artifacts. Scale bar: 1 mm; L: lateral; A: anterior.

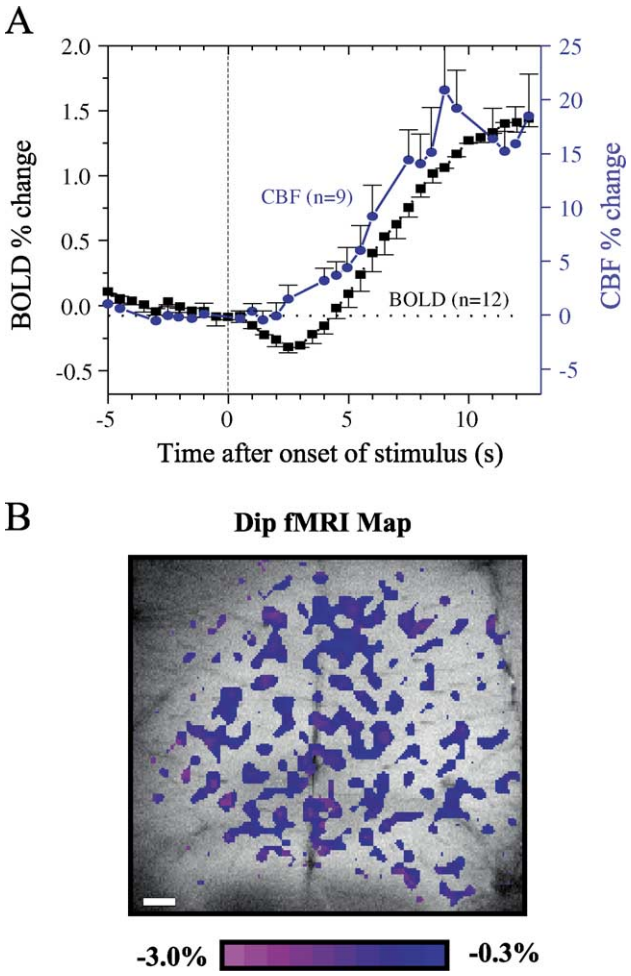

Fig. 2. (A) Comparison of the BOLD (squares) and CBF (circles) dynamic responses. Both time courses were obtained from entire area 18. The error bars are standard deviations of means. The early negative BOLD signal was observed when the CBF response was small. (B) Improvement of spatial specificity of the dip. Functional map was generated by using the first 2 s of early negative BOLD (dip) signal. Patchy activation areas appear column-like. The scale indicates the negative percent change. Scale bar: 1 mm.

activation maps obtained in response to moving gratings of four different orientations yielded similar spatial distributions that were barely distinguishable from each other.

Dynamics of the BOLD signal changes are closely dependent on an arterial pCO_2; the early-negative BOLD signal (referred to as the "dip") was not observed at a pCO_2 level of $\sim$30 mm Hg, while the dip was clearly observed at an end-tidal CO_2 level of $\sim$40 mm Hg [12]. Thus, animal physiological condition was adjusted for maximizing the magnitude of the dip (i.e., setting an end-tidal CO_2 level of 35–40 mm Hg). Typically, the dip reaches a minimum of ~ -0.2 to -0.4% at $\sim$3 s after the stimulus onset. At this condition, the CBF response is relatively slow, resulting in the early-negative BOLD response. Fig. 2A shows the time courses of the CBF and early-negative BOLD dynamics. Both studies were performed at an end-tidal CO_2 level of 35–40 mm Hg. Onset times (defined as time to reach 10% of the maximal signal from baseline) of CBF, early-negative, delayed-positive BOLD signals were 2.0, 0.5 and 4.0 s, respectively. The difference between the onset times of the early-negative and the CBF response is $\sim$1.5 s, suggesting that the first 2-s BOLD signal has minor CBF (and thus CBV) contribution. Thus, the early-negative BOLD signal within 2 s arises predominantly from an increase in dHb concentration. The dip signal can improve fMRI signal specificity because of its close relation to oxidative metabolism.

To investigate the accuracy of the early-negative BOLD signal, the functional maps were generated using images acquired at 0.5–2.0 s following the onset of stimulation [9,10]. In contrast to the conventional positive BOLD maps, the early (0.5–2.0 s) negative map showed predominantly patchy activation patterns in area 18 but not around the sinus (Fig. 2B). These patchy patterns appeared to be column-like based on size and spacing of patches. The average cluster size of the activated pixels was $\sim$300–500 μm and the distance between neighboring pixel clusters was $\sim 1.3 \pm 0.2$ mm [9], consistent with the dimensions of orientation columns visualized with the 2-DG technique ($\sim$500 μm and $\sim$1.1–1.4 mm, respectively [8]). The clustered pixels are irregularly shaped, also consistent with those observed using the 2-DG and optical imaging methods [4,8]. Further, functional maps of two orthogonal orientations were indeed occupied at complementary cortical territory [9], suggesting that the map based on the early dip is genuine.

Although the dip has been successfully applied for mapping columnar structures in anesthetized animals [9,10], it has only small changes on a percentage basis and requires high spatial and temporal resolution. Consequently, the early dip has small overall contrast-to-noise ratio and is highly susceptible to subtle changes of basal physiological condition [12]. Thus, this technique cannot be easily applied to human columnar mapping.

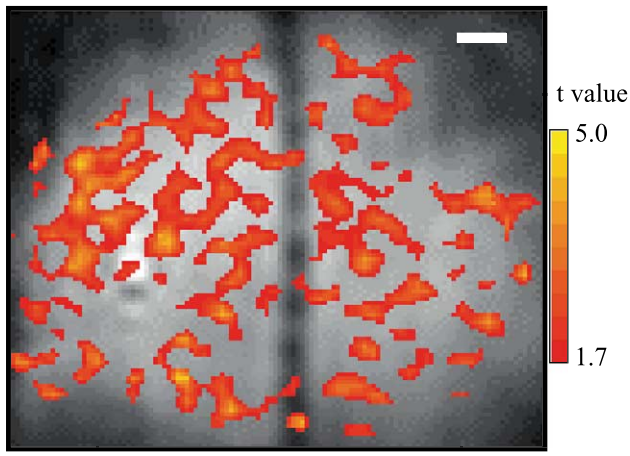

Fig. 3. A representative CBF-based functional map during a single orientation stimulus. Localized activation was observed as patchy, irregular column-like structures without any large vessel contribution. A color bar indicates a t value. Scale bar: 1 mm.

3.2. CBF-based fMRI

Fig. 3 shows one representative CBF map of the cat visual cortex obtained following visual presentation of a single orientation stimulus [11]. Increased CBF activity was observed predominantly in tissue area, avoiding the superior sagittal sinus. Most importantly, the CBF functional map shows patchy layouts with semi-regular cluster shapes, a prominent topological characteristic of orientation columns as observed using 2-DG, optical imaging and the early-negative BOLD techniques [4,8,9]. This is in a marked contrast to the delayed positive BOLD response where large draining vessels were heavily labeled and the mapping signals appeared too diffuse for resolving the patchy layouts (see Fig. 1). The spacing between CBF-responded clusters was 1.1 ± 0.2 mm ($n = 14$) [11], consistent with those obtained by using 2-DG [8], optical imaging [4] and early-negative BOLD [9]. Furthermore, CBF maps of two orthogonal stimuli were found to be spatially complementary [11]. This suggests that the stimulus-evoked CBF response is spatially localized to individual submillimeter cortical orientation columns. This finding contradicts previous findings based on optical imaging [4] and the delayed-positive BOLD measurements [9] possibly due to high susceptibility of the latter to large draining vessel contamination.

4. Conclusions

Hemodynamic-based functional imaging techniques can be used for submillimeter column-resolution brain mapping if large vascular contribution is minimized. Large vascular contribution can be suppressed by using the early-negative BOLD or perfusion techniques. The tissue-specific fMRI techniques can be an excellent tool for understanding cortical columnar structures. Especially, the CBF-based fMRI method can be applied to human studies.

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