High-Resolution fMRI Maps of Cortical Activation in Nonhuman Primates: Correlation with Intrinsic Signal Optical Images

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Abstract

One of the most widely used functional brain mapping tools is blood oxygen level–dependent (BOLD) functional magnetic resonance imaging (fMRI). This method has contributed to new understandings of the functional roles of different areas in the human brain. However, its ability to map cerebral cortex at high spatial (submillimeter) resolution is still unknown. Other methods such as single- and multiunit electrophysiology and intrinsic signal optical imaging have revealed submillimeter resolution of sensory topography and cortical columnar activations. However, they are limited either by spatial scale (electrophysiology characterizes only local groups of neurons) or by the inability to monitor deep structures in the brain (i.e., cortical regions buried in sulci or subcortical structures). A method that could monitor all regions of the brain at high spatial resolution would be ideal. This capacity would open the doors to investigating, for example, how networks of cerebral cortical columns relate to or produce behavior. In this article we demonstrate that, without benefit of contrast agents, at a magnetic field strength of 9.4 tesla, BOLD fMRI can reveal millimeter-sized topographic maps of digit representation in the somatosensory cortex of the anesthetized squirrel monkey. Furthermore, by mapping the “funneling illusion,” it is possible to detect even submillimeter shifts in activation in the cortex. Our data suggest that at high magnetic field strength, the positive BOLD signal can be used to reveal high spatial resolution maps of brain activity, a finding that weakens previous notions about the ultimate spatial specificity of the positive BOLD signal.

Key Words: digits; fMRI; optical imaging; primate; somatosensory cortex; topography

Introduction

The size and complexity of the human brain enable many behaviors that differentiate humans from other animals (for reviews see Kaas 2007; Preuss 2007; Rakic and Kornack 2007). To study the neural basis of these behaviors, brain imaging science has grown at a rapid pace in the past few decades. With the development of brain imaging technologies such as blood oxygen level–dependent (BOLD) functional magnetic resonance imaging (fMRI\(^1\)), scientists can now monitor activity in many different brain regions and study how these regions participate in sensation, motor response, attention, memory, cognition, and emotion.

BOLD fMRI is based on the idea that when neurons are active, their metabolic demands increase oxygen use, which leads to changes in blood oxygenation and in cerebral blood flow (CBF) and volume (CBV). These changes contribute to differences in magnetization between oxy- and deoxyhemoglobin, and the MRI scanner detects these differences. For example, the act of moving a finger increases oxygen use because of neural activations in the brain regions that are responsible for finger movement. The MRI detects the change in blood oxygen level and provides a map revealing all areas in which the balance between oxy- and deoxyhemoglobin changes during finger movement. The map reveals that each behavior corresponds to the activation of a network of cortical areas. This finding not only confirms our knowledge from animal studies but also extends our knowledge to human cognitive behaviors that are difficult to study in animals. Indeed, this powerful mapping technique has revolutionized the field of cognitive neuroscience.

However, these gains are not enough. Some patterns of brain activation, such as cortical columns or fine sensory topographies, require submillimeter resolution to detect, and existing imaging modalities have the capacity to capture some, but not all, of these patterns. Historically, investigators have used anatomical and functional methods (such as electrophysiology and intrinsic signal optical imaging) to study these smaller structures in animals (e.g., Van Essen 1985; Zepeda et al. 2004). Single- and multiunit recording methods are capable of monitoring responses of single or...
local groups of neurons. Optical imaging is advantageous for monitoring large areas at high spatial resolution but is unable to monitor deep structures. fMRI has the potential to monitor any region of the brain (superficial or deep), but has traditionally been limited by spatial resolution. Because low spatial resolution translates into a blurring (or averaging) of the signals across multiple cortical columns, it can lead to a crude or misleading understanding of cortical activation patterns. The development of higher spatial resolution methods is therefore imperative. Higher magnetic field ($B_0$) strengths improve the sensitivity of the fMRI signal and thus produce higher spatial and temporal resolutions. However, the ultimate functional spatial specificity of the positive BOLD signal and the extent to which these activation maps correlate with underlying neuronal activity remain open questions.

Higher field strength fMRI enables higher spatial and temporal resolution mapping in both animals and humans (Cheng et al. 2001; Duong et al. 2001; for review see Harel et al. 2006). At the submillimeter level, for example, previous studies have shown that the initial negative BOLD (the “initial dip”) (Duong et al. 2000, 2001), CBF signal (Duong et al. 2001; Kim and Duong 2002), and CBV-based fMRI (Zhao et al. 2005) can resolve columnar and laminar organization in sensory cortices and retina (Cheng et al. 2001; Fukuda et al. 2006; Harel et al. 2006; Logothetis et al. 2002; Lu et al. 2004; Sheth et al. 2004; Silva and Koretsky 2002; Zhao et al. 2005). fMRI studies in nonhuman primates, which share many of the same brain organizations and behavioral repertoires as humans and can be readily trained on behavioral tasks, are now being performed in the fMRI (e.g., Kayser et al. 2007; Pinsk et al. 2005; Sawamura et al. 2005; Tsao et al. 2006). These studies forge a valuable link between a large body of animal studies and functional imaging in humans (e.g., Disbrow et al. 2000).

In this review, we present data showing that high spatial (submillimeter) resolution is possible with fMRI technology in nonhuman primates without the use of any external contrast agents.

## Optical Imaging of Cortical Columns

Millimeter- and submillimeter-scale in vivo imaging has largely been the domain of other high spatial resolution techniques such as optical imaging of intrinsic signals (OIS$^1$) or optical imaging with voltage-sensitive dyes. The OIS method, based on the activity-dependent reflectance changes of cortical tissue, detects these changes through a “window on the brain” (see Figure 1A; Chen et al. 2002; Roe 2007). The imaging procedure uses a CCD (charge-coupled device) camera to record minute changes in the optical absorption that accompanies cortical activity (Bonhoeffer and Grinvald 1996). The 2- to 3-second time course (to peak) of the intrinsic signal correlates with early deoxygenation of tissue in response to neuronal response and is thought to correspond to the “initial dip” (Cannestra et al. 2001; Devor et al. 2003; Duong and Kim 2002; Thompson

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### Figure 1

Optical imaging of squirrel monkey somatosensory cortex maps millimeter-sized activations. (A) Optical window on the brain. When open, this window permits direct viewing of the cortex by a CCD camera. (B) Stimulus paradigm: vibrotactile stimulation of digit tips with plastic probes maneuvered by computer-controlled motors. (C) Summary map of activations shown in (D1-5). Each activation is indicated by an outline and overlaid on image of cortical vasculature. A lateral-to-medial D1 (thumb), D2, D3, D4, and D5 fingerpad topography is clearly evident. Dots indicate the electrode penetration sites where neurons with D1 and D2 receptive fields were recorded electrophysiologically. (D) Single-condition OIS activation maps of 8 Hz vibrotactile stimulation of D1-D5. A, anterior; M, medial. Scale bar = 1 mm.
et al. 2003). By presenting specific sensory stimuli during optical imaging, it is possible to map the functional organizations of the cerebral cortex at high (∼100 μm) resolution. This method thus offers the ability to reveal quickly and with high spatial resolution the activity of neural ensembles in vivo. Voltage-sensitive dyes offer similar spatial resolution but have a much higher temporal resolution, as the signal correlates primarily with changes in membrane potential and spiking activity. The drawback of voltage-sensitive dyes is that the dye must be absorbed by the brain tissue and activation of the dye may produce phototoxicity effects.

Researchers have used optical imaging methodology to study a number of sensory cortical areas in the primate. In visual cortex, optical imaging has revealed functional organizations such as maps for ocular dominance, orientation, color, motion, and depth (e.g., Grinvald et al. 1986; Lu and Roe 2007a,b; Roe and Ts’o 1995; Ts’o et al. 1990; Vnek et al. 1999; Xiao et al. 2003; Xu et al. 2004). In somatosensory (e.g., Chen et al. 2001; Tommerdahl et al. 1998) and auditory (in rodents, Bakin et al. 1996; Harel et al. 2000; Kalatsky et al. 2005; in cats, Ojima et al. 2005; Spitzer et al. 2001) cortices, this method has also revealed topographic and functional maps. Ground-breaking studies incorporating the use of voltage-sensitive dyes (which improve temporal resolution to the millisecond time scale) have enabled the study of fast-changing behavioral events (Jancke et al. 2004; Slovin et al. 2002).

Optical imaging has also been effective in studies of cognitive functions such as the organization for spatial working memory in the prefrontal cortex of monkeys (Roe et al. 2004; Seidemann et al. 2002). Other studies using intrinsic signal imaging in awake, behaving monkeys have examined the organization of gaze direction (Siegel and Read 1997) and spatial attention (Raffi and Siegel 2005) in the parietal cortex. To some extent, investigators have also used optical imaging methods to explore cortical organizations in humans (Cannestra et al. 2001; Pouratian et al. 2002a; Sato et al. 2002; Suh et al. 2005). In our laboratory, using OIS in anesthetized (Chen et al. 2001, 2003; Friedman et al. 2004) and awake (Chen et al. 2005) squirrel monkeys, we have demonstrated that cortical activations during individual fingerpad vibrotactile stimulation are about 1 mm in size and are organized topographically in the somatosensory cortex (Figure 1C, D). The digit activations are arranged lateral to medial in the expected topographic order, consis-

![Figure 2](image-url) **Figure 2** Time course of the optical signal. (A) The intrinsic optical signal or “initial dip” is the early (1–3 sec) part of the signal that corresponds to deoxygenation of the blood and thus a darkening of the tissue, resulting in a negative reflectance. (B) The late positive BOLD (4–15 sec) corresponds to influx of newly oxygenated blood and thus a brightening of the tissue, resulting in a large positive reflectance.

![Figure 3](image-url) **Figure 3** Anatomic MRI images for studying the primary somatosensory cortex (SI). (A) A high-resolution coronal image is collected to locate somatosensory cortices and to guide placement of three oblique slices parallel to SI cortex (locations indicated by rectangles). This oblique orientation was used for both high-resolution anatomical and functional imaging. (B) Major landmarks (central and lateral sulci) used to identify SI are visible on squirrel monkey brain tissue. (C) In three images acquired with T2* weighting, sulci and vascular structures appear dark. Central sulcus and lateral sulcus are indicated in the most superficial slice. Scale bar = 10 mm.
tent with the maps identified by single-electrode mapping methods (Sur et al. 1982).

One important drawback of the optical imaging method is that it can reveal only organizations that are on the surface of the brain (the cerebral cortex) and cannot image deep structures (such as cortical areas buried in sulci or subcortical structures). This limitation further motivates the development of high spatial resolution fMRI methods. To explore the spatial limitations of the positive BOLD, we used the established single-digit activation model in somatosensory cortex of anesthetized squirrel monkeys (Chen et al. 2001, 2005) to investigate whether millimeter-sized single-digit activations can be resolved and whether maps revealed by the OI and the fMRI methods spatially correlate.

Early versus Late Bloodflow Signals

Many studies suggest that the optical intrinsic signal corresponds to the early negative BOLD signal (the so-called “initial dip”) (Cannestra et al. 2001; Franceschini et al. 2003; Malonok and Grinvald 1997; Pouratian et al. 2002b; Sheth et al. 2003; Toth et al. 1996). But the latter is more focal and is believed to more closely correspond to underlying neural activity: this activity causes a deoxygenation of and thus darkening of the local blood supply, leading to a decrease in reflectance (Figure 2A), followed by an influx of newly oxygenated (i.e., brightened) blood that creates a large increase in reflectance (Figure 2B). The “initial dip” (or early negative BOLD) refers to the early signal, and late positive BOLD refers to the later signal.

The early negative BOLD signal is quite small and not reliably detected with standard fMRI methods (Cannestra et al. 2001; Duong et al. 2000). The late positive BOLD, on the other hand, corresponds to the large influx of oxygenated blood that is much less spatially specific. The early, more spatially specific signal leads to higher-resolution maps than the late signal. Although alternative fMRI approaches, such as cerebral blood flow (CBF) and volume (CBV) methods, can reveal submillimeter-sized columnar and laminar organizations of cortex (Duong et al. 2000, 2001; Harel et al. 2006; Lu et al. 2004; Pouratian et al. 2002b; Sheth et al. 2004; Vanzetta et al. 2004) and retina (Cheng et al. 2001), whether such spatial resolution is attainable with positive BOLD signal is unknown.

Imaging Cortical Columns with Positive BOLD fMRI

To enable the imaging of cortical columns with positive BOLD fMRI, we deviated from common fMRI brain scan methods. Instead of scanning in the traditional coronal, sagittal, or horizontal planes, we used a surface coil coupled with a high-field 9.4 tesla (T) magnet. This higher level of power focuses the magnetic field on a smaller area of interest and at the same time permits imaging in a plane that is parallel to the cortical surface. As shown in Figure 3, this

![Figure 4](image-url)

Figure 4 BOLD fMRI imaging of squirrel monkey somatosensory cortex maps showing millimeter-sized activations. (A-C) Alignment of anatomical MRI maps (A, shown enlarged in B) and optical maps (C) using blood vessel landmarks. Arrows indicate corresponding locations (e.g., central sulcus, lateral sulcus). (D) Single-condition BOLD fMRI activation maps in response to D2, D3, and D4 stimulation. (E) Overlaid activation maps reveal millimeter-sized activations with appropriate topography (compare Figure 1). A, anterior; M, medial. Scale bars: A, 5mm; B-C, 1 mm.
approach permits the viewing of different depths in the cortex as well as correlation with brain landmarks such as major sulci and vascular patterns. We conducted somatosensory mapping experiments in anesthetized squirrel monkeys with these fMRI methods.

As shown in Figure 4, BOLD fMRI is capable of revealing the same digit maps as are possible with optical imaging. Positive BOLD revealed discrete and focal activation during vibrotactile stimulation of the fingerpads (Figure 4D,E). The activation size and topography are consistent with previous studies. The somatotopic organizations of fingerpads were similar across seven monkeys examined with BOLD fMRI. Changing the statistical threshold in the fMRI and optical activation maps led to relatively small changes in the area of activation and did not alter their locations. Furthermore, imaging of the same animal with both optical and fMRI methods produced maps showing a high degree of alignment (Chen et al. 2007). This result gives us confidence that noninvasive fMRI methods (without the use of any injectable contrast agents!) can achieve high spatial resolution.

Submillimeter Resolution with BOLD fMRI

We then asked whether BOLD fMRI was capable of achieving submillimeter resolution. We turned to a finding we had made about the cortical representation of a “tactile funneling illusion,” a sensory illusion in which touching the skin at multiple points produces a single focal sensation at the center of the stimulus pattern even when no physical stimulus occurs at that site (Chen et al. 2003; Gardner and Spencer 1972; Gardner and Tast 1981). The illusion is the perception of spatial mislocalization: when adjacent fingers are simultaneously stimulated, subjects report the sensation of a stimulus between or “bridging” the fingers (Chen et al. 2003). Using intrinsic signal optical imaging, we had previously discovered that this illusion is mapped in the somatosensory cortex. Normally, stimulation of digit 3 (D3) leads to a focal activation at the cortical location representing D3, and that of D4 leads to an activation at cortical location D4 (see Figure 1). The stimulation of both together should result in two activation spots. However, we observed the activation of only a central spot, mimicking the illusory percept (Figure 5A). Thus, cortical maps represent not merely skin surface topography but our perceptions of sensory events.

This observation became our testbed for high spatial resolution mapping with fMRI because the center of the observed funneling activation was only 0.5 mm from the D3 and D4 activation locations. Could BOLD fMRI detect this small submillimeter shift in activation? We repeated the stimulation paradigm in anesthetized squirrel monkeys and observed the results with fMRI methods. Consistent with our optical imaging results (Figure 5A), we found that simultaneous stimulation of D3 and D4 produced a single central focal cortical activation located roughly 0.5 mm from the individual D3 and D4 activations (Figure 5B). This result suggests that BOLD fMRI technology is capable of submillimeter spatial resolutions.

Further examination of the similarity between fMRI and OIS maps obtained in the same animal indicated that the activation patterns that result from the two methods are the same. As shown in Figure 6, activation shape and peak locations identified in fMRI and OIS activation maps for four digits from two animals (Figure 6A-D for animal 1, and 6E-H for animal 2) revealed that the locations of OIS and fMRI activation were not significantly different. Given the differences in method, the likelihood of residual anatomic co-registration errors arising from slight differences in plane of imaging, and different signal-to-noise ratios, this degree of alignment is quite remarkable and suggests the equivalence of somatotopic maps generated by high-field BOLD fMRI and optical imaging.
Possible Contributing Factors to High Spatial Resolution fMRI Maps

We attribute our findings to several methodological factors that were essential for generating stable reproducible high spatial resolution fMRI maps. The first factor relates to the stimulus used. In the BOLD images, there was little evidence of contamination from draining veins and venules. This was perhaps due to the relatively subtle nature of the stimuli (low amplitude of the vibrotactile stimuli), which, in turn, would lead to very small changes in deoxyhemoglobin concentration in draining veins.

Second, at the high magnetic field used, the short T2 (the time constant of signal decay) of the intravascular signal (∼9 ms versus ∼40 ms for tissue; Lee et al. 1999) greatly reduces the contribution from large and small vessels to the BOLD response. Thus, the increased spatial resolution available with high-field BOLD fMRI is attributable in part to the increased signal-to-noise ratio, which allows reduced voxel volumes, and in part to the increased detectability of focal tissue-level BOLD signals not obscured or blurred by intravascular signals from draining vessels.

The third factor is our attention to fine-tuned anesthesia levels and to the animal’s stable physiological conditions. During imaging acquisition, we constantly monitored and adjusted the expired CO2, blood oxygenation, and repetition time (TR) in the fMRI sequence to provide a stable BOLD signal baseline. Animals were maintained at a level of anesthesia that did not overly suppress cortical activity and yet provided stable physiology and minimal variation in heart rate and blood pressure. Under these conditions, the BOLD signal amplitude (0.5% to 1%) remained stable across runs within single sessions, across multiple sessions, and across subjects, and was comparable to previous studies at 9.4 T (Schafer et al. 2006).

Conclusion

Our studies have shown that the positive BOLD signal can be used to achieve submillimeter spatial resolution without the use of exogenous contrast agents. As fMRI techniques continue to move toward higher field strengths, it will be possible to achieve higher spatial and temporal resolution mapping in both animals and humans (Cheng et al. 2001; Duong et al. 2000; for review see Harel et al. 2006). Efforts at imaging the initial negative BOLD (the “initial dip”; Duong et al. 2000, 2001), CBF signal (Duong et al. 2001; Kim and Duong 2002), and CBV-based fMRI (Zhao et al. 2005) show promise for resolving columnar and laminar organization in sensory cortices and in retina (Aoki et al. 2004; Cheng et al. 2001; Fukuda et al. 2006; Harel et al. 2006; Logothetis et al. 2002; Lu et al. 2004; Sheth et al. 2004; Zhao et al. 2005). In humans, the resolution of millimeter-scale ocular dominance and orientation domains in primary visual cortex (V1) has been demonstrated (at 4 T) in subjects with optimal cortical geometries using optimized surface coils and head stabilization efforts (Cheng et al. 2001).

Further refinement of these approaches to high-resolution functional mapping in animals and humans (Harel et al. 2006; Norris 2006; Yacoub et al. 2005) prom-
ises to provide answers to many functional organizational and evolutionary questions about cortical organization. Indeed, whether submillimeter functional units exist in human cerebral cortex remains an open question, which we hope can be answered in the near future with improved fMRI techniques.

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