What We Have Learned about Human Primary Visual Cortex from High Resolution Functional Magnetic Resonance Imaging

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This article reviews our exploration of structures and functions of the human visual cortex using high resolution (submillimeter) functional magnetic resonance imaging (fMRI). It discusses factors that restrict the spatial resolution of blood oxygenation-level dependent (BOLD) fMRI—the point-spread function of the BOLD signal, limited by both imaging techniques to be used and neurovascular units to be studied, and the signal-to-noise ratio. I offer personal thoughts regarding optimal solutions for dealing with these issues, summarize techniques we have developed over the years for using high resolution fMRI to visualize functional architectures and explore physiological properties in the primary visual cortex of humans, including choices of imaging hardware and pulse sequences, experimental procedures, and stimulation paradigms, and finally offer my personal opinions regarding the future of high resolution fMRI.

Keywords: blood oxygenation-level dependent (BOLD), columns, high resolution fMRI, point spread function, primary visual cortex

Introduction

Our interest in pursuing high resolution, hereafter referred to as submillimeter in-plane resolution, functional magnetic resonance imaging (fMRI) was heavily influenced by our explorations of functional architectures in the visual cortices of monkeys in the decade spanning the late 1980s and early 1990s. Using experimental approaches, including single-unit recording, intrinsic optical imaging, and anatomical tracing, we discovered the clustering of neurons that preferred similar visual object features in the inferotemporal cortex of monkeys.1–3 We also established that the similarity of clustered neurons in their stimulus preferences is relatively consistent across cortical layers but more variable across the cortical surface (at a spatial scale of ~0.5 mm). These findings led us to propose the existence of a columnar organization in the inferotemporal cortex of monkeys (and perhaps in humans) that is responsible for the flexible coding of visual objects.4 The theoretical implications of these intriguing discoveries were exciting, but criticisms and uncertainties naturally arose, primarily from the limitations of the techniques that had been utilized. With single-unit recording, the characterization of a neuron’s preference for particular object features generally takes a huge amount of time, and the limited features that can actually be tested restrict our ability to address issues related to the precision and spatial organization of object-selective columns. Though intrinsic optical imaging allows better characterization of spatial features of the columnar organization, the technique is limited in its penetration capability.

We were initially cautious at the introduction of blood oxygenation-level dependent (BOLD) fMRI to neuroscience early in the 1990s.5–7 Nevertheless, as our understanding improved of the signal source of the BOLD fMRI as well as its temporal and spatial characteristics, we were convinced that this might be the technique that would allow the efficient and noninvasive investigation of representa-
tional issues related to object vision in humans. Subsequently, we were fortunate to acquire a state-of-the-art Varian (now Agilent Technologies) Unity Inova 4-tesla whole-body MR imaging system and, a few years later, a high performance Magnex head gradient system (Santa Clara, CA, United States of America). However, initial technical struggles with these fancy instruments led to the quick realization it would be a lot longer before they could be used to address seriously representational issues in the human occipitotemporal cortex (homologous to the monkey inferotemporal cortex). Questions that remained included the point-spread function (PSF) that defines the spatial precision of BOLD fMRI and the optimization of the signal-to-noise ratio (SNR) for high resolution studies. Also needed were effective measures to restrict rigid head motion and efficient experimental procedures and stimulation paradigms, all of which are indispensable for high resolution exploration of functional architectures in humans.

We first tackled these issues in the primary visual cortex (V1), where receptive-field properties are well established in nonhuman primate models and several distinct columnar organizations, including ocular dominance columns (ODCs) and orientation columns (OCs), are known or expected to exist in humans. We continue to work extensively in the V1 and have just begun to explore original questions regarding issues of object representation in the human occipitotemporal cortex.

Quantifying Spatial Precision of BOLD fMRI

The spatial resolution in an fMRI experiment is ultimately restricted by the SNR, which is proportional to the strength of the static magnetic field, voxel size, and total scan time. The SNR in an fMRI study with conventional resolution with typical in-plane resolution of 3 to 4 mm (usual slice thickness, 2 to 4 mm) is sufficiently high and barely a concern, but in a high resolution study with submillimeter in-plane resolution, much effort is needed to retain a sufficiently high SNR. However, it should be remembered that though high resolution fMRI can reveal more detail, the resolution per se does not determine the spatial precision of the BOLD signal.

Two factors must be considered. First, the nominal spatial resolution in an fMRI study is not the real resolution of a functional image. Depending on the pulse sequence and imaging parameters of the acquisition, $T_2^*$ blurring can produce substantial spatial smoothing of an image. Second, because BOLD fMRI measures changes in cerebral blood flow (CBF), cerebral blood volume (CBV), and the cerebral metabolic rate of oxygen following changes in neuronal activity, its spatial precision is biologically restricted by the underlying vasculature.

To elucidate the spatial precision of BOLD fMRI, we estimated the PSF of the BOLD signal using a segmented (4 segments) gradient-recalled echo-planar imaging (GRE-EPI) pulse sequence with spatially localized and size-varied visual stimuli (Fig. 1). The experiment was conducted using our 4T MR imaging system with a spatial resolution of $1.1 \times 1.1 \times 2.5 \, mm^3$. By measuring the full width at half maximum (FWHM) of the BOLD response profile (from the center of activation to the periphery) as a function of the stimulus size, we found a PSF of $\sim 1.85 \, mm$ of the BOLD signal in the cortex devoid of large veins running on the cortical surface. Our observation was similar to that reported in a study conducted at 7T, in which large surface veins were also identified and removed. These results indicate that the PSF of BOLD fMRI is smaller than 2 mm independent of the field strength at which the measurement is made if the measurement is restricted within the cortex containing no large surface veins. The underlying microvasculature is thought to be the primary cause of this spread, which is likely inflated slightly due to $T_2^*$ blurring. A classical study of the cortical blood vessels of the human brain described the distribution of emerging venules from the cortex in spatially distinct territories, each occupying a draining area of one to 1.5 mm in diameter. This microvascular module and neurons encompassed in the module, forming the so-called neurovascular unit, perhaps define the upper limit of the spatial precision of BOLD fMRI.

Better spatial specificities may be obtained using other fMRI techniques, such as spin-echo (SE) EPI and fMRI measurements sensitized to changes in CBF and CBV. However, the general low sensitivity (SE-EPI and CBF-based fMRI) or toxic nature (CBV-based fMRI) of these methods continues to limit their use in the study of human brain functions.

Improving SNR for High Resolution fMRI

One fundamental feature of the cortical architecture is its columnar organization, in which neurons with similar functional properties cluster to form repeated units (columns) across the horizontal extent of the cortex. The columns are an elemental cortical processing unit. One of the clearest examples of the columnar organization of the cortex is the system of ODCs, in which each column receives input from either the left or right eye (Fig. 2a).
In the human V1, alternating stripes of ODCs running through the cortical layers extend roughly parallel to isoeccentricity lines across the cortex, but the width of their narrow dimension in the orthogonal direction measures only \( \approx \) one to 1.5 mm in humans. Capturing such fine structures would require an in-plane resolution of less than half of the ODC width to avoid partial-voluming effect (Fig. 2b). Fortunately, we can take advantage of the relative uniformity of the columns across cortical layers by subscribing a slice of 2 to 3 mm in thickness. In brief, we must deal with the issue of low SNR for a voxel as small as \( \approx 0.5 \) to 1.7 mm\(^3\).

To maximize the SNR for mapping cortical columns in the human V1, we developed a segmented GRE-EPI approach that uses a matrix size of 256 \( \times \) 256 typically or of 512 \( \times \) 512 in rare cases and 4 to 32 segments, depending on the structures to be mapped. The segmented EPI shortens the echo train length and reduces image blurring (T\(_2^*\) blurring).
At the beginning of each segment, we acquired a navigator echo, which was used to correct intersegment phase and magnitude variations and thereby reduce signal fluctuations from pulsatile and rigid head motion. In some cases, the strength of the readout gradient was also lowered to maintain a narrow bandwidth (~100 KHz). We were thus able to achieve a satisfactory spatial SNR greater than 50:1 in most of our studies.  

In recent years, advancements in radiofrequency (RF) coil and parallel imaging technologies have permitted further improvement of the SNR for high resolution fMRI. We have integrated these new features in current studies exploring functional archi-
Developing Experimental Procedures for High Resolution fMRI

Over the years, we have developed many practical experimental procedures for individual studies. All our high resolution experiments utilize 3 procedures considered critical for optimal results—head fixation to prevent excessive rigid motion, correction for physiological fluctuations, and the prescription of slices for optimal coverage of the brain structures to be mapped.

Rigid head motion during image acquisition probably most damages the quality of a time course, and excessive head motion prevents ideal resolution of columnar structures. Although there are many ways to correct for motion, none satisfactorily addresses motion artifacts that appear in the middle of image acquisition. This is especially problematic in high resolution experiments, in which the time of repetition (TR) is much longer. Padding with soft materials often leads to unwarranted low frequency shift in head position that generates complex translational and rotational motion artifacts. We experimented with various head fixation devices and concluded that a bite-bar tailored to the subject’s teeth best serves this purpose (Fig. 3). Pulsation causes movement of the brain during prolonged acquisition, but the pulsatile movement in the brain parenchyma is minimal, estimated as less than 0.5 mm. Thus, the bite-bar provides a reliable reference, with all images in a time series aligned along the bar.

Monitoring and removing physiological (cardiac and respiratory) fluctuations contained in time-series images can further improve the quality of a time course. This can be performed in the k-space, using well established estimation and correction methods, such as the Physiofix method proposed by Hu and colleagues. We have found that use of this procedure can significantly improve the stability of a time course, that is, the temporal SNR, which is what one really cares about in a functional study.

Perhaps the biggest lesson we have learned over the years is the proper prescription of a slice for optimal coverage of the underlying columnar structures to be mapped. Because most slices used in today’s fMRI experiments are flat, this procedure starts with the selection of subjects with well defined morphology in the region of the brain to be studied. A slice is then prescribed to maximally contain the columnar structure, much like what is schematically illustrated in Fig. 2b. A review article by the author describes how we incidentally came to this conclusion. Several other high resolution studies have also used this procedure successfully.

Revealing Columnar Architectures in the Human V1 by High Resolution fMRI

After initial technical struggles with our 4T system, we were ready to conduct decent high resolution experiments in late 1998. We thought that mapping ODCs in the human V1 would be an excellent first project after Menon’s group demonstrated the revelation of ocular dominance using fMRI at a high field (4T) and a report of the partial reconstruction of human ODCs using a postmortem specimen provided an invaluable source for understanding the layout of ODCs in relation to the shape and geometry of the V1. However, completion of the quick project we planned took 3 years. In addition to working out a number of experimental procedures in the study process, we had to address several important questions. We had to convince ourselves and the community that a prolonged stimulation, albeit suboptimal, could be used to resolve ODCs, and we had to determine how to deal with large surface veins that contaminate the spatial specificity of the BOLD signal.

Eventually, we were able to resolve ODCs in the V1 of living human brains (Fig. 4a, b). The orientation and width of ODC patterns mapped using BOLD fMRI were found to be very similar to ODCs revealed in postmortem brains, in which columns corresponding to the surgically enucleated eye and to the remaining eye were differentially stained with cytochrome oxidase (CO). Importantly, we demonstrated the reproducibility of mapped ODC patterns between successive scans in a session and even between sessions months apart.

There is a need to point out a critical detail in our analysis method. Even without an eye’s stimulation, the BOLD signal in the ODCs (voxels) corresponding to that eye increased significantly (Fig. 4c, d), indicating the extension of the change in BOLD response beyond the stimulated columns and spread of the signal to neighboring columns. We now know this is unavoidable because, as discussed, the PSF (~1.85 mm) of the BOLD signal is wider than the average width of the average ODC (~one to 1.5 mm). Indeed, we failed in our initial attempts to reveal ODC-like patterns using the so-called single-condition mapping method (i.e., comparing the condition of the eye with and without stimulation). Only when we adopted the differen-
tial mapping method (i.e., comparing the condition when one eye is stimulated with that when the opposite eye is stimulated)\textsuperscript{26} could we differentiate eye-specific, alternating stripes. We used this differential mapping method in many of our other studies and will mention it again below.

In an unrelated study that combined magnetoencephalography and fMRI to probe localization consistency and precision using these 2 techniques, we happened to find similar response strength and size of the portion of the V1 activated by a contrast-reversing stimulus when slow and fast reversal frequencies were used.\textsuperscript{27} This observation was inconsistent with the belief then that the V1 responds to such a stimulus in a frequency-dependent manner\textsuperscript{28} and suggests that V1 neurons coding different frequencies are either mixed together or located in separate domains. We tested these hypotheses in a high resolution (0.75 $\times$ 0.75 $\times$ 3 mm$^3$) study and discovered the segregation in the V1 of domains preferring low (0.75 Hz) and high (15 Hz) temporal frequencies into patch-like temporal frequency dominant columns (TFDCs) (Fig. 4e, f).\textsuperscript{19} The absence of reports of TFDCs in monkey studies demonstrates the usefulness of high resolution fMRI in our exploration of functional architectures in primates. Such segregated, frequency-dependent domains are likely related to the system of CO blobs and interblobs, a hypothesis that remains to be tested.

Exploring Physiological Properties of the Human V1 using High Resolution fMRI

High resolution fMRI does not just reveal the maps of functional architectures. Indeed, though ODCs are readily resolvable thanks to their binary anatomical origin, the mapping of other columnar structures, such as OCs, which result from intracortical neural processing and are spatially more finely organized,\textsuperscript{29} is more difficult using fMRI without extensive postprocessing measures.\textsuperscript{23}

In recent years, we have worked to devise novel stimulation paradigms to reveal cortical modular organizations in the human visual cortex, and orientation-selective responses at the single-voxel level have been revealed using high resolution fMRI.\textsuperscript{20} The paradigm was motivated by taking advantage of 2 discoveries from a large number of studies using single-unit recording and intrinsic optical imaging techniques (Fig. 5a–c).

First, changes in orientation preference in pinwheel structures in OCs are gradual and periodic at a spatial scale similar to or even larger than the width of ODCs.\textsuperscript{30} This implies that an orientation-
selective BOLD response could be identified using a submillimeter voxel, not a voxel at conventional low resolution, if the voxel were located around the linear zone of the pinwheel preferring the orientation.

Second, due to the orthogonal nature of orientation selectivity for a stimulus in a given orientation and the orientation selectivity for a second stimulus that differs in orientation by 90°, a continuous modular BOLD response, i.e., an orientation-selective response, could be revealed by comparing BOLD responses to a pair of stimuli that continuously change their orientations but maintain their 90° difference in orientation (e.g., see Fig. 5e). Using this continuous stimulation paradigm with a data-driven analysis method, we have demonstrated directly the differential BOLD response temporally modulated by the change in stimulus orientation. The preferred orientation of the voxel can be determined by the phase of the peak response following a sinusoidal fit to the differential time course (red curve, for the 180° indicated by the shaded area).
Using this approach, we quantified orientation selectivity for tens of thousands of submillimeter voxels in the human V1. By analyzing the orientation preference at a population level and correlating the orientation preferences of individual voxels with their retinotopic positions, we demonstrated for the first time that more voxels preferred horizontal and vertical orientations, a physiological substrate underlying the oblique effect, particularly near the cortex representing the horizontal and vertical meridians. This phenomenon is closely related to and supports in part the existence of the still hotly debated radial bias in orientation perception. However, we also observed no significant radial bias for the voxels around the oblique meridian, which indicates a spatial heterogeneity in the radial bias, the behavioral consequence of which remains to be investigated.

The variants of this continuous stimulation and differential analysis method have also been applied to address other outstanding issues related to contextual modulation and color selectivity in the human early visual cortex (Kuriki et al., unpublished data, 2015).

The Future of High Resolution fMRI

Our results demonstrate that high resolution (submillimeter) fMRI can be conducted using currently available high field systems, such as the 4T system used in our studies. Through many years of effort, we have come to understand much better the spatial specificity of the BOLD signal, developed practical measures for improving the SNR, and devised effective stimulation paradigms and analysis methods for the visualization of functional architectures and exploration of physiological properties in the human V1. Our accumulated experience and knowledge are of great value in our ongoing endeavor to elucidate the functional roles of the human occipitotemporal cortex in object vision. In this respect, high resolution fMRI, especially submillimeter fMRI, is expected to be crucial in clarifying certain new but sometimes contradictory claims regarding object representations. For instance, using fMRI with a resolution of $1.4 \times 1.4 \times 2 \text{mm}^3$, Schwarzlose and colleagues claimed that the fusiform face area (FFA) is selective only for faces and the neighboring fusiform body area is selective only for bodies.\footnote{Tsao’s group found most cells to be face-selective in a region in monkeys analogous to the FFA in humans.\footnote{These observations have been confirmed in a high resolution ($1 \times 1 \times 1 \text{mm}^3$) fMRI study\cite{Schwarzlose2015} (see also the corrigendum) in which the authors noted intermingling of regions of high and low selectivity for faces within the FFA. In a more recent study using the same resolution ($1 \times 1 \times 1 \text{mm}^3$) and scanning parameters, however, Hanson and Schmidt contended that the FFA is a structure in which neurons preferring different categories of objects are distributed and argued that selective response to faces in the FFA revealed in fMRI studies using conventional resolution may have resulted from an averaging artifact.\footnote{Preliminary results from one of our recent studies conducted at both conventional ($3 \times 3 \times 3 \text{mm}^3$) and high ($0.75 \times 0.75 \times 3 \text{mm}^3$) resolutions, on the other hand, indicate that as the spatial resolution increases, selectivity for faces in the FFA also increases. This lends support to the selective, but not distributed, coding scheme in the FFA (Tanskanen et al., unpublished data, 2014). High resolution fMRI should also contribute more to the resolution of hotly debated issues related to the source of information used in multivariate pattern analysis (MVPA),\cite{Kriegeskorte2008, Kriegeskorte2010, Kriegeskorte2011} an undoubtedly powerful tool for the study of brain functions. For example, one interpretation regarding the ability to decode perceived stimulus orientation from data obtained using fMRI with conventional resolution is that multiple submillimeter OCs within an isotropic 3-mm voxel may produce a small bias across multiple voxels that leads to a reliable representation for that orientation, which can be decoded using MVPA-based classifiers.\cite{Kriegeskorte2008, Kriegeskorte2010, Kriegeskorte2011} However, this interpretation needs to be made with caution. The fact that classification performance saturates with only several tens of voxels\cite{Kriegeskorte2011} suggests that there are single voxels that contain a more reliable signal that is selective for the stimulus orientation. Indeed, we have provided evidence in high resolution ($0.75 \times 0.75 \times 3 \text{mm}^3$) fMRI that high performance in classifying stimulus orientations is achieved by a small number of V1 voxels tuned to different orientations that are clustered and aligned with large surface veins.\cite{Schwarzlose2015, Rorden2010} In addition, better classifications have consistently resulted from data sampled at higher than lower resolution fMRI.\cite{Schwarzlose2015, Rorden2010, Rorden2011} This strongly suggests that whenever the SNR, brain coverage, and scan speed allow, MVPA should be performed at higher resolutions. The field of MR imaging is moving rapidly toward ultra high fields (7T and above). Recent technological innovations have provided spectacular solutions to problems associated with ultra high field fMRI, such as slow scan speed and limited brain coverage. Recently, a GRE-EPI approach at 7T with partial parallel imaging and 16-fold acceleration has been developed to achieve whole-brain imaging.}}

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coverage with a spatial resolution of $1 \times 1 \times 2$ mm$^3$ and TR of merely 1.5 s.\textsuperscript{47} Such techniques are well suited for mapping functional architectures and will play greater roles in high spatial and temporal exploration of human brain functions in the near future with further improvement of spatial resolution.

Newly emerged imaging techniques and capabilities have also improved characterization of the organization of the cerebral vasculature, in particular, that of the cortical microvasculature.\textsuperscript{48} Much more effort is needed to obtain this knowledge from both human and animal studies to provide a quantitative description of neurovascular units on an area-specific basis. Such microvascular information, along with mapped patterns of functional architectures, should greatly advance our understanding of the relation of neuronal to vascular units and their organization across different brain regions.

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