Published in final edited form as:

Neuroscientist. 2008 June ; 14(3): 287–299. doi:10.1177/1073858407309541.

Lessons from fMRI about mapping cortical columns

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Abstract

Recently-developed functional magnetic resonance imaging (fMRI) can map small functional structures non-invasively and repeatedly without any depth limitation. However, there has been a persistent concern as to whether the high-resolution fMRI signals actually mark the sites of increased neural activity. To examine this outstanding issue, we used iso-orientation columns of isoflurane-anesthetized cats as a biological model and confirmed the neural correlation of fMRI iso-orientation maps by comparing with intrinsic optical imaging maps. Our results suggest that highest fMRI signals indeed indicate the sites of increased neuronal activity. Now fMRI can be used to determine plastic and/or developmental change of functional columnar structure possibly on a layer to layer basis. In this review we focus mainly on what technical aspects should be considered when mapping functional cortical columns, including imaging techniques and experimental design.

Keywords

orientation columns; hemodynamics; high-resolution; BOLD; optical imaging

Introduction

Neurons with common functional properties in mammalian cortex are often found to be clustered into sub-millimeter-scale columns, which span the entire cortical plate (Mountcastle, 1957; Hubel and Wiesel, 1962). Although a direct relationship between this clustering and function is repeatedly questioned (Swindale, 1990; Purves et al., 1992; Horton and Adams, 2005), the structure, function, and plasticity of cortical columns have no doubt been of great interest. Thus, many efforts are dedicated to visualizing functional structures. Initially, the existence of cortical columns was determined by single-unit recordings (Hubel and Wiesel, 1962, 1963). Since unit-recordings measure on a point-by-point basis, it takes an enormous time to record a sufficient number of neurons to obtain a partial picture of columnar arrangement. In order to map cortical columns effectively, techniques must be capable of acquiring high resolution and of covering large cortical areas; 2-deoxyglucose (2-DG) autoradiography and optical imaging meet these requirements. The 2-DG autoradiography measures increases in glucose consumption rate during stimulation (Kennedy et al., 1976). However, *in vivo* imaging is not possible, and only the map corresponding to one stimulus can be obtained. The first high-resolution mapping of functional columns in live animals was accomplished by Blasdel and Salama (Blasdel and Salama, 1986) using a video camera and a voltage sensitive dye. Their seminal study triggered the use of optical signals for mapping functional columns (Tso et al., 1990; Bonhoeffer and Grinvald, 1991; Grinvald, 1992; Malach et al., 1993). The optical imaging of intrinsic signals detects a change in reflection of the incident light through the cortex induced by a change in light-absorbing hemoglobin contents

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(Grinvald et al., 1986; Frostig et al., 1990). Since endogenous hemoglobin is used as a tracer, it allows mapping during repeated measurements. Even though this technique has been used extensively for mapping columnar structures, its application is limited to the upper layers of exposed cortex due to a limited penetration of light and to animal models due to the required invasive surgical preparation. An extension of mapping functional columns to humans awaits the progress of recently-developed functional magnetic resonance imaging (fMRI). Using high-resolution fMRI, ocular dominance column-like maps in humans have been obtained by subtracting images acquired during right-eye and left-eye only stimulation (Menon et al., 1997; Menon and Goodyear, 1999; Cheng et al., 2001; Goodyear and Menon, 2001).

Setting aside the biological meaning of functional columns, the columnar structure is an ideal biological model to evaluate the spatial resolution of functional imaging techniques. There has been a persistent concern as to whether the high-resolution fMRI signals actually mark the sites of increased neural activity. This skepticism is not only based on a lack of a direct confirmation by other techniques, but also because of possibly a broad point spread function (PSF) of hemodynamic-based fMRI signals. Although an increase in fMRI signal is closely correlated with an increase in neuronal activity on a large supra-millimeter scale (Heeger et al., 2000; Logothetis et al., 2001), this finding may not be applicable on a sub-millimeter columnar scale (Duong et al., 2000; Cheng et al., 2001; Kim et al., 2004). Thus, it is critical to examine whether fMRI can pinpoint sub-millimeter functional sites. This outstanding issue motivates us to study reproducibility, specificity and confirmation of fMRI columnar maps (Fukuda et al., 2006a; Moon et al., 2007). To do this, we selected a well-established orientation column model in cats, which has been extensively investigated by single-unit recordings, 2-DG autoradiography and optical imaging of intrinsic (OIS) signals (Payne and Peters, 2002). This review focuses mainly on what technical aspects should be considered when mapping orientation columns using fMRI.

Vascular physiological changes induced by neural activity

Since fMRI measures vascular hemodynamic responses induced by increased neural activity, it is important to understand the chain of events from task to fMRI signals. Task and/or stimulation induce neural activities at localized regions, which will trigger an increase in cerebral metabolic rate of oxygen (CMRO₂), cerebral blood flow (CBF) and cerebral blood volume (CBV). Since CMRO₂ change occurs in neuronal active tissue (Thompson et al., 2003) and precedes vascular responses (Ances et al., 2001; Shibuki et al., 2003) its measurement will provide the high spatial specificity (Malonek and Grinvald, 1996). On the contrary, CBF and CBV are vascular parameters; CBF is defined as milliliters (*ml*) blood delivered to the region of interest per gram (*g*) tissue and per minute, while CBV is an intraluminal space of blood vessels within the region of interest (unit of *ml* blood per *g* tissue). Typically, gray matter in normal humans has CBF of 50–70 *ml*/100 *g*/min and CBV of 2–5 *ml*/100 *g* (Leenders et al., 1990). When CBF is measured by a diffusible tracer such as water, the change in blood flow can be observed in both vasculature and tissue (i.e., extra-vasculature). Both CBF and CBV are physiological parameters independent of imaging techniques.

It is thought that neural activation leads to an increase in oxygen delivery without a commensurate elevation in cerebral oxygen consumption (Fox and Raichle, 1986; Fox et al., 1988), which results in hyperoxygenation and a decrease in the capillary and venous deoxyhemoglobin (dHb) contents (see Fig. 1 for a schematic). Since dHb acts as an endogenous paramagnetic contrast agent (Pauling and Coryell, 1936), a decrease in the local dHb content in the brain induced by neural activity leads to an increase in the intensity of MRI signal (Ogawa et al., 1990b; Ogawa et al., 1990a; Ogawa and Lee, 1990). This imaging contrast is dubbed as the “blood oxygenation-level dependent” (BOLD) contrast, originating from small and large venous vessels and neighboring tissue (Ogawa et al., 1990b). The BOLD fMRI

technique has been widely used because of its high sensitivity and easy implementation. The BOLD signal is dependent on various anatomical, physiological, and imaging parameters (Ogawa et al., 1993).

Sensitivity and specificity of hemodynamic-based fMRI

To obtain high-resolution fMRI, two major issues have to be considered: i) sensitivity of techniques and ii) specificity of the signal source (i.e., the spatial extent of the signal at the site of neuronal activity). Both factors will determine the accuracy of the functional map. High resolution fMRI suffers poor sensitivity. For example, a voxel dimension of $0.15 \times 0.15 \times 1 \text{ mm}^3$ for our high-resolution fMRI is 1,200 times smaller than $3 \times 3 \times 3 \text{ mm}^3$ resolution used in typical human studies, and requires 1200^2 more averages to achieve similar sensitivity with the same equipment. Thus, it is crucial to optimize fMRI hardware and software as well as the stimulation paradigm and data analysis to maximize the sensitivity. Since sensitivity increases are roughly linearly dependent on magnetic field strength, and inversely related to receiver coil size, an ultrahigh magnetic field of 9.4 Tesla and a small coil of 1.6-cm diameter were mostly used for our cat orientation column studies (note that 1 Tesla is equivalent to 10,000 gauss, and earth magnetic field is 0.5 gauss). What signal source should then be measured with fMRI to achieve a high spatial specificity?

Considering spatially well-localized metabolic responses, measurement of CMRO₂ changes can provide a high specificity of fMRI signals. Since stimulation-induced CMRO₂ change precedes CBF and CBV responses, hypo-oxygenation may be initially experienced until the CBF response catches up with CMRO₂ demand. Optical imaging of intrinsic signals showed that the initial increase of “metabolism-based” deoxyhemoglobin signal (Vanzetta and Grinvald, 1999) can be used to resolve functionally active, individual orientation columns in the cat visual cortex (Malonek and Grinvald, 1996). An early increase in dHb contents will lead to the early negative BOLD signal (referred to as an “early dip”) (Kim et al., 2000), which likely indicates an early oxygen consumption change (Nagaoka et al., 2006). However, to detect this early BOLD response and utilize the high spatial specificity of the early dip signal, images must be acquired with high spatial and temporal resolution, which results in a significant reduction in sensitivity. Therefore, the measurement of early dip is not practical. Alternatively, hemodynamic-based responses (i.e., positive BOLD, CBV, CBF) should be considered for high-resolution fMRI.

The specificity of hemodynamic-based fMRI signals is dependent on both vascular regulation and the sensitivity of the imaging technique to different vessel types and sizes. To obtain high-resolution fMRI with high specificity, it is important to obtain signals from microvessels such as capillaries and/or tissue, not from large vessels. The microvasculature signal is believed to be close to the site of increased neural activity (e.g., distance between capillaries and neurons is $<25 \text{ }\mu\text{m}$ since an average inter-capillary distance is $\sim 25 \text{ }\mu\text{m}$ (Pawlik et al., 1981)), while functional maps based on the macrovasculature can be significantly distant from the actual site of neuronal activity.

The conventional positive BOLD fMRI signal is sensitive to all sizes of venous vessels and surrounding tissue. The principal intracortical veins with an average of $\sim 100 \text{ }\mu\text{m}$ diameter, which lead directly to the cortical surface, drain blood from surrounding capillaries up to 2 mm away and intermediate size veins (with $<80 \text{ }\mu\text{m}$ diameter) drain up to 0.5 mm (Duvernoy et al., 1981). Since conventional BOLD signals are sensitive to changes in these large intracortical veins, its spatial resolution is limited by the density of large vessels and extents of draining effects. In order to obtain columnar-resolution functional images, the signal from large vessels should be minimized using post-processing techniques and improved data acquisition methods. Post-processing approaches detect large draining vessels by using various BOLD signal

characteristics such as a large intensity (Kim et al., 1994) or a delayed response (Lee et al., 1995). Although these subjective criteria are effective (Cheng et al., 2001), it may not be sufficient to detect and remove all large vessels contaminations. Remaining 'common' large vessel contributions to the BOLD signal can be removed by the differential imaging approach if functional territories are complementary during orthogonal stimuli (Menon et al., 1997; Cheng et al., 2001). However, this differential method cannot be used in most fMRI studies because of unknown orthogonal stimulation conditions. Thus, it is critical to remove or minimize the draining vessel contribution during data acquisition. For this, perfusion-based techniques can be used because of their high sensitivity to changes in capillaries and tissue.

CBF measurements have been extensively performed using ^{15}O -labeled water as an exogenous positron emission flow tracer, although inherent resolution limitations due to sensitivity, positron travel distances and detector arrangements prevent sub-millimeter imaging. Alternatively, CBF-weighted images can be obtained using MRI by employing arterial blood water as an endogenous flow tracer. Arterial spin labeling (ASL) can be achieved by radiofrequency pulse(s), then magnetically labeled spins move into capillaries in the imaging slice and exchange with tissue water spins. Since background water signal exists, it is important to remove non-flow related water signals. Thus, two images are typically acquired; one with arterial spin labeling and the other without labeling (Detre et al., 1992; Edelman et al., 1994; Kim, 1995; Kwong et al., 1995). The difference between the two images is directly related to CBF, and relative CBF changes due to physiological perturbations can be measured. The perfusion-based signal change as measured by MRI predominantly arises from changes in local CBF at the level of arterioles and capillaries/tissues.

Alternatively, an exogenous intravascular contrast agent with high susceptibility can be utilized in animal studies to improve sensitivity and specificity relative to BOLD fMRI. Dextran-coated iron oxide nanoparticles have been used as a long half-life intravascular susceptibility contrast agent (Kennan et al., 1998; Mandeville et al., 1998; van Bruggen et al., 1998), similar to endogenous irons in dHb molecules (used for the BOLD contrast). The half-life of the iron oxides in blood is dependent on particle size and species; the typical half-life of 20–30 μm diameter particles is >4 hours in rats (Mandeville et al., 1998), 3–6 hours in cats (in our observation), and >18 hours in non-human primates (Vanduffel et al., 2001). During a steady-state condition established following the infusion of an intravascular contrast agent (reached within a few minutes), an increase in CBV during stimulation will induce an increase in intravoxel contrast agent, and consequently a decrease in MRI signal. Unlike in the BOLD case, iron oxide concentration in blood does not change with alterations in blood flow and oxygen consumption induced by neuronal activity. Although the change in dHb (used in the BOLD signal) will contribute to the MRI signal, exogenous contrast agents with sufficiently high doses dominate the effect compared to dHb. Thus, fMRI following the injection of contrast agents is predominantly weighted by the CBV change. Small-sized vessels including pre-capillary arterioles dilate rigorously during stimulation, while large vessels do not dilate much (Lee et al., 2001). Further, the MRI signal is very weak in a voxel containing large vessels due to a large amount of contrast agent, thus its change induced by dHb changes may not be detected (Mandeville and Marota, 1999). Thus, the specificity of CBV-weighted fMRI can be improved to small-sized vessels and surrounding tissue. The sensitivity gain of the CBV measurement over the BOLD technique is dependent on the dose of the contrast agent, the imaging parameters, and the magnetic field. When 5–15 mg iron/kg body weight is used, the sensitivity gain is about 1.3 to 5-fold (Mandeville et al., 1998; Mandeville et al., 2001; Kim and Ugurbil, 2003; Zhao et al., 2006).

Experimental design and data analysis

Since MRI is obtained from an imaging plane(s), the selection of an imaging slice relative to column directions will make columnar maps different. Figure 2 shows a schematic of apparent 'column' shape in a two-dimensional image. When the slice direction is perfectly orthogonal to the column direction (see red slice), activation patterns in fMRI appear as circles (red), which are same as the dorsal view of columns. When the slice direction is oblique to the column direction (see green slice), columnar shapes are elongated and larger than actual size of columns. If the imaging slice is parallel to the column direction (yellow slice), columns in 2-D images appear as bars in fMRI maps. Thus, it is crucial to determine the imaging slices properly.

The selection of the imaging slice can be aided by 3-D MRI since the column direction is orthogonal to the surface of the flat cortex. The imaging slice can be selected to parallel the surface of the cortex. Moreover, since columns and intracortical vessels run roughly orthogonal to the cortical surface, the visualization of intracortical vessels can be utilized. In our studies, a 3-D high-resolution venographic image with $\sim 80 \mu\text{m}$ resolution was acquired from cat brain (Fig. 3). The voxel containing intracortical veins appear to be hypointense because paramagnetic deoxyhemoglobin gathers into draining venous vessels. Dark spots indicate that intracortical veins run through the plane, while dark lines suggest that veins run parallel or obliquely within the imaging plane. The primary visual cortex is located in the marginal gyrus, and thus orientation columns should exist between the midline (dashed and dotted line) and lateral sulcus (dashed line).

For fMRI studies, isoflurane-anesthetized cats are restrained in a normal postural position using a custom-designed non-magnetic stereotaxic frame. Visual stimuli consisted of high-contrast square-wave moving gratings (0.15 cycle/degree, 2 cycle/sec) of different orientations to elicit neuronal activity in cat area 17 and 18 (Movshon et al., 1978). Two different stimulation paradigms can be used: block-design and continuous stimulation paradigms. Conventional block-design stimulation paradigm consists of repeated baseline control and orientation stimulation periods (see Fig. 4A). Control can be either stationary gratings or stationary solid gray (blank). Temporal resolution of fMRI is limited by hardware of MR equipment and MR physics. After the excitation of water proton spins (for MRI data collection), these spins will return to a normal status, which takes typically 1 – 3 seconds, depending on magnetic field strength and tissue type. Thus, rapid repetitions of excitations will significantly reduce the sensitivity of MR signals due to insufficient recovery from the preceding excitation. In our high-resolution fMRI studies with temporal resolution of 2 – 6 sec, 10 – 60 seconds stimulation duration was used for obtaining sufficient number of data points in each period. Data analysis can be performed by simple subtraction of averaged control image from the averaged stimulated image or by using general linear models. This simple analysis produces 'single-condition' functional maps. When orthogonal stimulus condition is known, the differential method can be utilized by subtraction of the single-condition map induced by one orientation from that induced by orthogonal orientation (Blasdel, 1992; Bonhoeffer and Grinvald, 1993), since orthogonal stimuli activate complementary territories. The differential approach has been extensively used by the optical imaging community to image cortical columns (referred to as 'differential map').

For orientation column mapping, continuous cyclic stimulation without control periods can also be adopted instead of block-design stimulation. For example, in our experiments, eight orientations were presented (0° (horizontal) to 157.5° , 22.5° increments, 10 s each) without gaps between stimuli, and the 80-s cyclical continuous stimulation paradigm was repeated 10 times (see Fig. 4B). The fMRI signal can be modeled using sinusoidal functions with multiple-frequency components. Thus, Fourier analysis can be applied to decompose signals

continuously recorded for 800 s (10 stimulation cycles) into different temporal frequency components (Engel et al., 1997; Kalatsky and Stryker, 2003). Then, orientation-specific signal at a frequency of 0.0125 Hz (i.e., 1/80 Hz) can be used to generate orientation-specific maps. This Fourier method is advantageous over the differential data analysis approach because the fMRI response following the orientation-specific stimulation cycle can be easily separated from other frequency components induced by vasomotion (Mayhew et al., 1996), respiration, heart beat, etc. The fMRI signal responds to the orientation changes in a gradual manner, which is well fitted by a cosine function model. Thus, the Fourier analysis method with continuous stimulation (Kalatsky and Stryker, 2003) is more efficient to detect functional response compared to a conventional block-design paradigm (Kim et al., 2000; Zhao et al., 2005a). However, the hemodynamic response time must be determined to accurately assign orientation preference to fMRI maps acquired with continuous stimulation since the hemodynamic response is not instantaneous, but rather sluggish. Note that the block-design paradigm will not have this problem. The hemodynamic response time can be determined using two different orientation presentation orders (Kalatsky and Stryker, 2003): one is the “forward” direction starting at 0° and increasing in orientation by 22.5°; the other is the “backward” direction starting at 157.5° and decreasing in orientation by 22.5°. The hemodynamic response time is the same in the forward and time-reversed backward stimulation data, but its direction is opposite; therefore, the effect of the hemodynamic response time can be removed from the functional maps.

Intrinsic spatial specificity of hemodynamic responses

Since the spatial extent of the hemodynamic response will impose a fundamental limitation on the spatial resolution of fMRI modalities, assessment of the intrinsic hemodynamic response function becomes imperative. According to intrinsic optical imaging studies, the increase in oxyhemoglobin signals, resulting from stimulus-evoked CBF and blood volume increases, was spatially diffuse and extend far beyond the true activation site (Malonek and Grinvald, 1996). Based on these findings, it is concluded that the delayed oxyhemoglobin and deoxyhemoglobin signal changes are not sufficiently localized to the actual areas of elevated neuronal activity to resolve cortical orientation columns. However, it is unknown whether the poor spatial specificity in the delayed optical signals is due to intrinsic properties of coupling between neuronal activity and blood flow or, alternatively, contamination from large downstream pial veins or upstream pial arteries. This can be directly examined using CBF or CBV-weighted fMRI since signal contributions from pial vessels can be avoided by appropriate slice selection unlike the surface optical imaging techniques.

The CBF-weighted fMRI technique was applied to determine the spatial specificity of CBF responses induced by orientation-selective stimulation (Duong et al., 2001). ASL images were acquired in a transverse view using block-design single-orientation stimulation. Figure 5 shows the CBF activation map of one representative animal. Increased CBF activity was observed predominantly in the tissue area, avoiding the superior sagittal sinus (indicated by the arrow). Most importantly, the CBF functional maps exhibit “patchy” layouts with semi-regular cluster shapes in area 18, a prominent topological characteristic of orientation columns as observed using 2-DG (Lowel et al., 1988), and optical imaging (Bonhoeffer and Grinvald, 1991, 1993). Time course of fMRI signals within the active regions shows ~20% increase during 0° stimulation (indicated by 60-s bars). Our data indicate that the previous optical imaging finding of poor CBF specificity is most likely due to large pial vessel contributions. Proper CBF contrast in ASL images is only achieved when enough time is allowed for the labeled arterial water to travel into the region of interest and exchange with tissue water. This makes it difficult to detect changes in CBF with a temporal resolution greater than the lifetime of the labeled water (~1.5 – 2.3 s). Acquisition of a pair of images can further reduce temporal resolution and consequently SNR. Also, the selection of CBF-based imaging slices is dictated by the direction

of flow. Thus, CBF-based imaging techniques have not been widely used for routine fMRI studies. Similar to the quantitative CBF approach, the CBV-weighted fMRI technique with iron oxide contrast agents may be applied for high-resolution mapping in animals.

CBV-weighted fMRI experiments were performed in a sagittal plane after injection of 10 mg iron/kg body weight using block-design stimulation. Raw stimulation-induced signal change (i.e., post-stimulus image minus pre-stimulus image) maps were obtained without any statistical threshold (illustrated in Fig. 6A and B for one animal of 45° vs. 135° gratings, respectively). Higher CBV increase results in larger negative signal changes. Patchy clusters were observed in complementary territories between single-condition 45° and 135° maps (Fig. 6A vs. 6B). To enable comparison with previous optical imaging studies (Bonhoeffer and Grinvald, 1991, 1993), differential maps (e.g., Fig. 6C) were obtained by subtracting the single-condition maps for two different orthogonal visual stimuli. In the differential iso-orientation map (Fig. 6C), regions sensitive to 45° orientation (“45° iso-orientation domain”) are shown as black, while pixels with preferential activity for the orthogonal stimulus (“135° iso-orientation domain”) are white. Clearly, patchy clusters preferential to the two orthogonal stimuli are segregated. The patterns and shapes of clusters in the differential maps are consistent with those found in intrinsic optical imaging (Bonhoeffer and Grinvald, 1993). The average anterior-posterior distance between iso-orientation domains in differential images is 1.37 ± 0.28 mm in all studies ($n = 10$ hemispheres) (Zhao et al., 2005b), which is also consistent with the result using 2-DG method (Lowel et al., 1987). Spatial profiles of signal changes along a single line of the entire field of view in the anterior-posterior direction (indicated by lines) were visualized (Fig. 6D). Difference between two single-condition profiles is also shown. The average ratio in all studies of CBV changes induced by preferred orientation vs. orthogonal orientation gratings is 1.7, indicating non-specific response is quite significant (in other words, 0.7 for orientation-specific vs. 1.0 for orientation-non-specific) (Zhao et al., 2005b). The non-orientation-specific signals can be due to a contribution from sub-threshold synaptic activity in inactive domains (Das and Gilbert, 1995), a less-specific CBV response compared to metabolic and neural activity (Malonek and Grinvald, 1996), non-optimal visual stimuli for some populations of neurons (due to the use of only two orientations), non-ideal slice selection relative to the direction of columnar organization, and/or limited spatial resolution (pixels contain neurons with varying responses to orientation of stimuli). Combining CBF and CBV data together, we can conclude that hemodynamic-based fMRI techniques *per se* can indeed be used to map functional columns if large-vessel contribution to the mapping signals can be minimized.

Confirmation of columnar-resolution fMRI maps

Even though column-like functional structures were mapped, it is not known whether higher CBF and CBV changes indicate neuronally active columns. When a single cortical column is activated, the highest fMRI signal will appear at the site of increased neuronal activity regardless of its PSF. However, when multiple columns are activated, successfully determining the location of active columns within fMRI maps will be dictated by the PSF width relative to the distance between neighboring active columns. Because of this limitation, it is conceivable that the highest CBV signals induced by bars of 0° (90°) orientation may appear at the location of 90° (0°) orientation columns. Thus, it is essential to confirm our fMRI observation using optical images with known neural correlates, since a good correlation between neural activity and OIS has already been observed in the visual cortex (Grinvald et al., 1986; Shmuel and Grinvald, 1996; Maldonado et al., 1997; Bosking et al., 2002). To achieve high sensitivity for orientation column mapping, we used CBV-weighted fMRI with iron oxide nanoparticles and the continuous stimulation paradigm. After fMRI data collection, OIS experiments were performed with the same stimulation paradigm on the same animal (Fukuda et al., 2006a).

Functional MRI and optical images were coregistered using pial vessel patterns; because the slice chosen for fMRI studies was below the cortical surface, the pial vessel pattern above the fMRI slice was visualized using a 2-D MR image. Then, the optical image was linearly transformed manually so that pial vessel patterns matched the MRI patterns. Since functional signals were obtained during 80-s cyclical stimulation, the peak of the functional response specific to a particular orientation can therefore be observed every 80 s (Fig. 7). Thus, this orientation-specific signal was extracted by selecting the orientation-specific stimulation frequency component in Fourier analysis. The non-orientation-specific signal induced by each stimulus presentation is almost saturated by continuous stimulation. Dark patches in fMRI maps have larger CBV changes responding to a presented orientation, and dark patches in optical images indicate active sites responding to a presented orientation (see Fig. 8). Iso-orientation maps of fMRI were similar to those of OIS; activation borders, columnar patterns (black and white patches and bands), and most loci show a good match between the two modalities. Thus, the highest CBV-weighted fMRI signal change is expected at the site of increased neuronal activity.

All human columnar mapping has been demonstrated using positive BOLD signals (Menon et al., 1997; Menon and Goodyear, 1999; Dechent and Frahm, 2000; Cheng et al., 2001; Goodyear and Menon, 2001). Since the BOLD PSF is determined by a mismatch between CMRO₂ and CBF responses, its assignment of neural activity sites into BOLD columnar maps is not clear. When multiple columns are active, the highest BOLD signals could originate from neurally active domains if CBF PSF is narrow relative to an inter-column distance (Duong et al., 2001), or the highest BOLD signal could originate from neurally inactive domains if the CBF response is broad as suggested by optical spectroscopic imaging (Malonek and Grinvald, 1996). To evaluate whether the highest BOLD response occurs in the active columns, we used CBV-related fMRI maps as a reference, where spatial neural correlations are known (Fukuda et al., 2006a). Orientation-specific modulations of the 80-s cycle are again extracted by Fourier analysis (Fig. 7). The orientation-specific modulation of 1/80 Hz for the BOLD signal begins at a positive deflection and is almost opposite to that of the CBV-weighted signal (Fig. 7A; note that negative CBV-weighted signal indicates an increase in CBV). Iso-orientation maps of the BOLD signal were significantly correlated to those of CBV-weighted fMRI (see Fig. 9). Thus, the highest orientation-selective BOLD signals indicate the sites of increased neural activity. In other words, the magnitude of BOLD response to preferred stimulation should be larger than the magnitude of response to non-preferred stimulation (Moon et al., 2007).

Column-specific BOLD vs. dHb-weighted OIS signals

Although a good match of orientation column maps between fMRI and optical imaging was observed, a discrepancy in orientation-specific modulation was seen between BOLD and dHb-weighted intrinsic signal (Fig. 7). Our results (see Figs. 7 and 9) clearly demonstrate that the preferred stimulation induces a larger positive BOLD signal than the nonpreferred stimulation. Thus, dHb content should decrease more during the preferred stimulation than during the nonpreferred stimulation (i.e., more hyperoxygenation during preferred stimulation). In contrast, the orientation-specific dHb-weighted OIS signal begins at a negative deflection as similar to the CBV-weighted OIS signal (Fig. 7B; note that negative reflected 570-nm signal indicates an increase in CBV). This result implies that the preferred stimulation induces a smaller decrease in the dHb amount than the nonpreferred stimulation (i.e., less hyperoxygenation during the preferred stimulation) (Malonek and Grinvald, 1996; Grinvald et al., 2000; Fukuda et al., 2005). What causes this apparent contradiction of the supposed dHb signals between fMRI and optical imaging?

When the CBF and CBV responses induced by neural activity are minimal at the vasodilator-induced hypotension condition (Nagaoka et al., 2006), similar to the condition of the early dip,

both negative BOLD signal (Fukuda et al., 2006a) and optical imaging signal (Fukuda et al., 2006b) have similarly larger hypooxygenated changes at active columns than inactive columns. This indicates that the dHb contribution is dominant in both BOLD and optical imaging signals when CBF and CBV responses are minimal. This suggests that the difference between BOLD and optical imaging signals at the normal condition appears to relate to CBF and/or CBV responses, which requires further systematic studies.

Conclusions

We hope our studies have helped to confirm that fMRI can convincingly map functional cortical columns. Both BOLD and CBV techniques can be used to determine functional columnar structure in cortical areas, which have not been investigated previously, such as regions deeply embedded in sulci (Fig. 10) or possibly on a layer to layer basis. Also, a plastic and/or developmental change of functional columns can be non-invasively tracked. Since sensitivity of the CBV-weighted fMRI signal is higher than that of the BOLD fMRI, CBV-weighted fMRI with contrast agent is preferable in animal studies.

Acknowledgements

This work was supported by the NIH (NS44589, EB003324, EB003375).

We thank Ping Wang for animal preparation, Michelle Tasker for proofreading and animal preparation, and Chan-Hong Moon for providing BOLD data.

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Fig. 1.

A schematic of vascular responses induced by neural activity. Blood containing oxyhemoglobin (red circles) and/or deoxyhemoglobin (blue circles) travel from arteries, arterioles, capillaries, venules, finally to veins. Oxygen delivered via oxyhemoglobins diffuses into (extravascular) tissue, and is used as metabolic substrates. At pre-stimulus baseline conditions, blood oxygen saturation is close to 100% in arteries, while it is ~60% in veins (even though it varies dependent on subject's physiological condition). When neural activities increase, they will trigger an increase in blood velocity (indicated by the size of arrows) and dilation of vessels directly or indirectly. Since the CBF increase induced by an increase in neural activity overcompensates an increase in oxygen consumption rate, venous blood is less oxygenated during stimulation (i.e., more oxyhemoglobin and less deoxyhemoglobin contents). Partially deoxygenated blood will drain from capillaries to large veins.

Cortical surface

Imaging slice

Fig. 2.

Schematics of slice selections and apparent patterns of columns. Columns orthogonal to the surface of the cortex run across the entire cortex. 2-dimensional MR imaging slice(s) can be chosen with a well-defined thickness at an arbitrary angle. Thus, appearance of columns in 2-D images is dependent on the slice selection relative to the column direction. When the imaging slice is orthogonal to the column direction, fMRI patterns are same as dorsal view of columns (red circles). If the imaging slice is oblique to the column direction, column patterns elongate depending on the oblique angle and slice thickness (green ovals). If the imaging slice is parallel to the column direction, bar-like patterns appear dependent on slice thickness (yellow bars).

Fig. 3.

A sketch of cat brain and 2-D slice view of various imaging slices. In the dorsal view of the cat brain (A), gray area indicates visual area 17 and 18, where orientation columns are expected to be present. To select imaging slices for columnar mapping, a 3-dimensional venographic image was acquired. From the 3-D image, a 2-D section can be selected for fMRI studies. In the coronal view (B), three different imaging slices indicated by 1-mm thick slabs can be chosen to cover visual area 17 or 18, transverse (c), oblique (d), and sagittal (e). Corresponding images are shown in C, D, and E. Dark lines or spots indicate venous blood vessels. Most intracortical vessels run nominal to the surface of the cortex. When vessels appear as small dark dots, then the imaging plane is selected roughly orthogonal to the vessel direction. LS (dashed line), lateral sulcus; ML (dashed and dotted line), midline; mg, marginal gyrus; D, dorsal; V, ventral; M, medial; L, lateral; A, anterior; P, posterior.

Fig. 4.

Stimulation paradigms used for mapping orientation columns. (A) Block-design. Orientation-selective stimulation period follows pre-stimulus control period. Red double-arrow in black-and-white gratings indicates a direction of motions. The same stimulus may be repeated or two orthogonal stimuli may be interleaved. (B) Continuous stimulation paradigm. Orientation-selective stimuli are presented without any control period. In this specific example, 8 different orientations are presented sequentially. Since no control period is used here, total response induced by one orientation cannot be determined, and the difference of signals induced by different stimuli will be observed.

Fig. 5.

Perfusion (CBF)-weighted fMRI map corresponding to 0° stimulation and time course of active pixels (Duong et al. 2002). An imaging slice was selected in a transverse plane, and arterial spin labeled images were acquired with temporal resolution of 6.0 s. Functional map was generated by using cross-correlation analysis between raw time course and a box-car reference function based on block-design stimulation paradigm. Functional map (color) was overlaid on an anatomic image. Color bar, cross-correlation value between 0.3 and 0.8; scale bar, 1 mm; gray bars, stimulation periods.

Fig. 6.

CBV-weighted fMRI. CBV-weighted fMRI was obtained in a sagittal plane after intravascular injection of superparamagnetic iron oxide nanoparticles. Negative signal change indicates an increase in CBV during stimulation. Single-condition maps corresponding to 45° and 135° stimulation (A and B, respectively) are shown as well as their differential map (C). Dorsal contour indicates the edge of the brain, while the ventral contour is the splenial sulcus. To easily visualize patches responding to 45°, green plus signs were obtained from the 45° single-condition map and then overlaid on 135° single-condition map (B) and the differential map (C). Clearly, functional territories responding to two orthogonal stimuli are complementary. Spatial profiles in a posterior-anterior direction indicated by a line were obtained (D). Clearly, negative signal changes indicating increases in CBV were observed from baseline (dashed horizontal line). The subtraction of 135 from 45° profiles enhances differential responses induced by two orthogonal stimuli; negative peaks represent the regions preferred to 45° stimulation, while positive peaks represent 135° stimulation. Interval between positive (or negative) peaks is related to inter-column distance.

Fig. 7.

Orientation-selective modulations during 800-s continuous stimulation. Eight 10-s long orientation-selective stimuli starting 0° and increasing 22.5° were repeated 10 times. CBV- (blue) and dHb-weighted (red) fMRI signals (A) and OIS responses (B) were obtained from 0° -specific columns. In optical imaging (B), reflected response of 570-nm wavelength light is weighted by CBV, while that of 610-nm wavelength is weighted by dHb. A decrease in CBV- and dHb-weighted signal indicates an increase in CBV and dHb, respectively. In both fMRI and OIS data, orientation-selective stimulation induces a decrease in CBV-weighted signals (i.e., an increase in CBV). BOLD response increases during orientation-selective stimulation, while 610-nm OIS response decreases. The time to peak of CBV and BOLD response slightly differs due to their different response characteristics.

Fig. 8.

Comparison between CBV-weighted fMRI and optical images (Fukuda et al., 2006a). An fMRI slice was selected in the middle of the cortex through the gyrus in the right hemisphere (indicated as a dashed slab), while optical imaging obtained from the upper cortical layers including the surface of the cortex. Thus, to co-register both modality images, pial vessel patterns just above the fMRI slice (indicated as a solid slab in A) were compared with optical image (B vs. C). Functional maps were obtained from continuous stimulation and Fourier analysis. In the functional maps, dark patches represent the region preferentially responding to the orientation stimulus shown in the right corner, while white patches represent orthogonal orientation stimulation. Plus signs were marked based on fMRI patches and overlaid on optical maps. Clearly, fMRI and optical images agree very well.

Fig. 9. CBV-weighted vs. BOLD fMRI. CBV-weighted fMRI followed conventional BOLD fMRI. Both maps were determined from continuous stimulation data and Fourier analysis. Higher signal change responding to 0° stimulation appears as black, while that responding to 90° appears white. Gray indicates either 45° or 135° response area. Green contours indicating 0° columns were marked based on CBV-weighted fMRI and overlaid on fMRI maps. Even though some regions mismatch between CBV-weighted and BOLD fMRI, there is generally good agreement between the two modalities.

Fig. 10.

CBV-weighted fMRI map in the medial region (Fukuda et al., 2006a). CBV-weighted fMRI was obtained on a sagittal imaging slice in the medial bank of cortical area 17 (A) with continuous stimulation paradigm. Vessel-weighted sagittal image (B) shows orientations of intracortical veins relative to the imaging slice; dark lines indicates vessels run within the imaging slice (as indicated between lines 1–2 and 3–4), while dark spots indicates vessels run through the imaging slice (lines 2–3). Spatially distinct activation patterns appear as irregular patches and bands. Patchy patterns appear in the region between dashed lines 2 and 3, where the imaging plane is parallel to the cortical surface. Band-shaped patterns appear in regions where the imaging plane is perpendicular to the cortical surface (between dashed lines 1 and 2, and between lines 3 and 4).