Frontiers of Brain Mapping Using MRI

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Over the past dozen years, the use of MRI techniques to map brain function (fMRI) has sparked a great deal of research. The ability of fMRI to image several different physiological processes concurrently (i.e., blood oxygenation, blood flow, metabolism) and noninvasively over large volumes make it the ideal choice for many different areas of neuroscience research in addition to countless applications in clinical settings. Furthermore, with the advent of high magnetic fields (and other hardware advancements, i.e., parallel imaging) for both human and animal research, spatial and temporal resolutions continue to be pushed to higher levels because of increases in the sensitivity as well as specificity of MR-detectable functional signals. fMRI methodology continues to grow and has the ability to cater to many different research applications. There seems to be no foreseeable end in sight to the advancement of fMRI techniques and its subsequent use in basic research as well as in clinical settings. In this work, fMRI techniques and the ongoing development of existing techniques are discussed with implications for the future of fMRI. 

Key Words: fMRI; BOLD; ASL; CBV; high resolution imaging; high magnetic field

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SINCE ITS INTRODUCTION IN 1992 (1–3) functional magnetic resonance imaging (fMRI) has grown explosively to become a standard and indispensable tool in neuroscience research. fMRI provides the ability to image regions of altered neuronal activity in animal and human brains. From a plethora of observations, we know brain function to be often subdivided and organized spatially so that neurons performing the same or similar tasks are clustered together. Therefore, the ability to image brain activity provides the means to probe mechanisms underlying brain function. In this respect, fMRI constitutes an enormous advance; in addition, it can cover the entire brain (albeit with some difficulty near air–tissue interfaces). Most importantly, fMRI is noninvasive and hence can be used to study the human brain. Functional images in the brain can be obtained using the blood oxygenation level dependent (BOLD) mechanism (1–3), measurement of cerebral blood flow (CBF) changes with arterial spin labeling (ASL) (e.g., Refs. 4–9 and references therein), intravoxel incoherent motion (10,11), and cerebral blood volume (CBV) alterations (e.g., Refs. 12–14).

In this short article, we summarize some of the recent evolutionary changes in fMRI methodology that may impact the way fMRI is performed in the future. In the spirit of this special issue that targets clinical applications, emphasis is placed on past and potentially future clinical utility and significance.

IMPROVING SPATIAL AND TEMPORAL RESOLUTION OF THE BOLD SIGNAL

The primary MRI technique used for fMRI exploits the BOLD effect (for a more comprehensive review of the BOLD signal, see article by Norris in this issue). The physiological basis of the BOLD effect is that CBF increases more than commensurately relative to the cerebral metabolic rate of oxygen (CMRO2) during increased neural activity. As a result the oxygen extraction fraction E is reduced and venous blood is more oxygenated. This physiological change is detectable with MRI because the MR signal is sensitive to microscopic magnetic field gradients, and deoxyhemoglobin and oxyhemoglobin have vastly different magnetic properties. Strongly paramagnetic deoxyhemoglobin, when sequestered in erythrocytes and blood vessels, creates magnetic field gradients in and around blood vessels whereas such gradients are absent for diamagnetic oxyhemoglobin. One of the complexities involved in interpreting the BOLD signal is that it depends on several physiological variables.

The hemodynamic response to stimulation evolves in seconds, whereas the neuronal changes are on the order of milliseconds. Thus, neuronal changes are averaged and temporally blurred in the vascular domain. In addition, until recently, the minimum spatial scale for the vascular control of blood flow changes was not clear. It is often assumed that CBF changes are actively regulated by the arterioles. An arteriole feeds a volume of tissue that is approximately a cylinder with 1-mm diameter (15). Therefore, different neuronal populations that span the submillimeter spatial scale and are contained in the “feeding field” of the same arteriole cannot be spatially separated.
Figure 1. Current state-of-the-art spatial resolution of fMRI. Single condition activation maps of sub-millimeter columnar structures of (a) iso-orientation and (b) single-eye ocular dominance columns in cat primary visual cortex obtained by CBF- and CBV-based fMRI techniques, respectively. c: Complementarity of CBF-activated pixels of orthogonal orientations (45° and 135°) marked with + and – signs, respectively. d: Differential map computed in area 17; marked in b. Yellow and blue regions represent alternating cortical areas exhibiting a larger signal change to left and right eye stimulation (black and white arrows), respectively. a,c: Modified from Duong TQ, Kim DS, Ugurbil K, Kim SG. Localized cerebral blood flow response at submillimeter columnar resolution. Proc Natl Acad Sci USA. 2001;98:10904–10909, with permission of the National Academy of Sciences USA. b,d: Modified from Harel N, Zhao F, Wang P, Kim S-G. Cortical layer specificity of BOLD and CBV fMRI signals at ultra-high resolution. In: Proceedings of the 10th Annual Meeting of ISMRM, Honolulu, HI, USA, 2002 p. 9, with permission.

Figure 2. Enhanced sensitivity of BOLD fMRI at high magnetic field. The BOLD response is strongly magnetic field dependent. a: BOLD activation maps from a single subject imaged at 4 T and at 7 T, both thresholded with the same significance. A significant increase in the number of activated voxels are detected at higher field. The ratio of 7-T vs. 4-T percent changes was $2.6 \pm 0.26$, which is close to the predicted value of 3 for a quadratic dependence on magnetic field. b: Due to the increase in SNR at higher magnetic fields, the spatial reproducibility of the detected signals is improved. An example of a test and retest of high resolution fMRI maps ($1 \times 1 \times 3$ mm³) acquired at 7 T are shown. c: Scatter plot of the cross correlation values obtained in the test and retest (b). a: Modified from Ref. 23. b,c: Modified from Ref. 8. (Reprinted from Shmuel A, Yacoub E, Pfeuffer J, Van de Moortele PF, Adriany G, Hu X, Ugurbil K. Sustained negative BOLD, blood flow and oxygen consumption response and its coupling to the positive response in the human brain. Neuron 2002;36:1195–210, with permission from Elsevier.)
by fMRI signals with arteriole control of CBF alone. Such physiological restrictions, as well as restrictions imposed by mechanisms by which blood vessels lead to MR detectable functional signals, impose limitations on spatial accuracy of fMRI signals. This is critical both in basic neuroscience applications and also in applications to diseased populations, such as presurgical evaluations.

**FUNDAMENTAL LIMITATIONS ON SPATIAL RESOLUTION**

Duong et al (6) demonstrated that it is possible to perform single-condition functional mapping of orientation columns in the cat visual cortex by mapping CBF changes using ASL (for the ASL method, see discussion below). This work demonstrated that while perfusion increases were not “perfectly” localized at the isoorientation column level, the difference between active and neighboring inactive columns was large and permitted single condition mapping (Fig. 1). This work indicated for the first time that there must be regulation of blood flow control in the submillimeter (~300 to 400 μm) scale. Subsequently, CBV-based fMRI studies, conducted using the extracellular contrast agent monocrystalline iron oxide nanoparticles (MION; see discussion below), have also demonstrated single-condition columnar resolution fMRI changes (Fig. 1; Ref. 16). Furthermore, with its superior sensitivity, CBV activation studies in animals have demonstrated that fMRI can detect and resolve laminar activity and that the larger intralaminar fMRI signal changes correspond to cortical layer 4 (Refs. 17 and 18 and references therein). This observation further indicates that capillary level control of blood flow and blood volume should exist. More recent work from several laboratories has confirmed these conclusions using optical imaging of intrinsic signals (e.g., Refs. 19–21).

Consistent with the results described above, the point spread function (PSF) of the CBF response to visual stimulation has been measured to be ~0.6 mm (full width half maximum (FWHM)) in the cat primary visual cortex (22). This number includes the neuronal contribution to the PSF as well. This PSF imposes a fundamental limitation on spatial specificity in all functional imaging techniques that directly (e.g., ASL or positron emission tomography (PET)) or indirectly (e.g., BOLD) rely on blood flow as the imaging signal.

In view of the submillimeter specificity of the blood flow signals, it is likely that in the near future current efforts will lead to functional mapping with submillimeter specificity and resolution in the whole brain or a large section of the brain in humans. One may conclude that we are already there with the ASL technique. However, as discussed below, ASL techniques have a poor contrast-to-noise ratio (CNR) for functional mapping compared to BOLD or CBV based techniques and encounter difficulties for whole brain coverage. Therefore, it is important to pursue alternative strategies.

**IMPROVING SPATIAL RESOLUTION AND SENSITIVITY WITH HIGH FIELD BOLD-BASED fMRI**

The BOLD response is strongly magnetic field–dependent and this dependence is complex. The increase in the signal-to-noise ratio (SNR) at higher magnetic fields has been demonstrated (23); more importantly, higher fields have been shown to have enhanced fMRI sensitivity, i.e., CNR (Fig. 2). Furthermore, with improved SNR, not only are the signal changes more robust, but also the reliability and reproducibility of the fMRI measurement increases (Fig. 2). At low magnetic fields, the response is dominated by large veins whereas at higher magnetic fields capillary mediated effects also become detectable (see Refs. 24–26 and references therein). Stimulus or task-induced alterations in the deoxyhemoglobin content leads to BOLD based functional signals originating from water molecules in blood as well as tissue in the extracellular space (Refs. 23,27 and references therein). Blood related signals are not highly specific because deoxyhemoglobin changes that occur at the site of altered neuronal activity are not static and propagate rapidly down the vascular tree due to blood flow. The blood contribution decreases dramatically with higher fields due to the rapidly decreasing $T_2$ of blood relative to the tissue $T_2$ at high fields. Thus, in principle, at high fields, the spatial accuracy of BOLD-based fMRI improves.

Conventionally used gradient echo (GE) BOLD remains the least accurate fMRI approach even at high magnetic fields. The PSF of this approach has been measured to be ~3.5 mm at 1.5 Tesla (28) in the human brain; the PSF appears to improve at higher fields; approximately 1.6 mm (FWHM) (22) and 2 mm (29) have been reported for 9.4 and 4.7 Tesla, respectively, in the cat brain. A rigorous comparison of the present data is difficult because of the different species used. Nevertheless, the (GE) BOLD PSF remains clearly in the supramillimeter range because vein contributions persist even at high fields. However, at ultrahigh fields such as 7 Tesla or higher, these nonspecific signals now coexist simultaneously with more specific functional signals of microvascular origin in GE BOLD fMRI (30). Thus, when very high resolution GE BOLD fMRI images (0.5 × 0.5 × 3 mm$^3$) are obtained in the human brain (Fig. 3), a diffuse activity overlapping gray matter is the dominant feature (30), whereas such high resolution images at 1.5 T result in activations maps that largely follow large blood vessels (e.g., Ref. 31). Therefore, the impact of the presence of nonspecific large vessel contribution at 7 T is not as consequential. With a PSF of ~2 mm FWHM, neuronal organizations of 1 mm dimensions can still be mapped, albeit with some blurring.

In view of this improved specificity at ultrahigh fields, coupled with the rapid proliferation of 7 Tesla instruments, it can be expected that GE fMRI with close to or at 1 mm isotropic resolution will become commonly used in cognitive neurosciences. This capability has already been demonstrated with successful mapping at 7 Tesla of the human tonotopic organization for the first time (32). Note that with sufficient averaging to compensate for the decreased SNR, it is possible to perform...
GE BOLD fMRI studies with \(~1\) mm isotropic image resolution at 3 T or even 1.5 T. However, the effective functional resolution is determined by the PSF of functional signals and not the intrinsic resolution of the acquired images.

The large vein contributions in BOLD fMRI can be suppressed further using Hahn spin echoes (HSE), leading to improved localization (e.g., Ref. 26 and references therein). However, this requires that experimental imperfections that bring potentially nonspecific con-
tributions (e.g., inflow effects associated with large vessels or $T_2^*$ BOLD effects due to finite image acquisition times) can be minimized (33). HSE fMRI is in principle composed of both blood and microvascular extravascular BOLD effects. The former is relatively nonspecific and can lead to false "activation" in large draining veins. For a given echo time, the blood contribution, however, diminishes as magnetic fields increase (27) and become small compared to the tissue signals at echo times optimal for detecting tissue signals (27). This leads to improved localization for ultrahigh-field HSE fMRI (for example see Fig. 3). In agreement with this expectation, the PSF of HSE fMRI at 9.4 T has been measured to be $\sim 0.6$ mm (FWHM) (34), the same as the CBF response within experimental error.

The use of an asymmetric spin echo (ASE) BOLD has been applied in order to capitalize on the sensitivity of GE BOLD while retaining the spatial specificity of conventional HSE. This is accomplished by imposing an offset time that increases the $T_2^*$-weighting in a spin echo (SE) sequence. These studies suggested that by adjusting the offset time, or essentially not completely refocusing the spins as in a typical HSE sequence, large vessel signals (commonly found in GE BOLD data) could still be suppressed (refocused), while increasing the CNR of smaller vessel signals via $T_2^*$ contrast. This technique has not been widely applied since it was initially suggested and furthermore, the advantages of this technique have only been reported at relatively low fields ($\leq 4$ T) at which it is now known that the intravascular BOLD signal contributes ($\geq 50\%$) of the total BOLD signal changes. At higher fields, at which the intravascular BOLD signal is greatly reduced, it was shown at high spatial resolutions that by increasing the offset time any additional contrast obtained with the ASE sequence arose primarily from large vessel regions. Further studies need to be done in order to accurately assess any potential advantages/disadvantages of ASE sequences and more specifically as it relates to higher fields.

With increasing interest in establishing ultrahigh field systems such as 7 and 9.4 Tesla, capable of human imaging, it is likely we will see an increasing use of HSE or analogous sequences (35) for high-resolution fMRI to attain better specificity. The major problem to be solved here is limitations on power deposition in human studies. However, parallel imaging methods for spatial encoding that work with greater efficiency at ultrahigh fields (\textit{e.g.}, inflow effects associated with large vascular compartments, which may be bringing nonspecific activation into functional maps, may be suppressed to a certain degree and therefore more accurate spatial localization can be achieved. For example, this method was successfully used for mapping human ocular dominance columns in V1 (39–41) using GE BOLD fMRI (see Fig. 4). Differential imaging is used routinely in optical imaging. However, it is unlikely that the nonspecific vascular component will be completely identical in the two stimulation conditions. Hence, cancellation may not be perfect or reproducible with techniques like GE BOLD, which is plagued the most by nonspecific vascular contributions. Using differential imaging in combination with methods such as ultrahigh-field HSE BOLD (42) or short stimulations (39) can improve the spatial specificity. In this case, compared to GE BOLD, any imperfections that exist in the data, are relatively smaller than the functional signals, and can be further suppressed with differential imaging.

**IMPROVING TEMPORAL RESOLUTION WITH ULTRASHORT STIMULATION**

In a typical fMRI experiment the stimulus duration is at least on the order of a few seconds. However, using two-pulse stimulation with different interstimulus intervals up to 100 msec, Ogawa et al (44) showed in rats that neuronal interaction—measured as suppression of the ERP magnitude to the second stimulus—is reflected by the amplitude of the BOLD signal. This experiment showed that although the hemodynamic response evolves in seconds, the magnitude of the hemodynamic response could encode events and interactions on a millisecond time scale.

**IMPROVING TEMPORAL RESOLUTION BY ANALYZING NONLINEARITIES OF THE BOLD RESPONSE**

Subtle changes of the vascular response might indicate neuronal influence on a time scale of tens of milliseconds. Kellman et al (45) presented two different stimulation designs, one being a slightly modified version of the other. In one design, a decorrelation delay was introduced between the stimuli. Using nonlinear systems analysis they found that nonlinearities of the BOLD...
response were only present in one of the designs. Because the hemodynamic response should be nearly identical for both cases, the authors concluded that the origin of the nonlinearities was neuronal.

In summary, clever analysis and experimental methods can be used to extract neuronal interactions on a fast time scale from the slow hemodynamic response.

**ASL**

An alternative method to BOLD fMRI to assess functional activity with MRI is ASL, which measures cerebral blood flow instead of blood oxygenation. CBF studies using $^{18}$O-labeled water as the contrast agent have been used extensively for functional mapping with PET. The MRI counterpart to this PET technique is to magnetically label arterial water with an applied radiofrequency (RF) pulse, implemented as either continuous (e.g., Refs. 46 and 47), modulated versions of continuous (48, 49), or pulsed (e.g., Refs. 5, 50, and 51) tagging approaches. In a typical implementation, the magnetization of proton spins of the blood is inverted on the proximal side of the slice, and after a delay of one to two seconds, depending on the magnetic field, to allow labeled spins to flow into the slice and then the image is acquired (a). The experiment is then repeated without the inversion (b), and a subtraction of these tagged and nontagged images yields an image directly reflects CBF.

Figure 5. Arterial spin labeling. a, b: Schematic illustration of the basic principle of ASL. The magnetization of proton spins in the blood is inverted on the proximal side of the slice, and after a delay of one to two seconds, depending on the magnetic field, to allow labeled spins to flow into the slice and then the image is acquired (a). The experiment is then repeated without the inversion (b), and a subtraction of these tagged and nontagged images yields an image directly reflects CBF.

**Figure 6.** CBV-weighted fMRI using exogenous contrast agent. Improvement in the spatial specificity of fMRI signals can be obtain by using an intravascular iron oxide contrast agent sensitive to CBV changes. High-resolution fMRI images (0.15 × 0.15 × 2 mm$^3$) obtained in cat visual cortex before (i.e., BOLD contrast) and following the administration of MION (i.e., CBV-weighted) images are shown. Note that the largest BOLD signals changes are observed on the surface of the cortex, while the largest CBV changes are located over the middle cortical layers. (Modified and reprinted from Harel N, Lin J, Moeller S, Ugurbil K, Yacoub E. Combined imaging-histological study of cortical laminar specificity of fMRI signals. *NeuroImage* 2006;29:879–887, with permission from Elsevier.)
implemented in continuous ASL (54), arterial large vessel presence in ASL images can also be suppressed. For these reasons, the ASL signal is localized in parenchyma rather than feeding arteries or draining veins. Images of CBF using the ASL technique result in improved spatial correlation with brain parenchyma compared with conventional BOLD fMRI, as already discussed. ASL also provides a quantitative means to study the mechanisms underlying the BOLD technique itself (e.g., Refs. 7, 55, and 56). However, compared to BOLD, the ASL signal has an inferior functional CNR, typically less by a factor of 3 to 5 and encounters difficulties with coverage of large or whole brain volumes.

The fact that the BOLD signal depends on a combination of changes in CBF, CBV, and CMRO2, and also on the baseline physiological state, makes it difficult to interpret the magnitude of the BOLD signal change unambiguously without further experimental information. For example, experiments have found that, when baseline CBF is increased by breathing CO2 or administering acetazolamide, the BOLD response is reduced (57,58). The implication of this sensitivity of the BOLD signal to the baseline state is that, potentially, many factors could alter the baseline state of a patient group (e.g., anxiety or vasoactive medications but also alcohol, caffeine, and nicotine) that could make their BOLD responses significantly different from a healthy population even if the neural responses in the two groups are identical. For instance, if diseased subjects have higher baseline CBF than healthy subjects, then the same change in neuronal activity results in a lower change in the BOLD signal and vice versa. However, typically an altered BOLD response is attributed to altered involvement of brain areas in the task, which might not be true in light of the aforementioned argument. Because inter- and intrasubject variability of the BOLD response to stimulation can partly be attributed to differences in baseline CBF, simultaneous CBF and BOLD measurements may prove to be a more robust approach for quantitative fMRI studies.

In many vascular and neuronal diseases, it is expected that baseline CBF is perturbed (for a review see Ref. 59). For example, reduced neuronal activity during baseline and cognitive tasks and hence reduced CBF—called hypofrontality—has been hypothesized to be correlated with schizophrenia. Various neuroimaging techniques (e.g., PET, and single-photon emission computed tomography (SPECT)) could reproducibly show altered CBF in schizophrenia patients (e.g., Refs. 60 and 61). However, contradictory findings have also been reported for the same patient group (62,63). Similarly contradictory findings have also been observed with fMRI with both decreased (64,65) and increased BOLD response relative to healthy controls (66,67) during working memory tasks. This problem can be a target for ASL and combined ASL and BOLD studies.

ASL has been applied in several cerebrovascular diseases, e.g., stroke, severe carotid stenosis, and transient ischemic attack (68,69). All of these diseases are expected to cause pathological changes in baseline CBF. Indeed, reduced global CBF in these diseases was observed as was a more pronounced reduction in CBF in the brain hemisphere fed by the partly occluded artery due to stenosis. ASL fMRI was also used in other neurodegenerative diseases, like Alzheimer’s disease (70,71) and epilepsy (72). Hence, ASL fMRI presents a noninvasive alternative method to a bolus-tracking approach to probe vascular and neuronal dysfunctions. The major shortcomings of this technique, namely the limitation on tagged spin lifetime, relatively poor CNR, and difficulty in whole brain coverage, are all alleviated in going to very high magnetic fields because of longer blood T1 and higher intrinsic image SNR. These advantages have already been demonstrated in pulsed ASL based fMRI studies conducted in the human brain at 7 T (8,9). Again, with the increasing availability of 7-T instruments for human studies in many laboratories, increased number of applications of this methodology to a variety of biomedical questions is certainly anticipated.

**CBV-WEIGHTED FMRI**

### Exogenous Contrast Agent

Historically, the first human fMRI experiment was based on cerebral blood volume measurement using an exogenous contrast agent (gadolinium diethyleneetriammine pentaacetic acid, GdDTPA) (73). However, data acquisition had to be performed transiently during bolus passage of the contrast agent GdDTPA; hence the temporal resolution and repeatability of such an approach was limited. Since then, more advanced contrast agents that alter the T2* relaxation times, with better sensitivity to CBV, have been developed. These agents are mainly composed of superparamagnetic particles of iron oxide, which have a blood half-life lasting up to several hours (74,75). Dextran-coated MION is an example of such a compound that is commercially available.

Following the intravenous injection of the contrast agent, the MR signal decreases due to the change in the magnetic susceptibility of vascular space, consequently magnetic field inhomogeneities are generated around blood vessels. During elevated neuronal activity, a local increase in CBV leads to an increase in the amount of contrast agent within a voxel, resulting in a further decrease in MRI signals. While changes in deoxyhemoglobin concentrations (i.e., BOLD) are concurrently present in a T2*-weighted sequence, the effects from the exogenous contrast agent, applied with a sufficient dose, will dominate, and the fMRI signal changes will be mainly weighted by CBV changes. Furthermore, quantitative CBV change can be obtained following a correction factor that includes contrast agent dose, basal CBV levels and taking into account the BOLD contribution to CBV-weighted signals. Furthermore, quantitative CBV change can be obtained following a correction factor that includes contrast agent dose, basal CBV levels, and takes into account the BOLD contribution to CBV-weighted signals.

There are two main advantages to the use of an exogenous contrast agent for measuring CBV changes: First, there is an enhanced CNR when compared to conventional BOLD, provided sufficiently high doses can be used, and second, there is an increase in the
spatial specificity of the mapping signals to the site of neuronal activity compared to GE BOLD.

The contrast agents at sufficiently high dose yield a higher CNR when compared to BOLD contrast due to the stronger intravascular-tissue susceptibility difference induced by the particles relative to deoxyhemoglobin. The enhanced CNR, however, is heavily dependent on factors such as dose and echo time used. At low fields (1.5–2T) the net gain in CNR is about five times (74) while at higher fields (3–4.7 T) the gain is approximately a factor of 3 relative to BOLD contrast (76). Thus, the gain in CNR when using an exogenous contrast agent is magnetic field dependent. The reason for this is that the BOLD contribution increases approximately supralinearly with the magnetic field (23,77), whereas the CBV change remains constant because the iron oxides are saturated at high fields (74). However, theoretical calculations and empirical data have shown that the benefits provided by exogenous agents will persist at fields as high as 9.4 T and beyond (78).

The second advantage of the contrast agent is the increase in the spatial specificity of the MR signal compared to GE BOLD (Fig. 6). In a high resolution fMRI study it has been shown that while the GE BOLD fMRI signal is mainly being detected at the cortical surface, a region containing large vessels, the CBV-weighted signals are centered over the supragranular and granular cortical layers (17,79). Due to the shortening of the T* of blood following the injection of the contrast agent, voxels containing large vessels (i.e., larger amount of contrast agent) exhibit a larger signal reduction, when compared to tissue signals. The SNR from these large vessel regions becomes so low to be undetectable after a relatively short echo time TE. As a result, the functional maps are “clean” from large vessel contamination even though blood volume changes occur in such large vessels (18,78). In contrast, large veins dominate the GE BOLD maps and are nonspecific to the site of neuronal activity.

With respect to the specificity of the CBV-weighted signal, another mechanism is working to reduce the vessels contribution in the functional maps as well. During activation, CBV increases leading to an increase in the content of MION in a voxel, and hence to a negative signal change. Countering this signal decrease is the BOLD contrast, which induces a positive signal change during activation. Therefore, the two mechanisms will compete. The effect is heavily spatially-dependent. The larger BOLD effect is observed around large vessels, mainly on the surface region, leading to a better cancellation of MION signals, while the tissue region is less affected by this phenomenon.

MION has been used successfully in animal models such as the mouse, rat, cat, and the monkey with very promising results. The advantages of the exogenous contrast agents should certainly be taken into account when high spatial and/or temporal fMRI studies are planned where CNR is a limiting factor. While exogenous CBV-weighted contrast agents exhibit obvious benefits over the currently available hemodynamic-based fMRI contrasts (e.g., BOLD, ASL) its use is currently limited to animal models due to the fact that the desired dose is not approved for human use.

Endogenous Contrast

In recent years, a number of noninvasive techniques for measuring CBV have been developed that use blood as an endogenous contrast agent. These methods are based on nulling the blood signal using inversion recovery techniques.

The vascular space occupancy (VASO) method (14) is based on eliminating the blood signal in a manner that is independent of blood oxygenation and flow. Since the T* of blood is independent of oxygenation, VASO aims to null out blood in all compartments of the microvasculature. The remaining VASO signal is composed of brain parenchyma and CSF. As CBV increases, the VASO signal should decrease as parenchyma and CSF is displaced by blood in a given voxel. As a consequence, changes in CBV can be assessed through changes in the remaining extravascular water signal.

Similar to the MION signal, during an increase in CBV the VASO signal shows an inverse correlation with the stimulus paradigm, e.g., decreased MR signal, consistent with local vasodilatation. Although the VASO signal provides an indication of the dynamics of CBV, it does not provide any quantitative measurement or absolute values of CBV changes.

Another approach was introduced by Stefanovic and Pike (80), who developed a new, noninvasive fMRI technique for direct quantification of venous CBV changes. Venous refsocusing for volume estimation (VERVE) isolates the deoxygenated blood signal by exploiting the dependence of the transverse relaxation rate in deoxygenated blood on the refocusing interval. In an fMRI study, an increase of 16 ± 2% of venous CBV was measured in visual cortex on healthy young adults (80).

In summary, there are several approaches that use exogenous and endogenous contrasts for measuring blood volume changes using MRI techniques. Potentially most promising are the development of the exogenous contrast agents that can yield, as in animal studies, significant improvements in both the CNR and the spatial specificity over the conventional BOLD contrast, and which can be used in humans. Development and refinement of these agents for human use and ultimately for clinical applications, will prove highly advantageous.

DIRECT NEURONAL CURRENT

The temporal and spatial resolution of BOLD and ASL fMRI is not only limited by the measurement technique but also by the coarseness of the hemodynamic response to the neuronal event. As discussed above, ongoing research is dedicated to bypass the limitations of the hemodynamics using clever experimental designs and analysis methods. In contrast, electroencephalogram (EEG) and magnetoencephalography (MEG) are more directly related to neuronal current but lack spatial accuracy and have their own methodological limitations. At present, no single method can be used to probe brain function with mm and msec accuracy and therefore, it is highly desired to measure neuronal currents directly with MRI (nc-MRI).

Recently, a few attempts have been made to measure nc-MRI on phantoms, cell cultures, healthy humans,
and epileptic patients (81–88). Time-varying currents inside the neurons and within the extracellular fluid induce small changes in magnetic field strength. If many neurons have the same orientation (such as parallel-oriented pyramidal cells) and fire coherently, then the concomitant magnetic and electric field changes can be measured noninvasively by MEG and EEG, respectively. The measurement squids are at a distance of 2 to 4 cm from the dipole source, and at this distance the magnetic field changes caused by evoked stimulus responses and spontaneous rhythmic activity (e.g., alpha oscillations) cover a range from $10^{-12}$ T to $10^{-13}$ T corresponding to an estimated dipole current of 10–100 nA. Proximal to the dipole source, the corresponding magnetic field strength is approximately $10^{-9}$ T.

As in other magnetic field disturbances in fMRI (e.g., deoxyhemoglobin in BOLD fMRI) this leads to changes in intensity due to altered relaxation time and to a phase shift between the undisturbed and disturbed regions of the probe. Simulations have shown that phase is more sensitive to neuronal currents than intensity with a CNR advantage of two orders of magnitude (85). The only question is: Is this effect big enough to be measurable with current technology?

To simulate neuronal firing, Bodurka et al (83) induced an electric current of different magnitudes in a wire in a water-filled container and measured phase changes using a GE sequence at 3 Tesla. They found that magnetic field strengths as small as $\Delta B_0 \sim 2 \times 10^{-9}$ T could be detected. This value is close to the aforementioned estimated magnetic field strength change occurring in the brain during evoked or spontaneous activity. In a follow-up study, Bodurka and Bandettini (82) compared the sensitivity of GE and SE sequences to detect the phase changes. In order to mimic physiological perturbations, e.g., respiration, a sinusoidal varying current at 0.28 Hz was superimposed on to the constant current with a CNR advantage of two orders of magnitude (85). The only question is: Is this effect big enough to be measurable with current technology?

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analysis method in order to make nc-MRI more reliable (see Ref. 86). Also crucial will be the correction method for physiological noise, which was not employed in the aforementioned studies.

**TARGETED EXOGENOUS CONTRAST AGENTS FOR FUNCTIONAL IMAGING**

Advances in MRI using endogenous contrast provided by spin density, and/or relaxation times $T_1$, $T_2$, and $T_2^*$ revolutionized the way in which cellular and physiological processes are being investigated. Functional imaging methods such as BOLD or ASL are all based on endogenous contrast. Exogenous MRI contrast agents are also utilized. To date, they have played a critical role in clinical diagnosis; such clinical applications have been based on a limited number of compounds (e.g., gadolinium chelates) with rather nonspecific mechanisms for biological processes. Susceptibility contrast agents, like iron oxide particles, have been clearly useful in CBV-based MRI. Again, the mechanism is rather nonspecific (i.e., an alteration in the susceptibility within blood vessels).

Recently, however, heightened interest and efforts can be noted in developing “targeted” MR contrast agents with specificity to biological events, such as the expression of β-galactosidase activity or intracellular Ca$^{2+}$ concentration (see Ref. 91 and references therein). Early agents that were developed for these purposes have utilized Gd$^{3+}$ complexes in which the access of water molecules to the paramagnetic gadolinium ion is modulated by the targeted chemistry. Recently, although still exploratory, another class of agents, called chemical exchange saturation transfer (CEST) or paramagnetic CEST (PARACEST) (92–96) have also been introduced; these agents rely on hydrogen that exchanges with bulk water but has a distinctly different chemical shift than water. With such an agent, the intensity of bulk water can be modulated by saturating the exchanging hydrogen, resulting in a contrast agent that can be turned on and off with the application of RF pulses.

A tantalizingly exciting prospect is to have such specifically-targeted agents employed in functional imaging. In principle, calcium agents can serve this purpose given the role played by calcium in a neurotransmitter release. This approach would overcome issues about specificity of secondary signals such as BOLD, and provide temporal resolution lacking in current MR methods of functional imaging. Other agents that aim for sensitivity to pH, glucose levels, and lactate concentrations are also potential candidates for functional imaging.

Clearly, such agents, when developed and used for neuroimaging, will remain in the domain of animal experiments in the foreseeable future. Human applications face the formidable task of delivery across the blood brain barrier. Intracellular delivery, if the agent is sensitive to intracellular processes, and toxicity. Nevertheless, it is clear that significant efforts will be spent in realizing this goal.

**MAPPING OXYGEN CONSUMPTION CHANGES DIRECTLY BY MR**

In the brain, the majority of energy consumption occurs by neuronal activity. This energy need is met predominantly through oxygen consumption mediated by the mitochondrial respiratory chain, which ultimately catalyzes the chemical reaction:

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O.$$  \hspace{1cm} (1)

The energy available from this reaction is subsequently utilized to generate the high-energy phosphorous metabolite, adenosine triphosphate.

Recent work (Refs. 97 and 98 and references therein) has demonstrated that the cerebral oxygen consumption needed to produce water can be quantitatively measured by using inhalation of oxygen gas enriched with the isotope $^{17}$O, which exhibits a nuclear magnetic resonance. The $^{17}$O gas is not easily detectable by MR but $H_2^{17}$O is. The time course of the buildup of $H_2^{17}$O signals in the brain during inhalation of $^{17}$O enriched gas is complex, and depends on the cerebral oxygen consumption rate (CMRO$_2$), the rate of $H_2^{17}$O washout from the brain and direct arterial supply due to recirculation and $H_2^{17}$O production in other aerobic organs such as the heart. Despite this complexity, however, recent experiments performed with detailed measurements of all parameters involved in this process confirmed that CMRO$_2$ dominates the process for inhalation times on the order of minutes, and that the $^{17}$O MR approach can measure the cerebral oxygen consumption rate accurately (Refs. 97 and 98 and references therein).

CMRO$_2$ is expected to change with neuronal activity, albeit the fractional change in CMRO$_2$ is less than the fractional change in CBF. These stimulus or task induced alterations in CMRO$_2$ can be expected to be spatially specific relative to the site of neuronal activity. Thus, in principle $^{17}$O MR imaging is potentially an accurate and quantitative methodology for functional imaging. Preliminary functional images obtained with $^{17}$O MR exist, and have been presented at conferences, although to date they remain unpublished.

The major drawback in this approach is the inherently low SNR of the low gyromagnetic ratio of the $^{17}$O nucleus and currently the exorbitant cost of $^{17}$O enriched gas. The SNR handicap can be alleviuated by the use of ultrahigh magnetic fields where the $^{17}$O SNR improves approximately quadratically with magnetic field (99). With the availability of 9.4-T systems for human imaging and the impending availability of 16.4-T systems for animal imaging, it is likely that this approach will be utilized for quantitative measurements of oxygen metabolism and functional mapping.

In conclusion, since its introduction, our understanding of MR-detectable functional signals in the brain has improved significantly. This is complemented by numerous developments in instrumentation, such as the introduction of ultrahigh fields, and methodologies that also substantially improve data quality. Some of these developments have not yet been incorporated
REFERENCES


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