Neuroimaging With Calibrated fMRI
Fahmeed Hyder, PhD

Abstract—The conventional functional MRI (fMRI) map offers information indirectly about localized changes in neuronal activity because it reflects changes in blood oxygenation, not actual neuronal activity. To provide a neurophysiological basis of fMRI, researchers have used electrophysiology to show correlations of fMRI and electric signals. However, quantitative interpretation of the degree to which neuronal activity has changed still cannot be made from conventional fMRI data. The fMRI signal has 2 parts: one describes the correlation between oxidative metabolism (cerebral metabolic rate of oxygen [CMRO2]) and cerebral blood flow (CBF), which supports the bioelectric work to sustain neuronal excitability; the other is the requisite dilation of blood vessels (cerebral blood volume [CBV]), which is the mechanical response involved in removal of waste while providing nutrients. Since changes in energy metabolism are related to bioelectric work, we tested whether spiking frequency of a neuronal ensemble (ν) is reflected by local energy metabolism (CMRO2) in rat brain. We used extracellular recordings to measure ∆ν/ν and calibrated fMRI (ie, using fMRI signal, CBF, and CBV maps) to measure ∆CMRO2/CMRO2 during sensory stimulation. We found that ∆CMRO2/CMRO2 is ≈ ∆ν/ν, which suggests efficient energy use during brain work. Thus, calibrated fMRI provides data on where and by how much the neuronal activity has changed. Possibilities of utilizing calibrated fMRI as a neuroimaging method are discussed. (Stroke. 2004;35[suppl 1]:2635-2641.)

Key Words: ATP ■ blood flow ■ energy metabolism ■ magnetic resonance spectroscopy ■ neurons ■ oxygen

Physiology of Neuroimaging
Recent electrophysiological studies1,2 suggest that collaboration among a large number of neurons within a functional unit or column, some of which are firing more quickly and others more slowly, is crucial for signaling in the brain. The ideal way to probe the activity of neuronal ensembles, however, is to measure electric signals directly with microelectrodes. However, simultaneous measurement of many neurons with microelectrodes is impractical for a variety of reasons. While noninvasive neuroimaging methods such as positron emission tomography (PET)3 and functional MRI (fMRI)4 are more practical, to localize neuronal processes it is assumed that local activity of a neuronal ensemble changes when a region is responding to a task.

The brain is composed of a restless collection of cells incessantly working (ie, receiving and distributing signals) to preserve functional integrity. Glucose is the main carbon source for the neuronal and glial cells in the adult mammalian brain, and its oxidation produces most of the ATP needed to sustain normal function.5,7,8 Since modern-day neuroimaging methods (eg, H216O-PET and H218O-MRI) measure components of CBF (or CBV), the inherent assumption is that these maps reflect changes in neuronal energy and hence alterations in neuronal activity. The fMRI method falls into this category4 and can be explained by functional hyperemia7 as follows:

\[ \text{OGI} = \frac{\text{CMRO}_2}{\text{CMR}_{\text{glc}}} \]  

Since the measured OGI is between 5 and 6 under a wide range of physiological conditions,6 it is generally accepted that glucose oxidation provides energy exclusively for normal cellular work,5,7 in which the OGI is only slightly decreased with increased workload6,7 (Equation 1), eg, for sensory tasks.

Because the brain in the adult human has a very high energetic demand, approximately 10 times that expected simply on the basis of its weight,8 even transient limitations in oxygen supply can critically harm the function of neuronal and glial cells. Since the oxygen reserve of the mammalian brain is not very significant,5 continuous supply of blood (providing nutrients and removing waste) is necessary for normal function if activities of neurons and glia are to be tightly correlated with their energy use9 (Figure 1). Thus, cerebral blood flow (CBF) or cerebral blood volume (CBV) may provide changes in ongoing neuronal function indirectly.5,8 Since modern-day neuroimaging methods (eg, H215O-PET and H216O-MRI) measure components of CBF (or CBV), the inherent assumption is that these maps reflect changes in neuronal energy and hence alterations in neuronal activity. The fMRI method falls into this category4 and can be explained by functional hyperemia7 as follows:

\[ \frac{\text{CMR}_{\text{O}_2}}{\text{CBF}} = C_s - C_v \]
which suggests that oxygen molecules permeating across the blood-brain barrier are related to the arteriovenous concentration difference (ie, $C_a - C_v$) in a quantitative manner. Since arterial blood is fully oxygenated, the arteriovenous difference reflects the deoxyhemoglobin concentration in the capillary bed. In an activated region, the difference between $C_a$ and $C_v$ becomes smaller because $C_v$ increases as a result of functional hyperemia (ie, increased blood oxygenation), which in turn is consistent with a larger fractional increase in CBF than in CMRO$_2$ (Equation 2). Thus, a metabolically driven increase in local perfusion is consistent with a drop in deoxyhemoglobin.

Two prior independent observations$^{10,11}$ provide insights about Equation 2. Hill$^{10}$ showed that saturation of hemoglobin with oxygen changed the color of erythrocytes: the fully oxygenated form is bright red, whereas the fully deoxygenated form is nearly black. Penfield$^{11}$ demonstrated that arterial ends of cerebral capillaries were almost always red, whereas venous ends were purple and veins became redder when the local cellular workload was regionally increased. These observations provide a link between functional hyperemia and changes in local neuronal activity. In an activated region, the arteriovenous difference becomes smaller and can be decreased only if the oxygenation of the capillary bed is increased. This is consistent with a drop in deoxyhemoglobin at the venous end of the capillary bed. Since deoxyhemoglobin is paramagnetic and attenuates the fMRI signal ($S$),$^4$ a small decrease in venous deoxyhemoglobin therefore enhances $S$ by $\Delta S$. Therefore, a qualitative understanding of functional hyperemia suggests raised blood oxygenation with increased neuronal activity, and a larger increase in CBF than CMRO$_2$ would be expected for a positive fMRI signal change,$^4$ as shown in the following equation:

$$\frac{\Delta S}{S} = M \left( \frac{\Delta CBF}{CBF} - \frac{\Delta CMRO_2}{CMRO_2} \right) - N \left( \frac{\Delta CBV}{CBV} \right)$$

where $M$ and $N$ are measurable physiological and magnetic constants and $\Delta$CMRO$_2$/CMRO$_2$, $\Delta$CBF/CBF, $\Delta$CBV/CBV, and $\Delta S$/S are changes induced by a task.

Calibrating fMRI: Energy Metabolism, Blood Perfusion, and Cellular Activity

Since Equations 1 and 2 provide the physiological basis of modern-day neuroimaging techniques, a pertinent question is the manner in which changes in CMRO$_2$, CMR$_{glc}$, and CBF (during sensory stimulation) relate to each other. Meta-analysis of PET data from awake humans$^{12-23}$ for a wide range of conditions (Table) can help to resolve this issue. The relationships among $\Delta$CMRO$_2$/CMRO$_2$, $\Delta$CMR$_{glc}$/CMR$_{glc}$, and $\Delta$CBF/CBF have been controversial for more than a decade because some PET studies$^{14,16}$ had shown that $\Delta$CMR$_{glc}$/CMR$_{glc}$ was negligible in comparison to $\Delta$CBF/CBF or $\Delta$CMRO$_2$/CMRO$_2$. The main proposal from these PET studies$^{14,16}$ was that the energy-producing pathway of the brain switched from an efficient oxidative mode (during unstimulated conditions) to a less efficient nonoxidative mode (during stimulated conditions).

First, with regard to energetic concerns, there is only a minor difference between fractional changes in CMR$_{glc}$ and CMRO$_2$ (Table), which suggests that glucose oxidation is still the abundant source of energy during activation in the awake, nonanesthetized human brain.$^5-7$ While the small difference between $\Delta$CMR$_{glc}$/CMR$_{glc}$ and $\Delta$CMRO$_2$/CMRO$_2$ gathered from other laboratories (Figure 2) qualitatively supports the suggestion from the PET studies$^{14,16}$ that $\Delta$CMR$_{glc}$/CMR$_{glc}$ is $\geq$CMRO$_2$/CMRO$_2$, glucose oxidation still provides $>90\%$ of the ATP during activation at steady state,$^{24,25}$ and the excess lactate generated may be effluxed into the blood to be used elsewhere.$^6,25$

Second, for the physiological basis of fMRI, there is a positive difference between fractional changes in CBF and CMRO$_2$ (Table). The small difference between $\Delta$CBF/CBF and $\Delta$CMRO$_2$/CMRO$_2$ gathered from other laboratories (Figure 2) qualitatively supports the suggestion from the PET studies$^{14,16}$ that $\Delta$CBF is $\geq$CMRO$_2$/CMRO$_2$. However, the (CMRO$_2$/CMRO$_2$)/($\Delta$CBF/CBF) ratios in humans$^{26}$ and rats$^{27}$ are generally similar ($\approx 0.7$) and are significantly different from the ratio from the PET studies of Fox and Raichle$^{14}$ and Fox et al$^{16}$ ($\approx 0.1$). Thus, most of the CBF-
CMRO\textsubscript{2} data are not in agreement with predictions from diffusion-limited models of oxygen transport,\textsuperscript{28,29} which suggest that \( \Delta \text{CBF/CBF} \gg \Delta \text{CMRO}_{2}/\text{CMRO}_{2} \). Rather, models that reveal a more efficient oxygen transport process\textsuperscript{26,27} are in better agreement with most of the CBF-CMRO\textsubscript{2} data.

While cognitive neuroscientists have embraced the indirect fMRI method because of its ease in application, biomedical engineers have been exploring the neuronal basis of fMRI by combining electrophysiological methods. The correlations that have been observed between fMRI and electric signals are based on aspects of spatial localization and/or temporal dynamics.\textsuperscript{30–34} Despite the large disparities in the temporal dynamics as well as spatial responses of each signal type, at best these correlations are delicate because one still cannot quantitatively infer from the conventional fMRI data the degree by which the neuronal activity has changed within an activated focus.

A major effort in our laboratory is to derive the neurophysiological basis of fMRI, which has its roots in biophysics.\textsuperscript{7,35} We exploited the fact that fMRI has 2 parts (Equation 3): one supports the extensive bioelectric work (described by the CBF-CMRO\textsubscript{2} correlation) needed to sustain the excitability of neurons, and the other is the required vasodilation (portrayed by the CBF-CBV correlation), which is the mechanical response involved in removal/delivery of waste/nutrients.\textsuperscript{9} The fMRI signal was calibrated by MRI and MR spectroscopy (MRS) measurements\textsuperscript{36,37} of the 2 underlying relationships, which in turn allowed calculation of changes in CMRO\textsubscript{2} with the use of S, CBF, and CBV maps (Figure 3).

While details of the multimodal MRI methods are discussed elsewhere,\textsuperscript{24} the \(^{13}\text{C}\)-MRS method for measuring CMRO\textsubscript{2} of the abundant excitatory and inhibitory neurotransmitters,\textsuperscript{25} glutamate and \( \gamma \)-aminobutyric acid (GABA), is important to highlight because these measurements validate the calibrated fMRI method. Intravenous infusion of a substrate (eg, glucose, acetate, or \( \beta \)-hydroxy butyrate) enriched with the nonradioactive, stable \(^{13}\text{C}\) isotope results in time-dependent appearance of the \(^{13}\text{C}\) label in metabolite pools, thereby allowing flux quantification under steady state conditions.\textsuperscript{7} Time courses of \(^{13}\text{C}\) label flow into pools of glutamate, glutamine, and GABA have been the primary in vivo measurements.\textsuperscript{7,25} The time course of \(^{13}\text{C}\) turnover of glutamate-C4 can be converted into a measure of the neuronal tricarboxylic acid (TCA) cycle flux, and CMRO\textsubscript{2} may be determined from this flux because of established stoichiometry between reducing equivalents generated by the TCA cycle and oxidative phosphorylation.\textsuperscript{7,25} Since energy is defined as the capability of doing work in a nondissipating system,\textsuperscript{38} we inquired whether brain cells utilize energy by an amount equal to the degree of work done. We used extracellular recordings to measure the spiking frequency of a neuronal ensemble (\( v \)) as a reliable and quantifiable measure of brain work.\textsuperscript{39} Our results showed that \( \Delta \text{CMRO}_{2}/\text{CMRO}_{2} \) was approximately equal to \( \Delta v/v \). These results suggest that the neuronal energy expended is commensurate with the additional work being done in a localized region; furthermore, the calibrated fMRI method used to

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Changes in CMRO\textsubscript{2}, CMRglc, and CBF of awake, nonanesthetized human brain measured by PET in different laboratories for a range of sensory stimulations are shown. Numbers in italics represent averaged values. See Hyder\textsuperscript{24} and Rothman et al\textsuperscript{25} for details.

Figure 2. Meta-analysis of PET data. Stimulation-induced changes in CMRO\textsubscript{2}, CMRglc, and CBF in the awake human brain from PET studies are shown.\textsuperscript{12–23} Inset shows distribution of \( \Delta \text{CMRO}_{2}/\text{CMRO}_{2}, \Delta \text{CMRglc}/\text{CMRglc} \) and \( \Delta \text{CBF/CBF} \) values from the PET studies (Table); exact values and their implications are discussed elsewhere.\textsuperscript{24,25} The difference between changes in CMR\textsubscript{glc} and CMRO\textsubscript{2} (ie, \( \Delta \text{CMRglc}/\text{CMRglc}−\Delta \text{CMRO}_{2}/\text{CMRO}_{2} \) on the vertical axis) reflects the degree of glucose oxidation; the lower the difference is, the greater is the degree of glucose oxidation (Equation 1). The difference between fractional changes in CBF and CMRO\textsubscript{2} (ie, \( \Delta \text{CBF/CBF}−\Delta \text{CMRO}_{2}/\text{CMRO}_{2} \) on the horizontal axis) reflects the degree of blood oxygenation; the higher the difference is, the greater is the fMRI signal (Equations 2 and 3). The pseudolinear relationship suggests that the fMRI signal changes are commensurate with nearly complete oxidation of glucose during functional activation.\textsuperscript{24} Therefore, the negligible increase in CMRO\textsubscript{2} during functional activation\textsuperscript{14,16} is not in agreement with trends observed in literature. Reprinted with permission from Wiley, copyright 2004.\textsuperscript{24}
obtain CMRO2 maps provides a view of the ensemble work within a voxel.40

Materials and Methods

Animal Preparation

Artificially ventilated male Sprague-Dawley rats (Charles River Laboratories, Mass) were prepared for physiological monitoring and infusion of an MRI contrast agent to measure CBV changes.36,37 Intraperitoneal lines were used for administration of α-chloralose (anesthetic agent) and D-tubocurarine chloride (paralyzing agent). The anesthesia was first maintained at a low dose (condition I) followed by a higher dose (condition II) to achieve high and low basal activity levels, respectively, in the same rat.39 The contralateral forelimb was stimulated (2 mA; 0.3 ms; 3 Hz) with 2 copper electrodes.

CMRO2 Measurements

All MRI and MRS data were obtained on a modified 7-T horizontal-bore animal system that has provided high fMRI sensitivity for a variety of rodent models.41–44 A multimodal fMRI method45 was used to measure ΔS/S and ΔCBF/CBF under fully relaxed conditions in an interleaved manner. An MRI contrast agent was slowly infused after collection of ΔS/S and ΔCBF/CBF data in both conditions I and II; ΔCBV/CBV was measured in condition II, and in condition I ΔCBV/CBV was estimated from the ΔCBF/CBF.36 The multimodal data were then used to calculate ΔCMRO2/CMRO2 in layer 4 of the somatosensory cortex40 for conditions I and II46,37 with the use of Equation 3.

Extracellular Recordings

High-impedance microelectrodes were placed at a depth of layer 4 within the somatosensory cortex in the contralateral and ipsilateral limb region.46 High signal-to-noise ratio spikes from the ensemble around the electrode tip were identified by shape recognition.39 The spikes recognized in the first experimental run of condition I were used to process the data collected in all subsequent experimental runs (in the same rat). The data were then converted to reflect the relative spiking frequency (ν) of the neuronal ensemble.39

Changes in Energetics and Redeployment of Neuronal Activity

The stimulation-induced changes in CMRO2 and ν for the contralateral side were significant. No significant stimulation-induced changes in CMRO2 and ν were observed in the ipsilateral side. The stimulation increased CMRO2 by ~40% from the basal activity level I prestimulus condition. The prestimulus condition of basal activity level II was lowered by ~32% from the same condition of basal activity level I, whereas the stimulation increased CMRO2 by ~102% from the prestimulus condition of basal activity level II. Similar magnitudes of changes in ν were observed. In the prestimulus condition of both basal activity levels, stimulation produced approximately the same total magnitude of activity in both CMRO2 and ν, while the increments varied accordingly (Figure 4). Dose-dependent decrease of basal activity level CMRglc has been observed under halothane,47 α-chloralose,48 and phenobarbital49 anesthesia. Similarly, stimulation-induced increments in neuronal activity have been observed from different basal activity levels with anesthesia.50–52

In this study,39 predominantly pyramidal neurons were measured in each rat. Since >90% of all microelectrode penetrations yielded at least 2 discrete and recognizable neurons, multiple microelectrode measurements provided a reasonable estimate of the activity for the entire neuronal ensemble. The histograms of ν for all stimulated conditions approached similar distributions (Figure 5), suggesting that the redeployment of the spiking frequency of the
ensemble was nearly identical during forepaw stimulation with α-chloralose anesthesia. Although comparable behavior of neuronal ensembles in the cortex has been reported previously,33,34 the quantitative redeployment of the same ensemble for different sensory workloads (ie, stimuli) provides novel insights for understanding the relationship between neuronal activity and energetics.35,40,46

Relating the fMRI signal to neuronal activity requires associating the fMRI signal with localized energetics and connecting energetics to neuronal processes (eg, rates of neurotransmitter release and action potential propagation). Our fMRI calibration studies in rat brain36,37 developed the first relationship, in which we related the oxidative energy consumed (CMRO2) and neuronal activity have provided phenomenological connections at best.30–34 In contrast, the present studies examining the dependence of spiking frequency of an ensemble (ν) on the localized energy metabolism (CMRO2) in the anesthetized rat brain.39,40 We found that ΔCMRO2/CMRO2 ≈ Δν/ν from both baseline activity levels on stimulation.46 Previous correlations between changes in the fMRI and electric signals of neuronal activity have provided phenomenological connections at best.30–34 In contrast, the present studies demonstrate that the appropriate way of using fMRI for neuroimaging would be by way of mapping changes in energy (ΔCMRO2/CMRO2) with the use of calibrated fMRI36,37 because that parameter represents a biophysical representation of neuronal work (Δν/ν and ΔVcyc/Vcyc).24,46 Since the increment in energy expended is commensurate with the required work, it is suggested that neurons operate in an efficient manner in which most of the energy expended is used for the type of work that they were designed to perform (ie, receiving and distributing signals).

Acknowledgments
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References

Summary
Since the electric activities that we have measured arise from layer 4 neurons in response to specific anesthetics and stimuli, far more detailed characterization of sensory and delocalized inputs (from other cortical layers and regions) contributing to total activity is needed.46 Detailed studies of neuronal populations in nonhuman primate models may be needed for extensions to cognitive function in the awake, nonanesthetized human. Additional rat experiments are needed because the contributions of individual neurons in the “voting population” (ie, individual neurons contributing to the ensemble activity) need to be evaluated elaborately during stimulated and unstimulated conditions for different experimental settings and/or paradigms (eg, anesthetic, stimuli, cortical depth). By manipulation of these parameters in anesthetized rat experiments, we would be able to extend the current results so that the total measured activity can be represented in terms of sensory and delocalized signaling.

Do brain cells utilize energy by an amount equal to the degree of work done? If so, then energy can be defined as the capability of doing work, a fundamental biophysical principle.38 We measured both CMRO2 and ν were under high and low basal activity levels to determine whether the fractional increases on stimulation were equal or different.39,40 We found that ΔCMRO2/CMRO2 ≈ Δν/ν from both baseline activity levels on stimulation.46 Previous correlations between changes in the fMRI and electric signals of neuronal activity have provided phenomenological connections at best.30–34 In contrast, the present studies demonstrate that the appropriate way of using fMRI for neuroimaging would be by way of mapping changes in energy (ΔCMRO2/CMRO2) with the use of calibrated fMRI because that parameter represents a biophysical representation of neuronal work (Δν/ν and ΔVcyc/Vcyc).24,46 Since the increment in energy expended is commensurate with the required work, it is suggested that neurons operate in an efficient manner in which most of the energy expended is used for the type of work that they were designed to perform (ie, receiving and distributing signals).

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