Interpreting oxygenation-based neuroimaging signals: the importance and the challenge of understanding brain oxygen metabolism

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Functional magnetic resonance imaging is widely used to map patterns of brain activation based on blood oxygenation level dependent (BOLD) signal changes associated with changes in neural activity. However, because oxygenation changes depend on the relative changes in cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO₂), a quantitative interpretation of BOLD signals, and also other functional neuroimaging signals related to blood or tissue oxygenation, is fundamentally limited until we better understand brain oxygen metabolism and how it is related to blood flow. However, the positive side of the complexity of oxygenation signals is that when combined with dynamic CBF measurements they potentially provide the best tool currently available for investigating the dynamics of CMRO₂. This review focuses on the problem of interpreting oxygenation-based signals, the challenges involved in measuring CMRO₂ in general, and what is needed to put oxygenation-based estimates of CMRO₂ on a firm foundation. The importance of developing a solid theoretical framework is emphasized, both as an essential tool for analyzing oxygenation-based multimodal measurements, and also potentially as a way to better understand the physiological phenomena themselves. The existing data, integrated within a simple theoretical framework of O₂ transport, suggests the hypothesis that an important functional role of the mismatch of CBF and CMRO₂ changes with neural activation is to prevent a fall of tissue pO₂. Future directions for better understanding brain oxygen metabolism are discussed.

Keywords: cerebral metabolic rate of oxygen, cerebral blood flow, functional magnetic resonance imaging, blood oxygenation level dependent, tissue oxygenation, positron emission tomography

INTRODUCTION

A brief neural stimulus produces a rapid and robust increase of cerebral blood flow (CBF), as illustrated in Figure 1A. Although this phenomenon is the basis for most current functional neuroimaging methods, it is still not clear what physiological function this serves. The CBF response is quite strong, with the fractional CBF change typically ~2–4 times larger than the associated fractional change in the cerebral metabolic rate of oxygen (CMRO₂). A useful way of describing this effect is that the oxygen extraction fraction (OEF) – the fraction of delivered oxygen that is extracted from the blood and metabolized – decreases with increased neural activity. This counterintuitive result, first reported by Fox and Raichle (1986), is surprising because at rest CBF and CMRO₂ appear to be tightly coupled, with a relatively uniform OEF across the brain despite a wide regional variation of blood flow (Gusnard and Raichle, 2001). Fortunately for the field of functional neuroimaging, the increase of local blood oxygenation with activation produces a small increase of the signal measured with functional magnetic resonance imaging (fMRI) because of the magnetic properties of deoxyhemoglobin, with higher deoxyhemoglobin levels tending to reduce the MR signal. Decreased deoxyhemoglobin due to the drop in OEF with activation then leads to an increase of the MR signal, the blood oxygenation level dependent (BOLD) response (Kwong et al., 1992; Ogawa et al., 1992; Buxton, 2009) (Figure 1B). Other techniques, including optical methods (Dunn et al., 2005; Grant et al., 2009; Roche-Labarbe et al., 2010) and tissue oxygenation measurements in animal studies (Caesar et al., 2008; Pilgaard and Lauritzen, 2009; Thomsen et al., 2009), also exploit the change in blood or tissue oxygenation due to the mismatch of CBF and CMRO₂ changes as a biomarker of altered neural activity.

Because oxygenation changes depend on the relative changes in CBF and CMRO₂, a quantitative interpretation of BOLD signals, and also other functional neuroimaging signals related to blood or tissue oxygenation, is fundamentally limited until we better understand brain oxygen metabolism and how it is related to blood flow. While there has been a large research effort focused on the links between neural activity and blood flow, our understanding of oxygen metabolism changes is limited by the intrinsic difficulty of measuring CMRO₂. However, the complexity of oxygenation signals also has a positive side: the fact that oxygenation signals are sensitive to both CBF and CMRO₂ changes offers the possibility of estimating CMRO₂ changes when these measurements are combined with additional measurements of CBF. In fact, a quantitative interpretation of oxygenation-based signals offers the best hope we have for measuring dynamic changes in CMRO₂ (Herman et al., 2009). This is intrinsically a multimodal imaging approach, and a key required element to make it work is a theoretical framework that accurately relates our measured signals to the underlying physiology.
This review focuses on the problem of interpreting oxygenation-based signals, the challenges involved in measuring CMRO$_2$ in general, and what is needed to put oxygenation-based estimates of CMRO$_2$ on a firm foundation. The importance of developing a solid theoretical framework is emphasized, both as an essential tool for analyzing oxygenation-based multimodal measurements, and also potentially as a way to better understand the physiological phenomenon that lies at the heart of BOLD-fMRI: the mismatch of CBF and CMRO$_2$ changes with activation.

**THE PROBLEM: CAN WE INTERPRET OXYGENATION-BASED SIGNALS AS A QUANTITATIVE REFLECTION OF PHYSIOLOGICAL ACTIVITY?**

Functional MRI based on mapping the BOLD response has developed into a widely used and sensitive tool for mapping patterns of activation in the human brain. Sophisticated statistical methods have been developed to identify significant signal changes associated with a stimulus, and these detected changes are taken as evidence for a change in neural activity at that location (with the caveat that the changes in deoxyhemoglobin may be dominated by downstream venous vessels that could be displaced from the precise site of neural activity change). This robust mapping approach addresses the basic question: where does activity occur? However, an important challenge facing functional neuroimaging is to move beyond mapping to meaningfully address the question of quantifying the activity change. This is a distinctly different, and much harder, problem than simply identifying where a change has occurred.

The key question is: does the magnitude of the BOLD response quantitatively reflect the magnitude of the underlying physiological changes? There are two primary effects that could alter the BOLD signal even for the same underlying change in neural activity: the baseline CBF state, and the coupling of CBF and CMRO$_2$ changes. The BOLD response has a ceiling, corresponding to complete removal of deoxyhemoglobin, and so the amount of deoxyhemoglobin present in the baseline state sets the possible dynamic range for the BOLD response (Brown et al., 2003). This baseline effect could be an important confounding factor when studying disease populations, where the baseline CBF and CMRO$_2$ may be altered by medications or by chronic aspects of the disease process itself (Fleisher et al., 2009). The second effect, that has received less attention to date, is that the BOLD response is sensitive to the exact coupling of CBF and CMRO$_2$ (Ances et al., 2008; Herman et al., 2009). That is, methods sensitive to blood or tissue oxygenation depend on the relative balance of the changes in CBF and CMRO$_2$. Increased neural activity drives increases in both blood flow and oxygen metabolism, but these physiological changes drive blood oxygenation in opposite directions.

The magnitude of the BOLD response thus depends on the CBF/CMRO$_2$ coupling ratio $n$, defined as the ratio of the fractional change in CBF to the fractional change in CMRO$_2$ for a given stimulus. This would not be a problem for interpreting the BOLD response if $n$ is fixed. For example, a simple picture would be that increased neural activity requires increased energy metabolism, and the increased CMRO$_2$ then drives an increase in CBF in a fixed ratio. However, a large body of evidence supports an alternative view that aspects of neural signaling, such as neurotransmitter release, nitric oxide (NO) generation, extracellular potassium changes, and activation of arachidonic acid pathways, can either dilate or constrict the blood vessels (Attwell and Iadecola, 2002; Hamel, 2006). In particular, the astrocytes, with processes projecting to both neuronal synapses and blood vessels, are thought to play a key role in detecting synaptic activity and modulating blood flow (Iadecola and Nedergaard, 2007; Koehler et al., 2009). In short, it is likely that the rapid control of CBF is driven by fast feed-forward mechanisms related to the neural activity itself, rather than feedback mechanisms related to energy metabolism. In other words, it appears that CBF and CMRO$_2$ changes are driven in parallel by neural activity.

This raises the critical question: which aspects of neural activity drive CMRO$_2$, which drive CBF, and are they always in the same ratio? The energy cost of neural activity is primarily in restoring ionic gradients following neural signaling, fueled by increased ATP consumption, and the ATP is replenished by the oxidative metabolism of glucose (Attwell and Laughlin, 2001). The focus here is on O$_2$ metabolism, because CMRO$_2$ is the relevant metabolic rate that combines with CBF to alter blood and tissue oxygenation. Clearly, though, CMRO$_2$ requires glucose metabolism, because glucose is the substrate that is oxidized. Positron emission tomography (PET) studies typically find that the fractional change of the cerebral metabolic rate of glucose (CMRGlc) more closely follows the fractional CBF change and is larger than the fractional CMRO$_2$ change (Fox et al., 1988). However, the link between CBF and CMRGlc is not tight, in that CMRGlc changes occur even when the CBF response is blocked (Cholet et al., 1997), and a recent study found...
an uncoupling of CBF and CMRGlc related to inhibition (Devor et al., 2008). A larger fractional change in CMRGlc compared to CMRO₂, suggests that there is some degree of aerobic glycolysis and a corresponding generation of lactate despite an abundance of available oxygen (Pritchard et al., 1991). The term aerobic glycolysis emphasizes the distinction to anaerobic glycolysis, in which reduced O₂ availability prevents oxidative metabolism of the pyruvate created by glycolysis, and the pyruvate is subsequently converted to lactate. When oxygen is not available, glycolysis can provide a source of ATP. However, glycolysis alone generates less than 1/15 of the ATP generated by full oxidative metabolism, so the additional ATP generated by the aerobic glycolysis in the well-oxygenated healthy brain is a small fraction of the increased ATP generated by the smaller increase of oxidative metabolism. It is still unclear how aerobic glycolysis fits into the metabolic side of neural activation (Paulson et al., 2010), particularly because the contribution to energy metabolism is small. The rest of this review focuses on CMRO₂ with the assumption that it is not limited by the availability of glucose.

It is likely that CMRO₂ simply responds as needed to match ATP production to ATP consumption, so that the increase of CMRO₂ reflects the overall energy cost of the evoked neural activity. In contrast, if the CBF response is driven by feed-forward mechanisms related to the neural signaling itself, this opens the possibility that the coupling index n is not fixed. As discussed below, a number of studies suggest that n varies regionally and even within the same region as the stimulus changes. Typical values are in the range n = 2–4, and unfortunately this is a range in which the BOLD response magnitude is expected to be sensitive to the exact value of n. For example, theoretical calculations indicate that for the same CMRO₂ change (and by assumption the same overall evoked neural activity), the BOLD response magnitude differs by 100% for n = 2 compared with n = 3 (Ances et al., 2008).

THE CHALLENGING TASK OF MEASURING CMRO₂ MEASURING CMRO₂ WITH LABELED OXYGEN

In contrast to CMRO₂, there are relatively direct and robust ways to measure CBF and CMRGlc. For these measurements, techniques have been developed in which a single agent is injected, allowed to circulate the brain, and a single measurement of the concentration of the agent then provides a direct measure of the appropriate physiological quantity (CBF or CMRGlc). The accepted standard for CBF measurements is a microsphere experiment, in which labeled microspheres are injected arterially. Because the microspheres are too large to pass through the capillaries, they stick in the tissue, and the local concentration of microspheres then directly reflects the local CBF. With an appropriate measurement of the injected arterial bolus, the local CBF can be quantified in absolute units of ml blood/ml tissue/min. While microspheres are not used in human subjects, microsphere techniques provide the classic “gold standard” for validating other methods for measuring CBF. For human studies with MRI, pulsed arterial spin labeling (ASL) methods approach the ideal microsphere experiment because the delivery of magnetically labeled blood is measured only ~1.5 s after creation of the labeled blood in the arteries, so there is little time for the labeled blood to pass through the capillary bed and clear from tissue (Buxton, 2005).

For measuring CMRGlc there is also an accepted standard based on injection of radioactively labeled deoxyglucose, a chemically modified form of glucose (Sokoloff et al., 1977; Phelps et al., 1981). Deoxyglucose is taken up from the blood like glucose and binds to the enzyme hexokinase, the first step in the glycolysis pathway. However, because of the structural difference between glucose and deoxyglucose, the latter cannot proceed to the later stages of glycolysis, and essentially sticks in the tissue. By waiting sufficiently long for unmetabolized deoxyglucose to diffuse back into blood and clear from the tissue, the remaining radioactive label in tissue directly reflects the local rate of metabolism of deoxyglucose, and can be quantified by also measuring the arterial concentration curve of the agent during delivery. This approach is applied in humans with PET using deoxyglucose labeled with¹⁸F, a positron emitting radionucleide. As with microspheres for CBF measurement, the reason this technique works so well is that a single measurement of the tissue concentration of the agent directly reflects the metabolic rate.

Oxygen dynamics in the tissue can be measured by injecting labeled O₂, either with¹⁸O for PET detection (Mintun et al., 1984) or¹⁵O for NMR detection (Arai et al., 1990, 1991; Fiat and Kang, 1992, 1993). However, deriving CMRO₂ from such measurements is surprisingly difficult. The essential problem is that one cannot tell whether the detected oxygen nucleus is in O₂, and thus not metabolized, or in water, and thus metabolized. Imagine measuring tissue concentration as a function of time during and following an injection of labeled O₂. Initially, the labeled oxygen nucleus detected in the tissue will be in the form O₂. Because the solubility of O₂ in extravascular tissue is very low compared to blood due to hemoglobin binding, most of the labeled oxygen is in the blood compartment of the tissue, so that cerebral blood volume (CBV) strongly affects the measured concentration of labeled oxygen. Over time some of the initially delivered O₂ will diffuse back to the blood without being metabolized and clear from the tissue. Another component will enter the mitochondria and be converted to water by oxygen metabolism. The labeled water will eventually diffuse to the blood and clear from the tissue. However, as blood continues to circulate the labeled water will be delivered back to the tissue, at a rate that depends on CBF, so that now the labeled oxygen enters as water as well as O₂. In short, the kinetics of the labeled oxygen nucleus depends strongly on CBF and blood volume in addition to CMRO₂, and there is no point in time when the tissue concentration cleanly reflects CMRO₂ alone.

For this reason, the current standard method for measuring CMRO₂ with PET requires three separate measurements following injection of¹⁵O labeled O₂, water, and carbon monoxide (for blood volume measurement), and analysis of the dynamic tissue concentration curves within a theoretical framework that accounts for the combined effects of CMRO₂, CBF and CBV on the observed tracer kinetics (Mintun et al., 1984). In addition, temporal sampling of arterial blood is needed to measure the arterial time course of labeled O₂ and labeled water. Even with these combined measurements it is still necessary to assume that there is no back flux of unmetabolized O₂ from tissue to blood, specifically that all the O₂ that leaves the capillary is metabolized. For both PET and NMR studies a number of ideas have been proposed for simplifying these measurements, but each of these is sensitive to particular
assumptions of the model (Ohta et al., 1992; Zhu et al., 2002, 2005, 2006; Ibaraki et al., 2004; Zhang et al., 2004; Kudomi et al., 2007, 2009). In short, the complexity of these studies reflects the intrinsic difficulty of measuring local O\textsubscript{2} metabolism, and emphasizes the need for an accurate theoretical framework to integrate the different measurements. Furthermore, as with all studies using injected tracers, the assumption is that the physiology is in a steady-state throughout the measurement so that the observed dynamics of the agent reflect the dynamics of the injected bolus and its transport to tissue. For this reason the measurements of CMRO\textsubscript{2} are necessarily steady-state rather than dynamic. Despite the complexities of the technique, PET measurement of CMRO\textsubscript{2} is now being done at a number of institutions, although usually as a baseline measurement in different clinical conditions. Applications looking at brain activation in healthy subjects are less common (Mintun et al., 2002).

Another NMR approach for measuring CMRO\textsubscript{2}, exploits a particular magnetic property of \textsuperscript{17}O: when incorporated into a water molecule, it acts as a relaxation agent, altering the relaxation times of hydrogen nuclei. Furthermore, this effect can be modulated by applying a radiofrequency pulse at the \textsuperscript{17}O resonance frequency (Ronen and Navon, 1994; Ronen et al., 1997, 1998). In this way the presence of \textsuperscript{17}O labeled water can be detected with standard proton MRI as a change in relaxation rate. However, when the \textsuperscript{17}O is incorporated into an \textsuperscript{16}O molecule it does not have this relaxation property. This creates the interesting scenario in which \textsuperscript{17}O inhaled as O\textsubscript{2} is not visible with standard proton MRI until the \textsuperscript{17}O has been incorporated into water by O\textsubscript{2} metabolism in the mitochondria. The rate of appearance of labeled water is then proportional to local CMRO\textsubscript{2}. To quantify this, however, requires additional measurement of the arterial input function (concentration of the label in arterial blood), and accounting for recirculation of the labeled water. Despite early interest in this approach, it has not been widely used to date due to the complexity involved in addressing these issues (similar to issues in the PET studies described earlier), combined with the high cost of \textsuperscript{17}O, although a recent study has extended the method to larger animals (Mellon et al., 2009).

**MEASURING CMRO\textsubscript{2} FROM BLOOD OR TISSUE OXYGENATION**

The alternative to the more classical tracer kinetics approaches described above is to use blood or tissue oxygenation in combination with a measurement of CBF to estimate a change in CMRO\textsubscript{2}. This section focuses on the calibrated-BOLD approach, the primary method used in human studies, and tissue pO\textsubscript{2} measurements used in animal studies. These two approaches are primarily sensitive to changes in CMRO\textsubscript{2}. Although these methods are not as sensitive to the baseline CMRO\textsubscript{2} value, they provide the best opportunity available to measure the dynamics of CMRO\textsubscript{2}.

Before discussing these methods, though, it is important to note briefly that two recent alternative MRI approaches have been proposed that potentially could provide estimates of baseline absolute CMRO\textsubscript{2}. Both focus on the question of estimating the venous oxygenation through its effect on the transverse relaxation rate \(R_2\). With TRUST, the signal in a draining vein such as the sagittal sinus is isolated with an approach similar to ASL, and the transverse relaxation rate \(R_2\) is measured (Lu and Ge, 2008; Xu et al., 2009). From calibration studies in blood samples, \(R_2\) varies with O\textsubscript{2} saturation. Combining this estimate of venous O\textsubscript{2} concentration with a separate measurement of CBF then provides an estimate of baseline CMRO\textsubscript{2}. When this measurement is done in the sagittal sinus the result is a whole-brain CMRO\textsubscript{2} estimate, but if this technique can be adapted for regional measurements it will provide important information on the distribution of baseline CMRO\textsubscript{2}. Even as a whole-brain measurement, baseline venous oxygenation can account for a significant amount of the variance of the BOLD response between subjects (Lu et al., 2010). The second method exploits a detailed model of how \(R_2\) changes with time as the MR signal decays, with the essential assumption that the additional signal decay in a gradient echo measurement compared with a spin echo measurement is entirely due to the effects of deoxyhemoglobin (Yablonskiy and Haacke, 1994; He and Yablonskiy, 2007; He et al., 2008). With additional assumptions about properties of the tissue compartments, this approach gave estimates of baseline O\textsubscript{2} extraction fraction that are in good agreement with PET measurements. As these methods mature they may provide an important complement to the dynamic methods discussed below by providing the link between fractional and absolute changes in CMRO\textsubscript{2}.

An essential component of BOLD and tissue pO\textsubscript{2} methods is a theoretical framework that accurately relates CBF and CMRO\textsubscript{2} changes to the measured oxygenation signal. Modeling these physiological variables, in terms of how they relate to each other and in terms of how they relate to the imaging signals, is an active area of research (Valabregue et al., 2003; Buxton et al., 2004; Gjedde, 2005a,b; Huppert et al., 2007, 2009; Uludag et al., 2009; Zheng et al., 2010). To clarify how such a theoretical framework can be used to estimate CMRO\textsubscript{2} changes, we focus here on the simple theoretical framework defined in **Table 1**, which relates, CBF, CMRO\textsubscript{2}, the BOLD signal, and tissue pO\textsubscript{2}. In this framework, changes in CBF and CMRO\textsubscript{2} are related to the BOLD response by Eqs 1,2, and to the tissue pO\textsubscript{2} response by Eqs 1, 3, and 4. In this framework, Eq. 1 is the most solid, essentially just reflecting mass balance. The product of flow and arterial O\textsubscript{2} concentration is the rate of delivery of O\textsubscript{2} to the capillary bed, and multiplying by the fraction of delivered O\textsubscript{2} that is metabolized gives CMRO\textsubscript{2}. The remaining equations, however, represent specific models.

**Modeling the BOLD response**

Equation 2 is a model for the BOLD response proposed by Davis et al. (1998), based on physical reasoning and Monte Carlo simulations. This is a simple model of a complex phenomenon, and yet this model has proven to be more robust than one might have thought given the restrictive assumptions that went into its derivation. Although originally derived just from consideration of extravascular signal changes, it captures the basic behavior of more detailed models that include additional effects (discussed below). The model reflects the primary dependence of the BOLD signal on changes in the O\textsubscript{2} extraction fraction, but also includes an additional term in \(f\) that is intended to model the effect of blood volume changes on the BOLD signal as a power law relation with CBF, with an empirical exponent \(\alpha = 0.4\) (Grubb et al., 1974). The role of blood volume changes in modulating the observed BOLD response is somewhat subtle, and we still do not have a satisfactory understanding. In principle, blood volume changes could influence the BOLD response by changing the local total deoxyhemoglobin in tissue independently of a change in blood oxygenation. That is, blood oxygenation determines the
Table 1 | Simple theoretical framework for estimating CMRO₂ from multimodal measurements.

Theoretical Framework

| VARIABLES | Description |
|-----------|-------------|
| \( f \) | CBF normalized to baseline |
| \( r \) | CMRO₂ normalized to baseline |
| \( s \) | BOLD signal normalized to baseline |
| \( E \) | \( O_2 \) extraction fraction |
| \( p_c \) | mean blood \( pO_2 \) (mmHg) |
| \( p_t \) | mean tissue \( pO_2 \) (mmHg) |

REQUIRED BASELINE PARAMETERS (TYPICAL VALUES)

| VAriABLES | Value |
|-----------|-------|
| \( E_o \) | 0.4 |
| \( p_{O_2} \) | 25 mmHg |

MODEL PARAMETERS

| Parameter | Description |
|-----------|-------------|
| \( \alpha \) | 0.4 (blood volume effects) |
| \( \beta \) | 1.5 (intravascular signal changes, diffusion) |
| \( M \) | local scaling factor, determined by calibration |
| \( p_{O_2} \) | 26 mmHg (\( O_2 \)-hemoglobin 50% saturation) |
| \( h \) | 2.8 (Hill exponent) |

MODEL EQUATIONS

1. Mass balance:
   \[ r = \frac{E}{E_o} f \]

2. BOLD signal (Davis et al., 1998):
   \[ s = M \left[ 1 - f \left( \frac{E}{E_o} \right)^h \right] \]

3. Blood/tissue \( O_2 \) gradient:
   \[ r = \frac{p_c - p_t}{p_{O_2} - p_{O_2}} \]

4. Mean blood \( pO_2 \) (Gjedde, 2005a,b):
   \[ p_t = p_{O_2} \left( \frac{2}{E} \right)^h \]

The equations describe: (1) Mass balance for \( O_2 \), expressed in terms of variables normalized to their baseline values; (2) Davis model for the BOLD signal, with the parameter \( f \) describing effects of volume changes, and \( \beta \) approximating differential diffusion effects around large and small vessels estimated from Monte Carlo simulations; (3) Assumption that the tissue/blood \( pO_2 \) gradient increases to match a change in CMRO₂, and that this gradient is determined solely by the difference of the mean \( O_2 \) concentrations in blood and tissue (no capillary recruitment); and (4) Expression for mean blood \( pO_2 \), assuming the Hill equation for the \( O_2 \)-hemoglobin saturation curve, with exponent \( h \) and half-saturation at a \( pO_2 \) of \( p_{O_2} \), and assuming that the \( pO_2 \) corresponding to half of the total extracted \( O_2 \) is the mean value for blood.

Deoxyhemoglobin concentration in blood, but total deoxyhemoglobin also depends on the volume of the blood compartments that contain partially deoxygenated blood. This is usually thought of as “venous” blood, but really also includes capillary and to some extent arteriolar blood, because it is thought that arteriolar blood is already slightly deoxygenated. Blood volume changes also can affect the measured BOLD response by a pure exchange of volumes, an effect that is not related to blood oxygenation. For example, a blood volume increase on the arterial side should have little effect on total deoxyhemoglobin, but if the increased blood volume occurs at the expense of a reduced extravascular volume, then the measured signal could change due to an intrinsic signal difference between the blood and the extravascular material that is displaced. This basic effect of deoxyhemoglobin is not purely oxygenation-dependent.

The BOLD signal model in Eq. 2 contains two additional parameters, \( \beta \) and \( M \). The exponent \( \beta \) is derived from Monte Carlo simulations of spins diffusing around magnetized cylinders (Boxerman et al., 1995a), and essentially captures the idea that the same amount of deoxyhemoglobin has less of an effect on the BOLD signal change when it is in capillaries compared to when it is in larger veins (Ogawa et al., 1993). The source of this effect is the random movement of water molecules due to diffusion. It happens that the magnitude of motion of a water molecule during an experiment is comparable to the diameter of a capillary, so near the smallest vessels each molecule will sample a range of field offsets. Because all the water molecules are essentially sampling the same range of field offsets, the net phase dispersion when the net signal is measured is not as great as it would be if the molecules had not moved around. The net phase dispersion translates directly into the measured signal change, so the signal change is larger around larger vessels, where diffusion is less important because of the larger spatial scale of the field offsets. From numerical simulations, \( \beta = 1.5 \) approximately captures these effects for 1.5 T, decreasing to a value near 1 at high fields. Although this simple model ignores several effects, such as intravascular signal changes (Boxerman et al., 1995a) and volume exchange effects, it agrees reasonably well with models that explicitly include these effects (Buxton et al., 1998, 2004; Obata et al., 2004; Leontiev et al., 2007).

The parameter \( M \) is an overall scaling factor that essentially depends on the amount of deoxyhemoglobin present in the baseline state, as well as details of the image acquisition method such as the field strength and echo time (Davis et al., 1998; Buxton et al., 2004). This parameter sets the maximum possible BOLD signal amplitude for that region of brain, corresponding to complete removal of deoxyhemoglobin. That is, it is important to remember that the BOLD response has a ceiling, and that ceiling is reflected in \( M \). Unlike the parameters \( \alpha \) and \( \beta \), which are usually assumed to be constant, the parameter \( M \) must be measured for each brain location for the particular experimental acquisition. This is done in the calibrated-BOLD approach introduced by Davis et al. (1998) by also measuring the BOLD and CBF responses to breathing \( CO_2 \). The CBF response is measured with an ASL technique (Detre et al., 1992; Wong et al., 1997) and the essential idea is to exploit the fact that the ASL signal depends just on CBF changes, while the BOLD signal depends on CBF and CMRO₂ changes. Mild hypercapnia is thought to produce a large change in CBF without changing CMRO₂ (Sicard and Duong, 2005) (although there is still some controversy about this assumption; Zappe et al., 2008), and Eq. 2 is used to calculate \( M \) for a particular brain region with the assumption that \( r = 1 \). With \( M \) estimated, the measured CBF and BOLD responses to stimulus activation are used with Eq. 2 to derive an estimate of \( r \), the fractional change in CMRO₂.
A useful way to look at the role played by the BOLD model is that it allows us to draw contours of equal BOLD response in a plane defined by the fractional CMRO$_2$ change on the x-axis and the fractional CBF change on the y-axis. Figure 2A shows an example for $M = 0.08$ (i.e., an 8% ceiling on the BOLD response magnitude). If the value of $M$ is not known, these contours would reflect equal but unknown increments of the BOLD response. Note that the non-linearity of the BOLD signal model primarily affects the spacing of the contour lines, while each contour is reasonably linear. If we are willing to assume that Eq. 2 holds for dynamic as well as steady-state changes (more on this question below), a hypothetical trajectory of CBF and CMRO$_2$ responses to a brief stimulus can be plotted in this plane along with the resulting BOLD response. An example is shown in Figure 2. Both CBF and CMRO$_2$ are assumed to exhibit a smoothed response, with two features added to illustrate how transients of the BOLD response could occur. The first feature is that the CMRO$_2$ response has a slightly faster rise time than the CBF response, producing an initial dip of the BOLD response. The second feature is a slight undershoot of the CBF response after the stimulus before it returns to baseline, producing a more pronounced undershoot of the BOLD response. This is just a simulated example, but it illustrates that the modeling framework predicts that relatively subtle differences in the CBF and CMRO$_2$ responses can produce transient features of the BOLD response that are similar to those that often have been reported (Perthen et al., 2008).

**Modeling tissue pO$_2$ changes**

In addition to signals related to blood oxygenation there is growing interest in using signals related to tissue oxygenation in animal models, including traditional microelectrode methods as well as newer methods (Ndubuizu and LaManna, 2007). Interestingly, though, blood and tissue oxygenation signals are not equivalent, and the theoretical framework helps to clarify the differences. The simple theoretical framework for interpreting tissue pO$_2$ shown in Table 1 is adapted from the work of Gjedde (2005a), although framed in a slightly different way (discussed below). The model is based on three key assumptions: (1) With increased activity there is no capillary recruitment, so the vascular geometry stays the same. Increased CMRO$_2$ requires an increased gradient of O$_2$ concentration between equal time increments in the evolving CBF and CMRO$_2$ curves in (C). (D) The resulting BOLD and tissue pO$_2$ responses are shown for the CBF and CMRO$_2$ responses in (C). Note that in this example the CBF and CMRO$_2$ responses are approximately in the ratio 2:1 (i.e., $n \sim 2$), and this causes the trajectory to pass over and under the zero change contour of p$_T$O$_2$, leading to the complex dynamics in (D). In addition, the CBF response was constructed with a weak post-stimulus undershoot to illustrate that this can create a more pronounced undershoot in the BOLD signal. In this example the ratio of the undershoot to the peak response is only $\sim 8\%$ for CBF, but $\sim 30\%$ for the BOLD response.
blood and tissue, and if the vessel geometry is fixed this increased gradient must be due to an increased difference between blood and tissue pO$_2$ values, leading to Eq. 3. (2) The O$_2$ saturation Y of hemoglobin as a function of pO$_2$ is described by the Hill equation with exponent $h$ and half-saturation value $p_{50}$, the pO$_2$ at which hemoglobin is 50% saturated: $Y = 1/[1 + (p_{50}/pO_2)^h]$. The values for $h$ and $p_{50}$ for human blood in Table 1 are taken from Mintun et al. (2001), but Gjedde (2005a) has suggested using higher values of $p_{50} = 35$ mmHg and $h = 3.5$ to approximate the Bohr effect. (3) The mean blood pO$_2$ driving diffusion into the tissue is the value derived from the Hill equation when the total O$_2$ concentration is halfway to the full extraction fraction from the blood given by $E$. Equations 1, 3, and 4 effectively provide two relationships between the four physiological variables CBF, CMRO$_2$, E, and tissue pO$_2$. In this way measuring any two of these variables allows one to calculate the remaining two from the equations. Specifically, measuring tissue pO$_2$ and CBF allows calculation of CMRO$_2$ and $E$.

At first glance the formulation in Table 1 may seem surprising, because it does not include any of the microscopic parameters that are usually introduced in analyzing O$_2$ diffusion into tissue (Gjedde, 2005a; Caesar et al., 2008). For example, the classic Krogh cylinder model is framed in terms of parameters such as the O$_2$ diffusion constant, capillary radius and capillary spacing (Mintun et al., 2001). Instead, the goal here was to define the framework in terms of macroscopic variables, with introduction of as few microscopic variables as possible, because the microscopic parameters may not be well known. For example, the exact value of the O$_2$ diffusivity does not enter the equations because it is assumed to be constant and effectively cancels out from Eq. 3. Instead of referencing the model parameters to microscopic parameters, the model is anchored by specifying baseline values of the macroscopic variables. In this case, one must specify $E$ and tissue pO$_2$ in the baseline state, and combined with Eq. 3 this gives the baseline mean blood pO$_2$. The key difference between the current formulation, and the previous formulation used to interpret pO$_2$ measurements (Caesar et al., 2008; Pilgaard and Lauritzen, 2009; Thomsen et al., 2009), is that this approach allows one to relate fractional changes in CMRO$_2$ to pO$_2$ without assuming values for microscopic variables, while the previous formulation focused on relating pO$_2$ to absolute CMRO$_2$, which requires accurate knowledge of microscopic O$_2$ diffusivity.

In short, the formulation in Table 1 exploits the general phenomenon of levels of description: not all of the variables that need to be specified in a microscopic modeling framework will carry through to affect macroscopic parameters. By anchoring the model to assumptions about the baseline values of the macroscopic parameters we essentially fine-tune the relevant microscopic parameters to produce that baseline state, without ever introducing those microscopic parameters explicitly. Importantly, this approach also effectively allows the model to be adjusted to the human brain without having to assume that microscopic parameters measured in animal models carry through precisely to humans. In this case, there are a number of PET measurements of $E$ in human subjects that suggest a baseline value of 0.4 is reasonable. For baseline tissue pO$_2$, there is considerable variability in the tissue (discussed further below), and the “typical” value given in Table 1 is consistent with recent measurements in animal models (Ances et al., 2001; Thompson et al., 2005).

The tissue pO$_2$ model allows us to draw contours of equal tissue pO$_2$ in the plane defined by the fractional changes in CMRO$_2$ and CBF (Figure 2B). One clear feature of these contour lines is that they are distinctly different from the contours of the BOLD response. Specifically, the line of constant BOLD signal and the line of constant tissue pO$_2$ have different slopes. This means that there is a range of possible CBF and CMRO$_2$ responses, lying between the two zero change contour lines, where the BOLD response and the tissue pO$_2$ response will have opposite signs (a negative tissue pO$_2$ response occurring with a positive BOLD response). Again assuming that this model accurately describes dynamic changes as well as steady-state changes, the predicted variation of tissue pO$_2$ for the hypothetical CBF and CMRO$_2$ responses is shown in Figure 2D. Note that this response is quite complicated, with a more pronounced initial dip compared with the BOLD response, and a positive response that only begins after the end of the stimulus. This complex response shows features similar to the interesting variety of tissue pO$_2$ responses that have recently been reported for different stimuli in animal models, including relatively simple positive responses to a strong stimulus (Ances et al., 2001), a pronounced initial dip (Offenhauser et al., 2005) and complex wiggles and slow post-stimulus undershoots (Thompson et al., 2003, 2004). The source of the complexity of the pO$_2$ response is that the simulated CBF and CMRO$_2$ responses have a ratio of about 2:1, and this means that the trajectory of the response passes back and forth across the zero contour of the tissue pO$_2$ response. This is consistent with the pO$_2$ measurements of Thompson and colleagues, in which all of the pO$_2$ fluctuations they reported were less than ~5% of the baseline value. The potentially important implications of this are discussed further below.

**IMPROVING AND VALIDATING THE THEORETICAL FRAMEWORK**

**Validating the accuracy of the models**

The discussion above illustrates that an appropriate modeling framework can play two important roles. First, it provides a way to combine multimodal measurements (in this case CBF and BOLD responses, or CBF and tissue pO$_2$ responses) to make a quantitative estimate of the CMRO$_2$ response. Second, it provides a framework for exploring how different responses (e.g., BOLD and tissue pO$_2$) will co-vary or diverge under different conditions. In particular, the theoretical framework brings out significant quantitative differences in the BOLD and tissue pO$_2$ responses that are not intuitively obvious. Examples are the predictions that: (1) a slight post-stimulus undershoot of CBF can produce a much more pronounced undershoot of the BOLD response; (2) there are possible changes in which the BOLD and tissue pO$_2$ responses will have opposite sign; and (3) the tissue pO$_2$ response can be significantly more complicated than the underlying CBF and CMRO$_2$ responses or the accompanying BOLD response. However, the theoretical framework outlined here is really just the starting point, and a number of possible limitations have to be tested, and the theoretical framework revised accordingly.

The BOLD signal model has several potentially important limitations. Although it was argued above that the current model is more robust than one might conclude from the restrictive assumptions involved in its derivation, this needs to be further tested. In particular, the most important aspect to nail down is the role...
played by blood volume changes (at all levels of the vascular tree) on the observed BOLD response. In applying the Davis model the exponent $\alpha = 0.4$ is almost always used, with the assumption that this captures the volume change of blood compartments containing deoxyhemoglobin (primarily venous). However, this value originally came from whole-brain measurements of total blood volume changes in an animal model. A more recent study using PET methods in human subjects found larger values (as high as $\alpha = 0.64$) (Rostrup et al., 2005), and a recent study in humans with the VASO technique also found values as high as $\alpha \sim 0.6$ (Lin et al., 2008, 2009). It should be noted, though, that the VASO technique measures an absolute total blood volume change with activation, and a baseline value for blood volume must be assumed to estimate $\alpha$. However, these methods measure total blood volume changes, and the primary quantity we need for interpreting the BOLD response is the venous volume change. A recent study using a novel method specifically sensitive to the venous blood found $\alpha \sim 0.2$ (Chen and Pike, 2009a), smaller than the previously reported values for total blood volume but still greater than 0.

A number of investigators are attacking the question of determining the volume changes in different vascular compartments with a variety of techniques in animal models. Two photon microscopy studies, which allow direct visualization of the vessels and accurate measurement of vessel diameters in the upper 0.5 mm of cortex in rats and mice, have found that the volume changes are primarily on the arterial side, with relatively small changes in venous blood volume (Hillman et al., 2007). In addition, a number of MRI techniques have been developed to estimate volume changes in different vascular compartments, but it remains a challenging task to clearly isolate specific compartments from each other and from confounding flow effects. Several of these studies in animal models also found that most of the blood volume change is on the arterial side (Kim and Kim, 2006; Kim et al., 2007). More studies are clearly needed to clarify how blood volume changes with activation, but a working model based on these results is that most of the change is arterial, with smaller fractional changes in the venous and possibly the capillary compartments.

As noted above, the parameter $\beta$ was initially introduced to approximate the net effect of smaller and larger vessels in describing the extravascular BOLD effect. It turns out fortuitously that this form also gives a reasonable description of the BOLD response when intravascular signal changes and volume exchange effects are included (Buxton et al., 2004; Leontiev et al., 2007). For this reason it may be better to look at $\beta$ as a parameter that captures multiple effects, and no longer think of it in terms of the original derivation. A more detailed model of the BOLD response including multiple vascular compartments and effects could be used to estimate the value of $\beta$ that maximizes the accuracy of the Davis model.

Although the concerns about which values of $\alpha$ and $\beta$ should be used need to be addressed, it turns out that estimates of fractional CMRO$_2$ change in a calibrated-BOLD experiment are relatively robust to uncertainties in $\alpha$ and $\beta$. The primary reason for this is that if the same model is used to analyze both the hypercapnia experiment and the activation experiment, the calculation is partially self-correcting (Davis et al., 1998; Ances et al., 2008). However, this robustness is based on the assumption that the relationship between volume changes and flow just depends on the magnitude of the flow change, and not on the details of how the flow change is evoked. A key question that needs to be addressed is whether this relationship is the same for CBF changes induced by hypercapnia and neural activity.

In contrast to $\alpha$ and $\beta$, the value of $M$ is critical. In general, the Davis model is robust because many of the physiological variables that could have a strong effect on the BOLD response magnitude are essentially lumped into the parameter $M$. This includes physiological parameters, such as baseline venous blood volume and hematocrit, but also aspects of the image acquisition, such as echo time and field strength, and even the way that a region of interest is selected for averaging. For this reason, the value of $M$ must be measured in each experiment, and a dangerous pitfall is to assume a value of $M$ reported in the literature and apply it to data collected in a different way (Chiarelli et al., 2007b; Leontiev et al., 2007; Lin et al., 2008).

The tissue pO$_2$ model also has a number of simplifying assumptions that need to be tested. Clearly this model ignores many of the complexities of O$_2$ transport in blood, such as a shift of p$_{50}$ as CO$_2$ diffuses into the blood, although these may be approximated by an appropriate choice of p$_{50}$ and the Hill coefficient $h$ (Gjedde, 2005a). Perhaps the most important question, though, is: Can the macroscopic effects be described in terms of the average pO$_2$ values in blood and tissue? That is, even for the simplest microscopic model, the Krogh cylinder, blood and tissue pO$_2$ values have a large spatial variability (Mintun et al., 2001), and experimental measurements show a wide range of pO$_2$ values, raising the question of whether it is even meaningful to talk about a mean tissue pO$_2$ (Ndubuizu and ManNan, 2007). Note, though, that the relevant question is not whether pO$_2$ is uniform in tissue, which would be a possible assumption that could be used to derive Eq. 3. Instead, the question is whether a much more detailed microscopic description leads to predictions for the macroscopic variables that differ significantly from the predictions of the simpler macroscopic models. A promising avenue for addressing questions like these is to construct detailed models of the vascular tree from animal imaging data and use such realistic geometries to calculate oxygen transport (Fang et al., 2008). These simulations also should include known effects of blood transport, such as the shift of p$_{50}$ and so need to model CO$_2$ production and clearance simultaneously with O$_2$ transport and consumption. In addition, such simulations could also test assumptions of the PET models used for estimation of CMRO$_2$, particularly the significance of the assumption of zero back flux of unmetabolized O$_2$ from the tissue. While such modeling is computationally demanding, it is not out of reach, and recent studies are encouraging.

**Dynamic responses**

One of the exciting aspects of oxygenation-based methods, compared with tracer methods, is the possibility of measuring dynamic CMRO$_2$ changes. However, the theoretical framework described in Table 1 is based on steady-state relationships, and it is an open question whether these equations are accurate for dynamic studies. For example, Eq. 1 is intrinsically a steady-state equation because the net extraction fraction is the result of the full passage of the blood through the vascular bed, which requires a finite time. To deal with this issue, more detailed models are required that carefully take into account the dynamics of different compartments,
or explicitly consider an extended vascular network (Boas et al., 2008). While such models may be too complex for routine use, they would provide a valuable test-bed for validating simpler models, such as the framework described here, and guide the development of more accurate macroscopic models that capture the dynamics. For these reasons, the dynamic curves in Figure 2 should be considered as suggestive of what the dynamics might look like, because they were calculated by assuming that the steady-state relationships apply at each time point, and this needs to be tested with the more detailed models.

In addition to these basic questions regarding the modeling, it is still an open question whether there are transient physiological changes that are not captured by the simple theoretical framework. There is a reasonable consensus that the initial dip of the BOLD response reflects a faster rise of CMRO₂ compared to CBF (Malonek and Grinvald, 1996; Devor et al., 2003), and the current framework captures that possibility (see Figure 2). However, there is no consensus on whether the post-stimulus BOLD undershoot is a neural, vascular or metabolic effect. Intriguing recent evidence suggests a neuronal modulation of the undershoot (Sadaghiani et al., 2009), but it has more commonly been hypothesized to be a biomechanical (Buxton et al., 1998; Mandeville et al., 1999; Chen and Pike, 2009b) or metabolic effect (Frahm et al., 1996, 2008; Lu et al., 2004). If the undershoot is due to a mismatch of CBF and CMRO₂, then the current framework will capture the effect. For example, the undershoot could be due to a slow recovery of CMRO₂, combined with a fast recovery of CBF, or a fast recovery of CMRO₂ combined with an undershoot of CBF (the latter possibility is illustrated in Figure 2). However, another possibility is that transients of blood volume and blood flow are different, so that blood volume recovers slowly even though CBF and CMRO₂, and thus intrinsic blood oxygenation, have returned to baseline. This effect is not captured by the current theoretical framework, because Eq. 2 assumes a fixed relationship between blood flow and blood volume given by a power law. For this reason, it is important to determine empirically whether this effect occurs.

Early MRI studies using MION, a magnetic agent that labels the blood plasma, found a slow recovery of blood volume with a duration that matched the BOLD post-stimulus undershoot and was longer than the recovery time of CBF (Mandeville et al., 1998). This observation prompted two similar models – the balloon model (Buxton et al., 1998) and the delayed compliance model (Mandeville et al., 1999) – hypothesizing that a slow recovery of venous blood volume, elevating total deoxyhemoglobin even though blood oxygenation had returned to baseline, could account for the BOLD post-stimulus undershoot. Later studies with MION and higher spatial resolution found that the slow recovery of blood volume was primarily in the middle layers of cortex (Yacoub et al., 2006; Zhao et al., 2007). Interestingly, though, the BOLD undershoot was present in the upper layers as well as the middle layers. This suggests that while a slow venous volume recovery could be a contributing factor, particularly in the middle layers, it cannot be a general explanation. The presence of a BOLD undershoot in the upper layers without a slow blood volume recovery suggests that there is also an alteration of blood oxygenation, presumably due to a mismatch of CBF and CMRO₂ in the post-stimulus period (Yacoub et al., 2006). In contrast to the MION studies, though, other studies using different techniques have not found evidence for a slow recovery of blood volume (Lu et al., 2004; Hillman et al., 2007; Frahm et al., 2008). The BOLD post-stimulus undershoot in the absence of a slow venous recovery could be due a slow recovery of CMRO₂, as noted above, although there is no direct evidence for this. Instead, this explanation remains when the possibilities of a slow blood volume recovery or a CBF undershoot are excluded. However, Figure 2 suggests that a weak undershoot of CBF would be sufficient to explain a more substantial BOLD undershoot, and in practice it may be difficult to exclude such a possibility because of the noise in the measurements. In short, an important physiological question that needs to be resolved in establishing a theoretical framework is whether blood volume changes are being accurately modeled.

**CURRENT EXPERIMENTAL MEASUREMENTS OF THE COUPLING OF CBF AND CMRO₂**

In addition to PET studies of the coupling of CBF and CMRO₂ (Fox and Raichle, 1986; Fox et al., 1988; Marret and Gjedde, 1997; Ito et al., 2005), several groups have adopted the calibrated-BOLD approach and reported values of the CBF/CMRO₂ coupling index n in the range of about 2–4 (Davis et al., 1998; Hoge et al., 1999; Kim et al., 1999; Kastrup et al., 2002; St Lawrence et al., 2003; Stefanovic et al., 2004, 2005; Uludag and Buxton, 2004; Fujita et al., 2006; Chiarelli et al., 2007a; Leontiev and Buxton, 2007; Leontiev et al., 2007; Ances et al., 2008, 2009; Perthen et al., 2008; Restom et al., 2008; Lin et al., 2009). Other studies have used the framework of the calibrated-BOLD approach to argue that CBF and CMRO₂ are coupled in a similar way in deactivations and activations (Shmuel et al., 2002; Stefanovic et al., 2004, 2005; Uludag et al., 2004; Pasley et al., 2007).

Figure 3 shows a sampling of current data in human subjects using either PET or calibrated-BOLD techniques. The plotted points are representative values from the studies, and the symbols used reflect the brain area and the technique. Also plotted are lines of constant values of n, the ratio of the CBF and CMRO₂ changes. Note that the word “coupling” is used here simply to describe the empirical ratio n, and does not necessarily imply a direct mechanical link between CBF and CMRO₂. Instead, current ideas point to CBF and CMRO₂ being driven in parallel by neural activity. For reference to Figure 2, the contour predicted for constant tissue pO₂ also is plotted.

There is a considerable spread of the coupling ratios, with most measurements lying in the range n ∼ 2–4. The calibrated-BOLD measurements show the highest changes in CBF and CMRO₂, and this could be due to reduced partial volume averaging of gray matter signals with the improved spatial resolution of MRI compared with PET. Overall, though, there does not appear to be a strong difference in the coupling ratio n between calibrated-BOLD and PET measurements. The original observation of the CBF/CMRO₂ mismatch by Fox and Raichle is one of the largest values of n found (∼6), but later PET studies found n ∼ 2 in other brain regions. Undoubtedly, some (perhaps most) of the variance in these data is due to the different methods used. However, there is a trend in this data for motor/somatosensory data to have the highest values of n, basal ganglia to have the lowest values, and visual areas to lie in the middle.
In short, the variability of $n$ poses a significant problem for the interpretation of BOLD signals. Specifically, the relative value of BOLD responses cannot be taken as a quantitative reflection of the relative values of the underlying physiological changes. Variability of $n$ is consistent with the current idea that neural activity drives CBF and CMRO$_2$ in parallel, and emphasizes the critical importance of understanding exactly which aspects of neural activity drive CBF and which create a demand for higher CMRO$_2$.

**Open Questions**

The phenomenon that a brief neural stimulus produces a robust, but delayed, increase of CBF (Figure 1) lies at the heart of many functional neuroimaging techniques, and yet we still do not have a clear picture of the mechanisms involved, or even of the physiological function served by this response: why is it so large, why is it so fast, and does it have anything to do with oxygen metabolism? The experimental data, integrated within the theoretical framework, may provide a possible explanation. These questions are considered in this section, emphasizing the idea that tissue pO$_2$ may be the key to understanding CBF/CMRO$_2$ coupling.

**Why is the CBF Change So Large?**

As noted above, recent studies of the tissue pO$_2$ response with neural activation have found interestingly complex wiggles. However, stepping back from these detailed fluctuations, a remarkable finding is that the fluctuations are relatively small. For example, with a visual stimulus in a cat model Thompson et al. (2003, 2004) found pO$_2$ fluctuations that were all less than about 5% of the baseline pO$_2$. Other studies with electrical stimulation in rats have found larger variations of $\sim$20% (Ances et al., 2001; Offenhauser et al., 2005),
but conceivably the stimuli used were exceptionally strong compared to normal physiological stimuli. In parallel to these animal studies, the PET and calibrated-BOLD studies shown in Figure 3 indicate that the CBF/CMRO₂ coupling ratio $n$ is ~2–4. The theoretical framework in Table 1 shows that these different experimental results are consistent with a simple picture: the observed CBF/CMRO₂ coupling ratio is approximately what is required to maintain a constant tissue pO₂ (the contour of constant pO₂ is plotted in Figure 3). Furthermore, the theoretical pO₂ curve in Figure 2 illustrates that complex fluctuations of tissue pO₂, but of small magnitude, can occur as the dynamic curves of CBF and CMRO₂ response pass over and under the contour of constant pO₂ in the CBF/CMRO₂ plane.

This observation suggests the hypothesis that the existence of the BOLD effect — the apparent mismatch of CBF and CMRO₂ changes — could be the result of a homeostatic mechanism acting to prevent a fall of tissue pO₂ despite changes in the metabolic rate. Based on the theoretical framework in Table 1, a CMRO₂ increase of 20% with no change in CBF would lead to a 30% drop of tissue pO₂, while a CBF change ~2.5 times larger than the CMRO₂ change would preserve tissue pO₂ ~25 mmHg. An earlier idea for why $n$ is large was that O₂ delivery is limited at baseline (Gjedde et al., 1991; Buxton and Frank, 1997), motivated by the previous assumption of the PET methods that the pO₂ in tissue is near 0 so that back flux of O₂ from tissue to blood is negligible (Mintun et al., 1984). In that case, because tissue pO₂ cannot drop, and because there is no capillary recruitment, the only way to increase the O₂ gradient from blood to tissue is to increase the blood O₂ by decreasing E. Valabregue et al. (2003) pointed out that relaxing the assumption of zero tissue pO₂ gave more flexibility for CBF and CMRO₂ to vary, and the pO₂ measurements cited above support the idea that tissue pO₂ is reasonably high. The current argument is in a sense an extension of the original oxygen limitation hypothesis, but now in terms of a non-zero tissue pO₂ that is prevented from dropping lower, rather than a zero tissue pO₂ that cannot drop. The required value of $n$ needed to maintain tissue pO₂ decreases for higher values of baseline tissue pO₂.

WHY IS THE CBF CHANGE SO FAST?

The argument above addresses the question of why $n$ is large, but does not necessarily address why the CBF response is so fast. The dynamic example in Figure 2 essentially assumes that the time constants for adjustments of blood and tissue oxygenation are on the order of 1 s, and this needs to be tested with more detailed models, as discussed above. As a start in this direction, it is useful to estimate the time constants for different aspects of energy metabolism as a guide to whether these components would require a fast CBF response, and whether they could potentially provide a rapid signal to drive the CBF response. It’s important to note that these time scales do not describe how quickly the glucose or O₂ metabolic rates could change. Both metabolic rates are likely to be able to change on a very short time scale with no changes in the concentrations of glucose or oxygen, through changes in the activity of rate-limiting enzymes. Instead, these time constants reflect the speed with which an abrupt change in metabolic rate would translate into a change in concentration of the metabolic substrates or products.

One clear function of blood flow is to deliver the substrates of energy metabolism, glucose and oxygen, and clear the waste products, carbon dioxide and heat. Metabolic rates are usually expressed as moles per minute per gram of tissue, but it is useful to consider these rates referenced to a unit volume of tissue, rather than a unit mass, and the metabolic rate is then a concentration divided by time (mM/min). In the adult human brain, a typical glucose metabolic rate is about 0.3 mM/min (Chugani et al., 1987), and a typical concentration of glucose in brain is about 2 mM at normal plasma glucose levels (Gruetter et al., 1998). The time constant for depleting the glucose is thus on the order of several minutes, suggesting that the regulation of blood flow on a time scale of seconds is not required to support glucose metabolism. Furthermore, if the O₂ supply was somehow abruptly stopped but CMRGlC continued at the normal rate, lactate would accumulate at a rate of about 0.6 mM/min, so a change of lactate concentration of only 1 mM would take on the order of 90 s to grow.

The CMRO₂ is higher, about 1.6 mM/min (Ito et al., 2004), and the tissue concentration of O₂ is lower. If the tissue pO₂ is ~25 mmHg, a solubility of 0.0013 mM/mM Hg gives an extravascular O₂ concentration of only ~0.03 mM. Arterial blood has an O₂ concentration of about 9 mM, so for a typical tissue blood volume fraction of 5% and a roughly 70% average hemoglobin saturation the intravascular O₂ content in tissue is about 0.26 mM. The total O₂ concentration in an element of tissue then is about 0.3 mM, with most of that in the blood compartment. The time constant for depleting the available O₂ is then on the order of 10 s, much shorter than the equivalent time for depletion of glucose. Excluding the O₂ in blood within a tissue element, the O₂ concentration associated with baseline tissue pO₂ would be depleted in ~1 s at the baseline metabolic rate, providing some support for applying the steady-state theoretical framework to dynamic changes.

Blood flow also carries away CO₂ and heat. Because CO₂ rapidly equilibrates with bicarbonate ions, the total pool of CO₂ in the brain is quite large, ~25 mM. The CO₂ production rate is close to the O₂ consumption rate, ~1.6 mM/min, so the time constant for changing total CO₂ is on the order of 10 min. Conceivably, though, pH changes could begin to become important on a shorter time scale, and a possible role for CO₂ in the post-stimulus undershoot has recently been proposed (Yücel et al., 2009). If the heat produced by the metabolic reactions is not dissipated, the temperature of the brain could rise to a level that could impair brain function. About 440 kJ of heat is released per mole of O₂ metabolized, and the heat capacity of water is ~4.2 J/g°C (Yablonskiy et al., 2000). If the heat of metabolism was not cleared, the temperature would rise only ~0.1°C in 40 s. Thus, while clearance of heat is an important function, it does not appear that this requires the fast CBF response observed with neural stimulation.

In short, if energy metabolism increased without a change in CBF, the time scales for significant changes in the concentrations of glucose, lactate, and CO₂, as well as the temperature rise due to added heat, appear to be too long to require a fast CBF response, or to provide the signal for increasing CBF. On the other hand, the time scale for changes in pO₂ is comparable, although perhaps a bit longer, than the observed CBF response, suggesting that a fast CBF response is necessary to prevent a drop of tissue pO₂.
WHAT DRIVES THE CBF CHANGE?

The arguments above raise two key questions: why does the brain need to maintain tissue $pO_2$, and what mechanisms could be involved in maintaining it? At first glance, it appears that the normal $pO_2$ level (~25 mmHg) is much higher than it needs to be, given that the effective $K_m$ for cytochrome oxidase, the $O_2$ concentration where $O_2$ availability limits $CMRO_2$, is below 1 mmHg (Gnaiger et al., 1998). That is, it appears that $CMRO_2$ should not be affected by transient dips in tissue $pO_2$. However, there are two potential reasons why a higher $pO_2$ may be important for healthy brain function. The first is that there are many other reactions for which $O_2$ is a substrate, and these have much larger $K_m$ values than cytochrome oxidase (Vanderkooi et al., 1991; Erecinska and Silver, 2001). For these reactions, fluctuations of tissue $pO_2$ could affect the reaction rate. A second possible reason why maintaining a high tissue $pO_2$ may be important is related to the thermodynamics of energy metabolism. The Gibbs free energy ($\Delta G$) available from oxidative metabolism of glucose depends on the $O_2$ concentration. This $\Delta G$ must be sufficiently negative to offset the strong positive $\Delta G$ required to produce ATP from ADP and Pi. If the $\Delta G$ of oxidative metabolism is reduced because of low tissue $pO_2$, there will be a point where $CMRO_2$ cannot proceed unless the $\Delta G$ related to producing ATP also is reduced. This would be a reduction of the phosphorylation potential, the ratio [ATP]/[ADP]Pi. This phenomenon was observed in cell cultures, where even though the rate of $O_2$ metabolism could be maintained down to very low levels of $pO_2$, the phosphorylation potential began to decrease at much higher levels (Wilson et al., 1979).

In the last decade there has been a surge of research on the mechanisms by which cells sense their oxygen level and respond with a wide range of physiological changes, particularly the cascade of events that follows stabilization of hypoxia-inducible factor-1 (HIF-1, Semenza, 2009). While often considered in the context of responses to hypoxia, it is interesting to note that some of the intermediate reactions are sensitive to the $pO_2$ at higher levels (Ward, 2008), and recent work suggests that these mechanisms may play a role in normoxia as well as hypoxia (Semenza, 2009). While it is conceivable that a $pO_2$ feedback system may be involved in driving the acute CBF changes with activation, it is not clear that these mechanisms could operate effectively on the time scale of a few seconds. Such mechanisms, like other metabolic influences, may operate on a longer time scale to match CBF with energy demands. For this reason, the immediate trigger for the CBF increase most likely operates in a feed-forward fashion, with aspects of neural activity triggering an initial response that is then modulated by slower feedback responses. A great deal of experimental evidence has supported a role for astrocytes in modulating CBF, acting as both an integrator of local synaptic activity and an effector of blood flow change (Iadecola and Nedergaard, 2007). However, it is still not clear whether astrocyte responses are fast enough to drive CBF changes on a time scale of seconds, as the time scale observed for vascular responses in slice preparations is much longer than what is observed in awake humans (Koehler et al., 2009).

One mechanism that has been observed to respond quickly enough to drive the CBF response is the production of NO (Buerk et al., 2003). While NO is produced by vascular and endothelial mechanisms, it also is produced by neurons. Interestingly, though, the neurons that produce NO have been isolated to a class of inhibitory GABA-ergic interneurons (Estrada and DeFelipe, 1998). This is an example of a mechanism that has an inhibitory effect on the neuronal population, and yet also increases blood flow. Adenosine is another prime example, reducing neuronal excitability while also acting as a vasodilator (Dirnagl et al., 1994; Haydon and Carmignoto, 2006). At first glance, this counterintuitive behavior is puzzling if the goal is to match blood flow to the level of neural activity. However, this combined effect makes sense as a protection against a potentially dangerous drop in tissue $pO_2$ by both increasing delivery and decreasing demand for $O_2$. Both effects would serve to raise tissue $pO_2$. This is consistent with the idea of oxygen conformance, that a primary defense of an organism when faced with hypoxia is to reduce the ATP consumption and $O_2$ metabolic rate (Hochachka et al., 1996).

The existence of mechanisms that drive neural activity down while increasing CBF suggests the possibility that a greater involvement of inhibitory activity could be associated with larger values of $n$. However, we should be cautious in interpreting these studies: the identification of a particular mechanism in simple preparations does not necessarily mean these mechanisms are dominant or even invoked in vivo. For example, interneurons also release neuropeptide Y (Abounader and Hamel, 1997), a vasoconstrictor, and astrocytes can trigger both vessel dilation and constriction (Zonta et al., 2003; Mulligan and MacVicar, 2004; Blanco et al., 2008), and we have no clear picture of how these different potential mechanisms are integrated into a responsive system in vivo. Nevertheless, the possibility of significant variations in CBF/$CMRO_2$ coupling related to inhibition is a potentially fruitful direction for future research.

In summary, the data and the theoretical modeling suggest the hypothesis that the underlying function served by $n \sim 2–3$ is to approximately maintain tissue $pO_2$ at a constant level. However, feed-forward mechanisms driven by the neural activity itself, such as NO production, are likely required to achieve the fast CBF response necessary to prevent a fall in $pO_2$. Furthermore, the specific interplay of different aspects of the evoked activity, such as the degree of evoked inhibitory activity, could alter the CBF/$CMRO_2$ coupling ratio. This speculative scenario needs to be tested with detailed studies in animal models of the specific aspects of neural activity that drive CBF changes, and how these relate to the overall energy demands of the full evoked activity. Finally, it is interesting to note that the hypothesis that tissue $pO_2$ is approximately conserved during neural activation is consistent with a broader evolutionary hypothesis. Baseline $pO_2$ levels in tissue are similar to what is thought to have been the atmospheric level of $O_2$ two billion years ago, when the machinery of oxidative metabolism evolved. It has been hypothesized that organisms try to preserve this level of $O_2$ in tissues as a balance between too little, limiting $O_2$ metabolism, and too much leading to increased generation of reactive oxygen species and potential cellular damage (Massabuau, 2003).

CONCLUSIONS

The basic picture of blood flow and oxygen metabolism being driven in parallel by neural activity – rather than a serial connection with neural activity driving energy metabolism which then drives blood flow changes – creates a significant challenge for interpreting many brain imaging signals in a quantitative way. The central
problem is that methods sensitive to blood or tissue oxygenation depend on the relative balance of the changes in blood flow and oxygen metabolism. For this reason, any quantitative interpretation of oxygenation signals such as the BOLD response in terms of the underlying physiological changes is problematic without a better understanding of O₂ metabolism. Because blood flow and glucose metabolism are easier to measure, there has been much more work done relating neural activity to CBF and CMRO₂. However, the positive side of the complexity of oxygenation signals is that they potentially provide the best methods we have for studying the dynamics of CMRO₂ when combined with separate CBF measurements. To make this a reality, though, requires a validated theoretical framework that accurately describes how oxygenation signals (such as BOLD or tissue pO₂ responses) depend on the changes in CBF and CMRO₂. The current framework may be sufficient, but this needs to be tested with experimental measurements and more detailed modeling. This goal is within reach, but will require a focused research effort.

In addition, an accurate theoretical framework allows us to explore the physiological consequences of different hypotheses. For example, the existing data integrated within the simple theoretical framework described here suggests the hypothesis that an important functional role of the mismatch of CBF and CMRO₂ changes with neural activation is to prevent a fall of tissue pO₂. This hypothesis would explain why the CBF response is as fast and as large as it is, and accounts for the complexity of the observed dynamics of pO₂ when dynamic CBF and CMRO₂ responses are not perfectly balanced. However, many basic questions remain unresolved, particularly the mechanisms that control CBF changes in the healthy, awake human brain and, most importantly, how these mechanisms may be altered in disease. Progress in answering these questions will lay a foundation for using oxygenation-dependent neuroimaging signals as a quantitative probe of brain function, rather than simply a mapping tool.

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