Cerebral Hemodynamics and Oxidative Metabolism
Dynamics Observed by Calibration of Functional MRI

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by

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Abstract

Task activation of brain cellular activity causes stimulus-dependent focal increases in perfusion out of proportion to oxidative metabolism increases, leading to the blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) brain mapping signal. The physiological basis for BOLD time course and magnitude in primary visual cortex was established by comparing BOLD and perfusion-sensitive responses for matched activation paradigms. Development of a novel integrated model, incorporating susceptibility contrast biophysical and oxygen transport principles, allowed prediction of transient fMRI responses based on the underlying time-varying physiology. Coupled measurements of BOLD and perfusion during transient and extended-duration visual stimulation demonstrated rapid changes in physiological parameters which remained tightly coupled to the stimuli over time.

MRI was extended to the measurement of changes in oxidative metabolism in the normal human during functionally-induced changes in cellular activity. A new method that is noninvasive and model independent calibrates the BOLD signal against perfusion-sensitive MRI, utilizing carbon dioxide breathing as a physiological reference standard. This calibration procedure provides a regional measurement of the expected sensitivity of the fMRI BOLD signal to changes in the cellular activity of the brain. Maps of the BOLD signal calibration factor showed regional heterogeneity, indicating that the magnitude of functionally-induced changes in the BOLD signal will be dependent upon both the local change in blood flow as well as the local baseline physiology of the cerebral cortex. BOLD signal is shown to be reduced by 32% from its expected level by the action of oxygen metabolism.

The calibrated fMRI technique was applied to stimulation of the human visual cortex with an alternating radial checkerboard pattern. With this stimulus oxygen consumption increased 16±1% whereas perfusion increased 45±4%. This finding indicates that oxidative metabolism is a significant component of the metabolic response of the brain to functionally-induced changes in cellular activity. Application of the model-based analysis to the human has clarified the physiological significance of transient functional MRI signals and has allowed new measurements of the metabolic changes which accompany neuronal activity.

Thesis Supervisor: Bruce R. Rosen, M.D., Ph.D.
Associate Professor of Radiology, Harvard Medical School
Director, Radiological Sciences Program, Massachusetts Institute of Technology
Co-Director, Massachusetts General Hospital NMR Center
to

Dana

my soul mate

mother of joy, my balance

I love the world because you loved me

first
Preface

*Wildcats!!!*

That was what my late grandfather John Lloyd Davis said nightly to himself as a child in the early years of this century, in order to get himself worked up enough to mount his high bed with a great running leap. It seems to help me too: here I am! Thanks, Grampa, for those stories on your knee.

As a man, I am naive enough to imagine that delivering a finished dissertation is not unlike delivering a baby. At least on the surface, all of the predictable attributes are present: desire, anticipation, testing, advice from specialists, excitement, abstinence, inklings of life, the first kick, nesting and pre-due-date hyperactivity. As is common, especially with first pregnancies, the first due date passes serenely by, and everyone becomes more tense as the baby becomes heavier and harder to bear. Eventually the inevitable arrives. Family and friends gather to fondle and coddle the wrinkled and battered skin, to glimpse the opening of big round eyes, to hear the feeble newborn cry, and of course to heap congratulations and observations of beauty, strength, and intelligence on the seemingly perfect baby. A new life has begun.

Such is the birth of this dissertation. Fortunately, I have not been alone. I thank God daily for his presence, and for the wonderful gift of family. Without you, Dana, I may have lost faith. Without you, Elizabeth and Andrew, I would not have been grounded. Without you, Carol and LeRoy, I might not be smiling. Without you, Mom, Dad, Martha, and Brad, I would never have explored my dreams. By encouraging me to dream and teaching me to pray, you gave me the keys to fulfillment. This dissertation stands as a testament to patience, to forgiveness, to charity, to kindness, and to sacrifice, by those who continue to stand beside me and who daily enable me to live out my dreams. I hope that this rather self-centered educational exercise serves our common future, and I pray that it will inaugurate a new epoch in my life, a time of acting for the greater good before thinking of my own ends. You all deserve my best: you have given of yourselves so deeply.

The Massachusetts General Hospital NMR Center has been a fantastic place to learn. As the size and importance of the lab continues to increase, I continue to be impressed with the spirit of open helpful communication, the vibrant enthusiasm of its members, the general *esprit de corps* characterizing all interactions. I believe this to be the result of active planning at the very top. Tom Brady has the magic formula for success, an important part of which seems to be encouragement of individual accomplishment for all members of the group. I thank Tom for setting the stage, and for encouraging me out onto it.

In spite of its positive attributes, the lab would have been useless to me without the shining beacon of Bruce Rosen. Bruce is equally adept on the podium, behind closed doors with a graduate student, and at home with his family. I have felt fortunate that Bruce and I share many priorities, opinions, goals, and dreams. I still do not understand why
Bruce placed my education equal with his other important goals. He must really have believed in me. I can only hope to carry on his tradition in my regard for colleagues and students. There aren’t many people of whom one can see all sides and still be envious; Bruce Rosen is one of those people.

My scientific mentoring came as a powerful package: the Bruce Rosen / Robert Weissskoff double header. I remember many meetings of the three of us, Bruce galvanizing us with the big picture, Robert revving up his analytical horsepower, and both challenging me to pitch in. Lots of gesturing, seemingly new and crazy ideas, chalk flying, keys clacking, and me, occasionally riding the wave and interjecting comments, and occasionally hopelessly capsizing, all the while furiously taking mental notes. Through it all, it has been Robert’s sense of direction, his technical acumen, his singularly interesting questions, which provided substance within the form; the centerboard in the storm, if you will. Robert is the best I have ever seen at applying physics to real problems. When I was on top of my game, theory discussions with him made me believe in the future of scientific inquiry. Thinking with him makes me think better. I can only hope that some of his alacrity and wisdom have seeped through to my core, as otherwise thinking will be hard without him.

My thesis committee has been patient and dogged in its pursuit of my completion. My highest regard goes to Roger Mark, first my master’s thesis supervisor and now my Ph.D. committee chair. I chose him because I knew his word was true, and because I knew he would see me through. His example has been a fitting model for my intellectual and moral development. Rox Anderson and Mike Moskowitz brought specialist knowledge, which they shared freely, along with laboratory resources and personnel. I thank each of my committee members for their seemingly boundless generosity, and for their eagerness, receptiveness, and insight. As I attempted to teach them about my work, they taught me even more. To me the mark of a true scholar is the ability to grasp a new subject with ease, to quickly understand the central issues, and to ask the most important questions. Each of my committee members has met the task graciously and effectively. For that I thank them.

My many lab mates were a source of constant inspiration. I must specifically go out of my way to thank Ken Kwong and Peter Bandettini for their selfless friendship, and for teaching me both of their unique experimental approaches. Thanks to Anders Dale and Randy Buckner for late night discussions, and in advance for their promise of sushi after my defense! Thanks also to Randy for the use of his apartment during two weeks of thesis cranking. Thanks to Jack Belliveau for whispering the word in my ear that summer night. Your faith matters more than you know. Thanks to Van Wedeen for his fits of genius, and for convincing me that I am capable of similar fits, even if they are less flamboyant. Thanks to Bob McKinstry, Bernice Hoppel, Brigitte Poncelet, Alex Guimares, Jerry Boxerman, Tueng Shen, Iris Chen, Sekhar Ramanathan, and others who came before me, for showing me how to do it. There are so many names and so many moments of learning through my time at the MGH, I cannot give all their due memory.

Thanks to the support crew: Carol Barnstead, who kept Bruce dancing with me; Dee Dee Correia, who was always ready with the check book; Keiko Oh, who knows more about foreign relations than Madeline Albright; Mike Vevea, who at first just kept my bits in line but over time became a real friend; Terry Campbell, who can really make the magnet sing. I am indebted to Professor Martha Gray, who helped me to keep my cool as I found my way through a maze of twisty passages, all alike, at MIT and MGH.
Thanks also to those who were ever ready to remind me of real world applications and the need to find solutions to clinical problems in the laboratory frame of reference: Hans Breiter, Randy Benson, Greg Sorensen, Rees Cosgrove, and now Dan Kido, Mark Bahn, Marcus Raichle, Randy Buckner, and Colin Derdeyn. I want to give you all something we can use together.

As I am now writing this as correspondent from the Mallinckrodt Institute of Radiology at Washington University in St. Louis, it would be a mistake to forget the home office. I have had tremendous support from a new mentor, Marcus Raichle. I am flattered by Marc’s enthusiasm for my continued career, and trust that our friendship and his mentoring role will continue to grow. It is by Marc’s good graces that I am able to finish this dissertation at all. I must also thank Drs. Evens, Balfe, and Brown for allowing me the flexibility, within the confines of one of the best radiology residencies in the country, to combine research with clinical training, and for being patient as I learn to juggle careers.

I am leaving behind in Boston a long list of too many people whose advice, friendship, and support were indispensable. Parts of me will remain moored in the Charlestown Navy Yard and in Cambridgeport because of you. I think of Jack and Lisa, who with their two children remain in Arlington pursuing the Boston dream while many of their close friends have left. We wait for Gary and Stephanie to find a post closer to us, or vice-versa, so that we can help raise our godson. If the distance is only physical, why do our hearts ache?

Research training is expensive. I have accumulated over $140,000 in Ph.D. tuition charges alone. In terms of output, that’s almost $10,000 per coauthored paper, or $14,000 per formal talk, or $4300 per meeting abstract. Clearly generous agencies have been paying the bills. I am indebted to the United States Air Force Laboratory Graduate Fellowship program which, through mentor Dr. Sherwood Samn at the School of Aerospace Medicine in San Antonio, supported me for three years as this project began. I am also indebted to Sterling-Winthrop Pharmaceuticals, the Isadore and Bertha Gudelsky family foundation, and the Walter Hennesey foundation, for graduate fellowships, and to the International Society for Magnetic Resonance in Medicine for travel support. The MGH NMR Center has graciously supported me as well, through its own resources and those of the National Institutes of Health and the Whitaker Foundation. I am grateful to the MIT Division of Residence and Campus Activities, who employed Dana and me for seven years as graduate resident tutors. The built-in social environment, free baby-sitting, and support groups added measurably to our success, and enabled life without financial ruin.

Although it is clearly expensive, I maintain a belief in basic science and education as investments in our shared future. It is critical that we foster continued dialog with these and other funding resources in order to maintain their belief as well.

St. Louis, December 1997
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Chapter 1

Functional MRI as a window on physiology

Often, the less there is to justify a traditional custom, the harder it is to get rid of it.

– Mark Twain
The thesis that functional activity in the brain is directly related to alterations in cerebral metabolism and blood supply was presented by Roy and Sherrington over one hundred years ago [1]. This relationship has been firmly established [2, 3, 4], and used as the basis for radionuclide maps of activation-induced changes in cerebral blood flow (CBF) and volume (CBV), and cerebral metabolic rates for glucose (CMRglyc) and oxygen (CMRO2) by subtracting resting from stimulated states [5, 6]. In the last several years NMR techniques have been developed which are sensitive to changes in CBF, CBV, and blood oxygenation [7, 8, 9]. By analogy to previous positron emission tomographic (PET) results, hemodynamic measurements with functional magnetic resonance imaging (fMRI) can also be used to map changes in cerebral neuronal activity.

This introductory chapter first reviews the opportunities presented by functional MRI for the study of hemodynamics, metabolism, and brain activation. A discussion of the theoretical and methodological challenges to physiological interpretation is presented, followed by an overview of the dissertation.

1.1 Opportunities and significance for functional neuroimaging

Understanding the link between dynamic fMRI signals and their underlying physiological origins would provide a distinct window on physiology, with increased temporal and spatial resolution over PET measurements. Already functional MRI shows great promise for brain mapping techniques, by allowing for the first time single event detection using repeated trials in the same individual [10], enhanced spatial discrimination for mapping fine detail within primary and secondary visual processing regions [11], and allowing discrimination between response times, all of which would have been impractical using other techniques. The combination of brain mapping techniques with high resolution ana-
tomical imaging has also fostered a new method for brain localization based on the topography of the unfolded cortical surface [11], as opposed to three dimensional standardized coordinates referenced to a standard, abnormal, folded cortex [12].

These clear advances are hampered by the questionable interpretability of the most widely utilized functional brain mapping technique: so-called blood oxygenation level dependent (BOLD) MRI, which is sensitive to static inhomogeneities in the local magnetic field through an effect on transverse NMR magnetic coherence. Despite advances in magnetic susceptibility contrast biophysics [13, 14, 15, 16], the link between neuronal activation and BOLD signal remains dependent on physiological assumptions which may vary between subjects and between brain regions, and which govern the BOLD response magnitude and time course. Hundreds to thousands of BOLD experimental paradigms have been designed over the past half decade without a clear physiological basis for interpreting magnitude or regional specificity of signal changes.

It is the missing link of physiology between cellular activity of the brain and MRI phenomenology which is the topic of this dissertation. By harnessing the unprecedented spatio-temporal resolution of functional MRI for physiological measurements, long-standing controversies surrounding possible nonoxidative metabolic processing [17], and possible slow conversion to oxidative metabolism over time [18], may be seen in fresh light, and possibly be put to rest.

Features of the time course of BOLD signals, namely an early transient dip before the positive activation signal, and a variably present post-stimulation undershoot and recovery phase, become interpretable in the context of a dynamic metabolic model. Once explained, their purported utility for better localizing brain function can be better addressed.

The magnitude of BOLD signal differences between regions is not directly interpretable. Although preliminary reports showed correspondence between magnitudes in a
given region and previous measures of graded neuronal activity [19], evaluation of magnitude variations between regions presupposes consistent baseline deoxyhemoglobin content and oxidative metabolism changes during activation. Uncertainty in the validity of this supposition limits comparability between disparate brain regions; moreover, any brain region in which changes in blood flow during activity are matched by changes in metabolic oxygen uptake would be rendered invisible to detection of activation. We will show how the utilization of flow-sensitive MRI would sensitize to such differences, and would clarify the interpretability of the BOLD signal.

1.2 Challenges for application of MRI to functional neuroimaging

Despite the superficial similarities between PET and fMRI, there are fundamental differences between the two modalities that provide both a challenge and significant potential for the MR-based techniques. At the heart of this challenge lies the complexity of the relationship between perturbations in cerebral physiology and the measurable changes in MRI signal. Unlike radionuclide measurements, for which the signal output from a voxel (the gamma ray emission) is directly proportional to the concentration of the tracer within that voxel, the signal changes observed in functional MR images are dependent upon many physical and physiological parameters.

Inherent to most fMRI techniques, which are sensitive to changes in blood oxygenation, are the complexities of the magnetic susceptibility contrast phenomenon. In tissue, the diffusion of protons in the presence of the magnetic field variations in or around deoxyhemoglobin is now recognized as the dominant source of the oxygenation dependence of the measured transverse relaxation time (T2) of blood [20]. Work continues on understanding the interaction between the full set of physiologic parameters (including capillary and macrovascular blood volume changes, water exchange between the vascular and
extravascular compartments, distribution and concentration of deoxyhemoglobin, tissue perfusion and macrovascular flow) and this common form of fMRI contrast.

Although less effectively exploited than the deoxyhemoglobin-sensitive contrast mechanism, blood flow-sensitive fMRI has a biophysical basis that is less complex. MRI sensitivity to inflowing spins was noted in the first published reports using the deoxyhemoglobin-sensitive techniques [8], and has since been used to track blood flow changes from arteries to veins [21]. Differences between direct flow signal and deoxyhemoglobin concentration time courses might be indicative of changes in blood volume or oxygen uptake. The difference between BOLD and CBF measurements forms a basis for comparison with PET observations of decoupling of oxidative metabolism from blood flow during activation [2, 22].

Blood oxygen sensitization and blood flow sensitization have been shown to produce dynamic mapping MRI signals. No such noninvasive method exists for the measurement of blood volume separate from blood oxygenation. This is because oxygenated blood has similar NMR characteristics to the surrounding tissue; an intravascular NMR-sensitive tracer is required. Intravascular contrast administration may provide a method for dynamic blood volume imaging in humans, and has recently been shown effective using a novel contrast agent in animals [23]. Because of the difficulty of measuring blood volume dynamics directly, we assume a flow-volume coupling as measured previously [24] and recently confirmed in animals using a novel contrast agent technique [23].

1.3 Dissertation Overview

The synthesis of blood oxygenation sensitive and blood flow sensitive MRI methods via biophysical models of MRI signal production offers unique opportunities for new understanding of changes in oxidative metabolism in the brain. It will inform approaches
to functional neuroimaging by MRI, and promises to allow extension of functional MRI interpretations and applications.

In Chapter 2, the background for later chapters is laid with a description of MRI contrast mechanisms. A dynamic model of oxygen delivery and cerebrovascular dynamics is described, and related to transient functional MRI signals. This model links blood flow, volume, and oxygenation, oxygen extraction to tissue, and metabolic oxygen demand. BOLD signal time course is predicted based on changes in interrelated physiological variables. Novel experiments comparing magnitude and time course of perfusion and deoxy-hemoglobin sensitive signals were performed and are discussed in relation to two major outstanding questions: (1) the visibility and origin of an early blood oxygen level dependent (BOLD) signal dip before the positive activation signal, and (2) the origin of a variably-observed post-stimulation undershoot in the BOLD signal which can take as long as one minute to return to baseline. Two prime candidates for physiological explanation of the post-stimulation undershoot are discussed: changes in metabolic rate for oxygen, and changes in blood volume separate from blood flow.

Chapter 3 describes activation signal characteristics for long-term stimulation, with the objective of identifying possible conversion from nonoxidative to oxidative metabolic processing as reported by others. Recent work has suggested that immediate brain activity increases are accompanied by nonoxidative glucose metabolism increases [17] and lactate generation [25, 26, 18], which is slowly reversed as oxidative metabolism adjusts over several minutes. This recent hypothesis is challenged by our observations of rapid and stable increases in oxygen uptake during photic stimulation. Our findings are concordant with dynamic optical spectroscopy measurements of hemoglobin accomplished through cranial windows [27, 3, 28, 29, 30].
Chapter 4 describes a novel calibration method which allows direct measurement of changes in oxidative metabolism by use of a physiological reference standard: hypercapnia. This final development in MRI methods cuts through the epiphenomenology otherwise inherent to fMRI by calibrating the blood oxygen level dependent signal, to allow mapping by MRI of true metabolic and hemodynamic variables. Several physiological implications of magnitude and variability in the BOLD calibration parameter and in the activation-induced metabolic rate for oxygen are discussed.

In order to maintain readability of the individual chapters separately, some basic introductory material is repeated, with the exception that general background and explanatory material applicable to any of the chapters is more detailed in the introduction to Chapter 2.
References


Chapter 2

Transient functional MRI signals:

physiological basis and biophysical models, with application to visual cortex

Chi troppo abbraccia nulla stringe.

He who embraces too much can squeeze nothing.

– Italian proverb
2.1 Introduction

What are the possible physiological mechanisms for task-related changes in brain MRI signals? It is the aim of this chapter to explore in detail the nature of transient blood oxygen level dependent (BOLD) MRI signals observed during the several seconds after changing brain state, and to identify and characterize physiological variables which may be responsible for these transient signals.

Our work is motivated partly by our observation that temporally resolved BOLD signals differ from dynamic cerebral blood flow (CBF) MRI, particularly in the post-stimulation period, when a profound rebound “undershoot” and recovery is observed which can last up to a minute. This BOLD undershoot is strong in primary visual cortex, but is not seen using CBF contrast [9, 10], and, as an unexplained change in hemoglobin oxygenation, has been postulated as a possible marker for oxidative metabolism [11]. Except for the calibrated functional MRI method described later (Chapter 4), it remains uncertain whether changes in metabolic demand for oxygen are related to brain activity, and whether they have a measurable effect on the BOLD signal magnitude or dynamics.

After laying the foundations of BOLD signal phenomenology and theory of functional MRI signal production, we report experiments which show differences in time courses and regions responding with BOLD signal, regions generating post-stimulation BOLD undershoot, and regions which show increased CBF signal in response to photic stimulation in primary visual cortex. To explain our primary data, and to further examine the dynamics of linkage between hemodynamics, metabolism, and functional MRI, we report a model of blood oxygen level dependent (BOLD) MRI signals in terms of cerebral blood flow (CBF) and volume (CBV), integrated with a kinetic model of oxygen transport and metabolic uptake, designed to predict the temporal evolution of MRI signals based on postulated physiological events. This dynamic modeling addresses limitations in the interpretation of
quasistatic estimates of change in metabolic rate for oxygen as described in Chapter 4. The dynamic departure from equilibrium between capillary, tissue, and venous oxygenation in response to changes in blood flow and oxygen demand require an approach which includes spatial and temporal distribution. This chapter also provides motivation for the calibration approach presented in Chapter 4 as a method to remove the remaining ambiguities.

### 2.2 Background: Characteristics of dynamic BOLD signals

![Characteristic BOLD signal transient behavior](image)

**Figure 2.1:** Characteristic BOLD signal transient behavior. The sequential phases of signal evolution are described in detail below.

The phenomenology of BOLD signal response to sensorimotor activation has been well characterized, as illustrated in Figure 2.1. Following an initial delay of 2 seconds, during which some have detected an *early dip* in signal, there is a steady *rise* to activated level which reaches its maximum in less than 8 seconds. The signal remains at a *plateau*, possibly losing some of its original magnitude during continued task activation, and promptly *decays* away within 5 s of cessation of stimulus. In many experiments and most prominent in primary visual cortex, there is a post-stimulation *undershoot* which has been measured up to 50% of the magnitude of the positive signal deflection, followed by a *recovery* with time constant on the order of 15 s. These phases of signal evolution are elaborated below.
2.2.1 Baseline

It may be taken for granted that the resting brain has a relatively constant blood flow and metabolism; this is seen as a necessary starting point for BOLD functional MRI activation experiments for detection of changes from a “resting” state. However, a plethora of intrinsic physiological rhythms, including electroencephalographically detected alpha and beta rhythms, respiratory fluctuations in central venous pressure and intracranial pressure, and vascular pulsations at the heart rate all may contribute physiological variation to the MRI signal [12]. Moreover, bilaterally symmetric correlated noise has been detected which conforms to borders within somatosensory cortices, suggesting that neuronal rhythms may generate hemodynamic rhythms detectable by fMRI [13, 14]. Fortunately for the purposes of stimulus-locked signal analysis, averaging over these relatively shallow modulated intrinsic rhythms allows comparison of activated to non-activated brain, albeit with increased physiological noise which might be removed with appropriate modeling and experimental modifications. It remains to be seen whether entraining of intrinsic rhythms, for instance entraining of respiratory rate by stimulus presentation, may adversely impact the identification of regions which may or may not correspond with brain directly subserving the processing of stimuli. In the remainder of this work, we make tacit assumption of a stationary baseline state.

2.2.2 Early dip

Optical spectroscopic experiments have described an early oxygen desaturation within active columns of striate cortex, and within active whisker barrels in primary somatosensory cortex, which occur within 100 ms of sensory stimulation, followed 2 s later by vascular engorgement and increased oxygen saturation in draining veins [15, 16]. BOLD MRI search for this early desaturation phenomenon has been surprisingly difficult. Hennig was the first to report an early signal in spectroscopic measurements of water free induction
decay within visual cortex which corresponds in temporal nature to the early dip [17]; however, these experiments would predict a larger BOLD MRI effect than is observed. Working at high field, Menon was able to detect an early dip in the population of pixels which also exhibit a large post-stimulation undershoot, leading to the assertion that post-stimulation undershoot and early dip signals may be produced by the same mechanism, namely, increased oxygen utilization [11]. If this were true, then the early dip and post-stimulation undershoot may be more specific markers for location of neuronal activation than the apparent luxury perfusion increase observed with BOLD. Simulations described in the current chapter of BOLD during changes in oxygen demand ahead of vascular response show putative origins and expected magnitudes of this small signal perturbation.

**2.2.3 Rise to peak and plateau**

The increase in BOLD-weighted MRI signal reflects decrease in deoxyhemoglobin, counter to early reports of hemoglobin-based contrast, which had originally assumed that active brain would cause increased oxygen utilization, and thus increased, rather than decreased, oxygen desaturation in blood [18]. This reversal of expected contrast has been attributed to uncoupled increased blood flow to active brain out of proportion to possible increased oxygen extraction, as described by Fox and Raichle who compared PET CBF and oxygen extraction measurements [5]. Maintenance of the BOLD signal requires maintenance of uncoupling between blood flow increase and oxidative metabolism, explored in detail in Chapter 3 and quantified in Chapter 4. We consider here the dynamics of this rise.

Early functional MRI reports describe a rise to peak time from 7 to 9 seconds [10]. These are concordant with impulse-response measurements [19, 20] which show a 2 second delay followed by a near gaussian cerebrovascular impulse response with peak at 6 seconds after stimulus onset within visual cortex. It is clear that the rise to peak is a func-
tion both of blood flow response time and deoxyhemoglobin washout time. Analysis of the plateau period during long-duration stimuli is the subject of Chapter 3.

2.2.4 Decay, undershoot, and recovery

The return of signal following cessation of stimulus may be more or less rapid than signal onset. The post-stimulus behavior is in fact widely variable across cortex. This may in part reflect variations in neuronal activity following external stimulus. An example is given by studies of the motion aftereffect: visual area MT response following an aftereffect-generating stimulus persists much longer than the response following a balanced-motion stimulus [21], possibly driven by latent apparent motion in the visual field rather than the external stimulus alone.

For most stimuli in primary visual cortex, however, the return to baseline is rapid. In fact, many stimuli, particularly high contrast flashing visual stimuli, produce a profound post-stimulation rebound in primary visual cortex which is up to half the magnitude of the initial plateau, and which returns to baseline over the following 30 s. Could neuronal deactivation be responsible? Is blood flow rebounding as well? If not, then can oxygen transport dynamics, dynamic changes in oxygen metabolism, or blood volume lag explain the findings? These questions form the main focus of the current chapter.

2.3 Theory

We begin by examining NMR signal production in physiological systems where perfusion, variations in perturbations by magnetic susceptibility, and compartments with different relaxation characteristics coexist in a single imaging volume. For human brain we describe the important compartments of interest and expected results of physiological maneuvers. Using a model of blood and oxygen transport, we then investigate the temporal relationship between hemodynamic changes and oxidative metabolism, and the result-
ing BOLD signal changes which would be expected based on a kinetic model of oxygen delivery.

2.3.1 Background: NMR Signal Production

The proton NMR signal is produced when the relaxed portion of hydrogen nuclei in a strong magnetic field are perturbed such that they exhibit coherent magnetization projecting into the plane transverse to the axis of main magnetic field. This projected coherent magnetization precesses at the Larmor frequency (42.57 MHz per Tesla for \(^1\text{H}\)), a phenomenon attributable in the classical mechanics model to the interaction between angular momentum and magnetic moment, just as a spinning top precesses in a gravitational field with a frequency dependent on both angular momentum and mass. Magnetization can be “tipped” from the longitudinal axis into the transverse plane or out of the transverse plane either aligned or anti-aligned in the longitudinal axis by coupling to an electromagnetic coil driven by current oscillating at or near the Larmor frequency. The oscillating magnetic field produced by the coil can be thought of as a second magnet precessing along with the spins, and with which the spins attempt to align during the duration of the oscillating pulse. Paul Lauterbur discovered that pulses can be selectively directed at anatomical regions (e.g. a slice of tissue in the transverse plane of the magnet) by applying a relatively small gradient of magnetic field (10 mT/m) during the delivery of a band-limited pulse. To excite a defined block of tissue uniformly, the fourier transform of a square wave \((\frac{\sin \omega t}{\omega t})\) is used to modulate the magnetic coil pulse around the Larmor frequency for the tissue at the center of the block. The same coil, inductively coupled to the tissue sample, can then be used to detect the small changes in magnetization from transverse oscillating spins. Demodulation of the resulting radiofrequency modulations in current within the coil forms the nuclear magnetic resonance (NMR) measurement.
**Relaxation phenomena:** The spins will gradually realign with the main magnetic field and thus regain the potential to deliver coherent magnetization when excited; this occurs in a manner well described in detail as an exponential recovery, with time constant $T_1$. The relaxation mechanism responsible for longitudinal magnetization recovery is called spin-lattice relaxation.

The magnetic spins will also gradually lose their measurable projection into the transverse plane. This results from several simultaneous processes: spin-lattice relaxation, spin-spin momentum transfers which cause the material to lose coherence, and loss of spin coherence due to local differences between magnetic fields as seen by the nucleus, which slightly change the effective Larmor frequency. If these local field differences are due to the local environment of the molecule to which the atom is attached, the shifts are reproducible and measurable, and termed *chemical shift*. The separation of resonance frequencies for species with different chemical shifts is the basis of NMR spectroscopy utilized in chemical structure analysis. For NMR imaging, however, the bulk of the signal comes from two biological sources: water and fat. While the chemical shift between the water spectral peak and the fat spectral peak can indeed cause misregistration between water and fat in MRI images, especially in echo-planar imaging, with this exception chemical shift does not generally come into play for imaging purposes.

**Hahn’s spin echo:** If the differences in observed Larmor frequencies are slowly varying in space and are constant, then these differences are a part of the potentially correctable macroscopic environment, and lead to observed shortening in the transverse relaxation which can be avoided. A method to avoid the effect of these static inhomogeneities was found by Hahn in 1950, termed the Hahn echo or spin echo [22]. Hahn found that an ensemble of spins with some magnetization visible in the transverse plane (due to previous pulse) could be inverted by application of an energy pulse twice that necessary for
maximum transverse magnetization. This is termed a 180 degree pulse, and can be described in the classical model as inverting the spins. Because of the nature of coherence in the precessions of spins, this causes spins which have a higher than average precession rate, and thus more accumulated spin phase per time, to be placed in an analogous precession lag. By the same token, spins which are slow to precess are placed ahead by reflecting their orientation about the transverse plane.

After the 180 degree pulse, the spin begins to catch up to its neighbors, or fall back to its neighbors, at the same rate which caused it to become incoherent with the ensemble since the excitation period. Thus the Hahn spin echo is produced as spins come back into alignment with one another in the transverse plane, exactly 2 time periods from the beginning of the excitation, following an inverting pulse delivered after one time period. Transverse magnetization decay is measurable as an exponential fall in signal as the Hahn echo time is increased in separate experiments. This time constant is termed $T_2$.

**Sensitivity to heterogeneous magnetic susceptibility:** Without application of a spin echo, magnetization from free induction decay falls off with a time constant $T_2^*$. In liquid spectroscopy, $T_2^*$ is assumed to be shorter than $T_2$ due to spectrometer magnet inhomogeneity and other experimental factors, rather than intrinsic properties of the sample. In contrast, in biological systems, the local variations in magnetic field are more often due to variations in magnetic susceptibility of tissues, and thus $T_2^*$ becomes an inhomogeneous material property just as are $T_1$, $T_2$, and the density of spins. Of note, $T_2^*$ is only a shorthand term for observed transverse relaxation, which occurs with a dependence on time which may not follow a precise exponential form, and must be addressed specifically for the evolution times (TE) of interest.
Sensitivity to diffusion: When spins diffuse through regions of magnetic inhomogeneity, the effect on precession is random, and thus not removable by use of the spin echo, because the path through the inhomogeneities is not retraced precisely before and after execution of the inverting pulse. Thus magnetic inhomogeneities on a diffusion scale (less than 20 microns for typical experiment times) contribute to the observed T_2 relaxation rather than only the T_2*.

The diffusion-mediated signal change also can be utilized directly to measure diffusion, by applying large magnetic field gradients to the spins in the direction for which diffusion sensitization is desired. Well-established theory relates field gradients and pulse timing to the observed diffusion coefficient of the tissue [23]. Anisotropy of macromolecular barriers to diffusion forms the basis of diffusion tensor brain white matter tract visualization [24, 25].

Oxygenation sensitivity: Deoxyhemoglobin in red blood cells, as well as capillaries containing deoxygenated red cells, cause local magnetic field inhomogeneities on the diffusion spatial scale; this blood oxygen level dependent (BOLD) contrast mechanism thus has both T_2 and T_2* effects.

Sensitivity to convective transport: A central feature of biological systems not yet accounted for is perfusion. The spins not only relax according to the above described mechanisms and diffuse around macromolecules, but they also are transported by flow in vascular structures. A major effect is noted due to perfusion after selective excitation using band-limited pulses and gradient magnetic fields. Spins which were not excited at their Larmor frequency, which were outside the targeted volume when the band-limited pulse was applied, do not receive the same excitation energy as those within the volume. As an example, suppose we invert the spins at equilibrium within a volume of interest, and then
after waiting a period \(-T_1 \log 0.5\), we excite those spins into the transverse plane. Because of \(T_1\) regrowth, the spins which see both the inverting and excitation pulse will have no signal, as their longitudinal magnetization is passing from negative to positive at that time precisely. However, spins which were not inverted, but simply flow into the imaging volume after the first pulse, will tip readily into the transverse plane with the second pulse. This forms the basis for measuring signal which is proportional to perfusion: the subtraction of a nonselective from a selective excitation pulse.

**Time-varying perfusion estimates:** We characterize apparent T1 relaxation during transient changes by solving the dynamical NMR signal equations for a time-varying blood flow input function. We write in terms of the magnetization difference \(m(t)\) from equilibrium \(M_0\): \(m(t) = (M_0 - M(t))/M_0\), such that \(m(t) = 0, 1, \) and \(2\) at spin equilibrium, saturation, and inversion, respectively.

In the absence of a large tissue-blood \(T_1\) difference, the modified Bloch equation with time-varying spin turnover at rate \(F(t)\), for which the incoming spins have a known magnetization \(m_a(t)\) and outflowing spins have well-mixed magnetization \(m(t)\), is

\[
\frac{d}{dt} m(t) = -\left( \frac{1}{T_1} + \frac{F(t)}{\lambda} \right) m(t) + \frac{F(t)}{\lambda} m_a(t)
\]

where \(\lambda\) is the blood to tissue water partition coefficient (\(\lambda = 0.9\)). For inversion recovery with a non-slice-selective inversion pulse, inflow of magnetization \(m_a(t) = 2e^{-t/T_1}\). For the case in which all RF pulses are slice selective (e.g. spin echo or inversion recovery), \(m_a(t) = 0\).

The general solution for time varying \(F\) can be written as the sum of an initial (zero-input) response and a convolution of time-varying input with the impulse response function \(h(t): m(t) = h(t)m(t_0) + \int_0^t \left((h(t)/h(t'))((F(t')/\lambda)m_a(t'))dt'\right)\) where the time varying impulse response \(h(t) = \exp\left(-\int_0^t (1/T_1 + F(t'')/\lambda)dt''\right)\) is dependent on the integrated history of the sys-
tem parameters. For a slice-selective inversion recovery pulse sequence, it follows that

\[ m(t) = 2\exp(-t(1/T_1 + (1/t_1)\int_0^t (F(t')/\lambda)dt')) \]

and thus

\[ \left(\frac{1}{T_1}\right)_{\text{apparent}} = \frac{1}{T_1} + \frac{1}{\lambda} \int_0^t F(t') dt' \quad (2.2) \]

The measured \( F \) in a time-varying system using T1-sensitive slice selective excitation is precisely the simple time-averaged \( F(t) \) over the interval between initial spin excitation and readout (e.g. the inversion-recovery TI). Variation in flow over the course of a measurement does not lead to incorrect results.

**Echo planar magnetic resonance imaging:** The technology of echo-planar imaging makes use of rapidly varying gradients of magnetic field during the readout period of the NMR spectroscopic experiment. By varying one orientation of gradient magnetic field quickly, and adding phase dispersion in a second direction between rapid gradient sweeps, an entire two dimensional representation of NMR spatial signal distribution is obtained from a single NMR excitation. The major impact on image content in terms of the relaxation parameters above is that, for the spin echo experiment, the Hahn echo is placed only at the center of spatial-frequency space; in other words, the bulk magnitudes of large structures in the image are weighted by T2 as for a spin echo experiment, whereas smaller structures with more high spatial frequency content (e.g. sharp edges) are imaged with more T2* weighting. This difference has not been problematic in experimental practice when fast oscillating gradients are used (readout window 32 ms), although the selective blurring of objects with short T2* in an EPI experiment must be kept in mind for analysis of echo-planar data, especially for slower acquisitions with longer readout windows.

**Summary:** Now that the physical basis of NMR signal generation has been described intuitively, let us examine it in mathematical form. For each compartment with its own T1,
T2* and spin density $M_0$, the NMR signal $S$ following a previous excitation TR time units previously, and measured TE time units after the current excitation, is weighted as such, assuming that all transverse magnetization is discarded or randomized at the end of each pulse sequence, and that transverse relaxation indeed follows the assumed exponential form:

$$S = M_0 e^{-\frac{TE}{T_2}} \left(1 - e^{-\frac{TR}{T_1}}\right)$$

(2.3)

Variation of TE and TR over appropriate scales (TE similar to T2*, TR similar to T1) allows the determination of T1, T2*, and $M_0$. If in addition a Hahn echo inversion pulse is applied, with expected echo at time TEh, then the equation becomes

$$S = M_0 e^{-\frac{TE-TEh}{T_2}} \left(e^{-\frac{TEh}{T_2}} - e^{-\frac{TR}{T_1}}\right)$$

(2.4)

Perfusion changes effective TR for a portion of the spins. Because of its exponential character and dependence on repetition interval, the perfusion portion of the signal can be viewed as changing the effective T1 of tissue: $\frac{1}{T_{1\text{effective}}} = \frac{1}{T_1} + \frac{F}{\lambda}$ (see Introduction) where lambda is the partition coefficient for water between blood and tissue. This effective T1 change can be magnified by use of an inversion recovery pulse sequence. Inversion recovery is similar to a spin echo, but instead of starting with fully relaxed longitudinal magnetization, a preparatory inversion pulse negates the sign of the longitudinal magnetization. As longitudinal magnetization then begins to recover at T1 time constant, the signal passes through zero and becomes positive. The doubling in variation of magnetization, from $-M_0$ to $M_0$, doubles the sensitivity over the short-TR spin echo sequence described above. Signal measured after an inversion time TI has the following characteristic:
With the exception of quantifying the effect of susceptibility perturbers on MRI signal, we now have a model of MRI signal production based on the phenomena of spin excitation and relaxation in a heterogeneous medium. It remains to identify the compartments of interest and to quantify the effects of changing blood oxygenation, blood volume, and perfusion on the MRI signal.

2.3.2 Blood oxygenation level dependence: signal magnitude estimation

The relationship between tissue deoxyhemoglobin content and signal attenuation has been established as primarily due to the effects of microscopic inhomogeneity of magnetic susceptibility between deoxygenated blood cells and surrounding plasma and tissue, affecting both extravascular and intravascular spins. We model the BOLD effect as due to changes in the magnetic susceptibility difference $\Delta \chi$ between intravascular and extravascular compartments. Details are given by Boxerman [26]; briefly, the heterogeneous magnetic susceptibility of tissues is dominated by the paramagnetic effect of deoxyhemoglobin in a mildly diamagnetic milieu occupied mostly by water. Deoxyhemoglobin is compartmentalized into erythrocytes; this compartmentalization causes large step changes in magnetic susceptibility between intracellular and extracellular spins in blood; the compartmentalization of blood within vessels also causes step changes in effective magnetic susceptibility between intravascular and extravascular spaces. In the presence of a large base magnetic field of the MRI system (1.5 T for our experiments), the variations in magnetic susceptibility lead to warping of magnetic field lines, and thus local gradients of magnetic field in the region of vessels and in the region of erythrocytes. These magnetic field perturbations extend for several vessel diameters into the surrounding tissues, and thus effect a much larger proportion of spins than simply the intravascular space alone.
The attenuation of tissue spins \((A_t)\), capillary spins \((A_c)\) and venous spins \((A_v)\) depend on their respective blood volume fractions \((f_t, f_c, f_v)\), the susceptibility gradients in the region, and vascular geometry, and can be combined as follows to produce signal \(S\) (ignoring for the moment \(T1\) related effects):

\[
S = S_0 A_{EV} \left( f_t \frac{-TE}{T_{1mo}} + (f_c A_c + f_v A_v) \frac{-TE}{T_{2blood}} \right)
\]  

(2.6)

**Extravascular attenuation**: \(A_t\): is tissue water signal attenuation due to \(\Delta \chi_{blood}\), the magnetic susceptibility difference of blood in nearby vessels. The function \(A_t(\Delta \chi_{blood})\) was determined by simulation for vascular sizes representative of capillaries (dia. 3\(\mu\)) and larger venules (dia. 25\(\mu\)) containing deoxyhemoglobin with a \(\Delta \chi\) up to \(\Delta \chi_0 = 1.8 \times 10^{-7}\), corresponding to 100% Hct fully desaturated red cells (cgs units) [27]. The extravascular contribution to signal change was computed from the accumulation of phase dispersion in a population of randomly diffusing water molecules near a randomly oriented collection of vessels of the given diameter, with volume fractions for capillary and vein \(V_{c,mc} = 2\%\) and \(V_{v,mc} = 2\%\). These Monte Carlo-based estimates were combined and scaled to the required volume fraction. Calculation of \(A_t\) utilized lookup tables translating \(\Delta \chi\) to signal attenuation for each of two vessel sizes, \(A_{t,c}\) and \(A_{t,v}\), and then weighted them according to volume fraction. \(A_t = (A_{t,c})^{V_{c,mc}} (A_{t,v})^{V_{v,mc}}\). The Boxerman-Weisskoff Monte Carlo susceptibility model has been shown to agree well with measurements in phantoms [28, 29].

**Intravascular attenuation** \(A_c\) and \(A_v\): Intravascular spins also contribute significantly to BOLD contrast, as has been shown by our experiments which remove the intravascular signal by application of velocity sensitizing gradients [26]. Monte Carlo simulations of intravascular water exposed to the susceptibility difference between red
blood cells and surrounding plasma, \( \Delta \chi_{\text{rc}} \), provide the intravascular signal change. The intravascular spins acquire a small difference in complex phase from the extravascular spins, so \( A_c \) and \( A_v \) are maintained as complex quantities, while \( A_i \) is real. The extravascular attenuation also affects intravascular spins.

**Effective tissue volume fraction** \( f_t \): The fractions \( f_c \), \( f_v \), and \( f_i \) in Eq (2.6) reflect the relative NMR signal proportion attributable to each compartment, a function of \( T_1 \), spin density, and volume fraction. If increases in \( f_c \) and \( f_v \) cause loss of volume \( f_t \), then the signal increases due to functional imaging are not as large as indicated previously [26]. However, if the volume that is lost has a long \( T_1 \) (e.g. from CSF partial-volume in the voxel) and the acquisition uses a relatively short repetition time, the reduction in tissue volume results in less visible loss of signal from tissue. The tunable parameter \( \epsilon \) reflects this, where \( f_t = 1 - \epsilon(f_v + f_i) \): \( \epsilon = 0 \) indicates no visible loss of tissue signal (assumed by Boxerman et al. [26]); \( \epsilon = 1 \) indicates that all of the blood volume increase is matched by tissue volume loss. Because of CSF-containing Virchow-Robin spaces in the region of vasculature which are likely attenuated during blood engorgement, we choose \( \epsilon = 0.5 \) to reflect loss of CSF signal. It is less than 1 because CSF would be attenuated to half its full magnitude by our repetition time \( TR=2 \) s with CSF \( T_1=3 \) s in our experiments \( (1 - e^{\frac{TR}{T_1}} = 0.5) \).
2.3.3 Sensitization to physiology in the pulse sequence

By appropriate choice of NMR pulse sequence parameters, specific sensitization to BOLD, CBF, or CBV effects can be maximized while minimizing the others.

**Blood oxygenation dependence:** To first approximation, the BOLD effect scales with $1/T2^*$ for echo times of interest [18]. The largest BOLD signal changes are obtained with long-TE gradient echo or asymmetric spin echo sequences. We estimate BOLD signal by assuming exponential transverse $T_2^*$ decay and scaling the Monte Carlo results at TE=50 to our experimental parameters.

**Blood flow dependence:** Experiments which set up a state of different magnetization in the arriving blood supply from the extravascular water spins within an imaging volume cause a degree of blood flow dependence. For most simple pulse sequences, blood flow dependence scales with the $1/T1$ contrast sensitivity of the sequence as described in the
Introduction. The measured change in apparent \( T_1 \) follows closely the block-averaged change in flow. For the case of repeated slice-selective excitation, signal deconvolution is not necessary: the measured \( T_1 \) change is representative of the change in CBF on a point-by-point basis, except when changes occur more rapidly than the measurement interval, in which case the \( T_1 \) changes reflect the average CBF over the preceding TR or TI period.

Application of this development to perfusion measurement in the heterogeneous biological system requires several assumptions. We assume monoexponential decay in \( T_1 \), implying similarities in \( T_1 \) between compartments or fast exchange between compartments. We assume that blood has time to relax with tissue before leaving the imaging volume, which is adequate for human imaging volumes and perfusion rates but not animal studies. We also assume that we have controlled for transfer of magnetization between NMR visible and NMR invisible spins, or that this effect is the same between subtracted image pairs.

**Blood volume dependence:** There is a component of blood volume sensitivity in the BOLD measurement, which according to theoretical and Monte Carlo results scales the BOLD signal attenuation linearly with blood volume. The situation is somewhat more complicated when the intravascular \( T_1 \) or \( T_2 \) is shortened through the introduction of extrinsic contrast agents [30], which may allow the independent measurement of changes in CBV. Dynamic independent CBV measurement is beyond the scope of this work.

Prior animal and human studies have shown dynamic blood volume changes in step with blood flow changes. A quantitative experiment set reported Rhesus monkey CBV changes as proportional to the blood flow raised to the power 0.38 [31]. Recent results by Mandeville in our laboratory have confirmed this exponent at 0.4 in rats using intravascular contrast agents to measure blood volume changes in response to hypercapnia, and comparing with laser doppler flowmetry (Mandeville, personal communication). However, this
recent result also demonstrated a lag of blood volume change with respect to blood flow change, with a time constant on the order of 14 s in the rat.

Possible transient uncoupling of blood flow from blood volume remains unmeasured in the human; quasistatic measurements are uniform in their reporting of tightly coupled blood flow and blood volume. In our simulations, we choose a tight coupling model (4 s time constant) for the majority of cases in order to maintain simplicity and consistency [31], and also perform simulations to show the effect of longer time constants and delays in blood volume change.

2.4 Dynamic Functional MRI Experiments
2.4.1 Experimental Methods

Echo-planar imaging: All imaging was done on a GE 1.5 T Signa retrofitted for EPI by Advanced NMR, Wilmington MA (ANMR), using either a 5 inch surface receive coil or the standard head coil. At the beginning of each imaging session, initial sagittal T1 weighted images were obtained at 5 mm intervals. The volume of central occipital cortex was identified and automated shimming performed based on an echo-planar asymmetric spin echo Dixon pair of images at each point in the image volume, from which magnetic field perturbation is measured (Reese, Davis, and Weisskoff [32, 33]). An idealized set of spherical harmonic gradient shims is fitted numerically to the residual field inhomogeneity, the shims are corrected, and the process iterated if necessary to achieve NMR water resonance linewidth on the shimming volume to the order of 5 Hz. We have shown that shimming is crucial to obtaining high quality echo-planar images with T2* weighting without excessive geometric distortion to superimpose on the conventional images [34].

Following shimming, the calcarine sulcus was identified morphologically and an oblique plane parallel either to the average of left and right sulci or oriented with the most
prominent of left and right sulci was selected for single-slice experiments. Dynamic series were obtained using sequences tailored to either inflow perfusion flow weighting or sensitive to local susceptibility-induced field inhomogeneities. Flow-weighted sequences were as follows: A) EPI inversion recovery, TI=0.9 s, TE=20 ms, TR=3 s, and B) EPI spin echo, TE=20 ms, TR = 1 s. Susceptibility-weighted sequences were as follows: C) EPI gradient recalled echo TE=40 ms, TR=3 s, and D) EPI asymmetric spin echo, -40 ms spin echo offset from readout window center, TE=70 ms, TR= 2 s.

Pulse programming language codes were specially designed for functional imaging experiments. Pulse sequences A, B, and C were minimally modified from those provided by ANMR for echo-planar spin echo and gradient echo experiments. Local enhancements included improved tuning, and improved fat saturation preparatory pulse. The EPI asymmetric spin echo sequence D was produced by modifying the standard EPI spin echo sequence to place the central 180 degree pulse and its surrounding crusher gradients and slice selection gradient at a user-specified offset from center of the gradient echo time. This modification allows user control of T2* weighting in the low k-space elements of images, controlling the bulk contrast between T2 weighting and added T2* weighting. For our experiments, we set the 180 degree pulse forward by 25 ms. This provides 50 ms of T2* equivalent echo time at the center of the 32 ms readout window.

All functional images were obtained using the standard ANMR resolution, with 500 ns per transverse k-space line with 256 point resolution over 40 cm field of view horizontally, and 64 vertical k-space lines obtained in a single NMR experiment during a 32 second readout window. Echo-planar imaging is accomplished on our hardware by using sinusoidally-varying whole-body transverse magnetic field gradient coils with 5 gauss/cm peak-to-peak amplitude variation coupled to a resonant power supply oscillating at 1 kHz. This design delivers magnetic field oscillations (dB/dt) near the physiological limit for induced
current-generated nerve and muscle endplate depolarization. In fact, body geometry dictates a shoulder-to-shoulder magnetic field oscillation orientation in order to minimize the anteroposterior distance across which Faraday-induced electrical field forms. If this geometry is violated, for instance by clasping hands in a conductive self-handshake, the oscillating gradients produce a strong electrical shock. No subject has reported a shock while laying flat with limbs uncrossed during transverse oscillation of the field.

**Psychophysical paradigm:** Subjects were repeatedly examined in primary visual cortex (V1) with imaging planes parallel to the calcarine sulcus during and after stimulation by 8 Hz alternating black and white radial checkerboard pattern projected onto a translucent screen in the magnet bore at 30 cm optical distance from the eyes. Stimulation periods ranged in log steps between 1 s and 60 s. At least 60 s of recovery time data (neutral gray field with a small focusing spot) were collected after stimulations. Response time course was verified to be indifferent to longer intervals between stimulation, or to ordering of stimulus durations.

Four individuals were examined with inversion recovery CBF (sequence A) and gradient echo BOLD (sequence C), using repeated one minute stimulation periods followed by 1 minute of rest. These methods correspond to those used for experiment 1A in Chapter 3. In order to reduce pulse sequence-dependent differences which might artifactually weight some voxels differently from others, an additional seven subjects were tested using CBF-weighted short-TR spin echo (sequence B) compared to BOLD-weighted asymmetric spin echo (sequence D). For this second pair of sequences, the stimulation period was varied between 1 s and 32 s with 60 intervening recovery periods. Average time courses were constructed across subjects for both CBF and BOLD experiments.

To minimize synchronization error, stimulus presentation was triggered by the scanner scope trigger TTL-level output port, which was monitored by a LabView signal processing
board on a Macintosh IIvx computer. Custom software (R. Savoy, personal communication), modified and maintained by the author, counted pulses of the scope trigger generated explicitly in the embedded-system MRI pulse program language provided by General Electric (PPL, 4.x and 5.x revisions). A Radius VideoVision 24 bit video output board on the Macintosh IIvx was used to drive a Sharp XG-2000U liquid crystal light-valve type NTSC color video projection unit which was positioned just outside the Faraday cage of the magnet room, directed through the cage screen to a biconvex refocusing lens focused on a frosted pexiglass projection screen mounted near the subject’s chin in the magnet bore. A 6x15 cm single surface mirror was placed at 45 degrees at the subject’s forehead and oriented such that the center of the projected image was at or near the center of the visual field at neutral eye position. The subject was instructed to focus on a black fixation dot at the center of the screen for the duration of the experiment, during which a radial checkered pattern of alternated black and white wedges oscillated at 8 Hz for varying periods of time on a neutral gray background.

**Data analysis:** Data processing system *xds* written by the author [35] was used for data analysis. Regions of interest were computed semiautomatically by using an iterative cross-correlation approach. First, a model time course was constructed to fit expected results: for stimulus onset, this was a step function delayed 2 s from stimulus onset. For post-stimulation undershoot identification, the model was a linear rising ramp, matched to the period beginning 8 s after the cessation of stimulus. This is termed the zeroth-generation time course.

The first-generation cross correlation of each image pixel was computed to this model signal, and a region of interest semiautomatically identified by thresholding to a correlation coefficient (typically 0.5) and eliminating pixels outside of visual cortex and at the high-contrast margins of brain tissue to eliminate any motion artifact contamination. The
average time course of this region of interest was then computed, and used to generate a second-generation correlation map. Figure 2.6 shows the second-generation map and the second-generation time course calculated from that map.

Macrovascular contamination of regions of interest was avoided by excluding voxels with low intensity, high variance, or bright regions identified in a flow-sensitized conventional gradient echo image of the same slice; however, these voxels accounted for less than 5% of a given region (typically 1-2 additional voxels deleted).

2.4.2 Experimental Results

CBF and BOLD signals: Average BOLD signal in primary visual cortex increased 3.8 ± 0.7% (mean ± s.e.) during 32 s stimulation, with a concomitant increase of 1.8 ± 0.4% in the CBF signal (N=7). Averaged time courses of BOLD and CBF-weighted experiments are shown in Figure 2.3. The T1 CBF model calculates from these data an increase in CBF of 50 ml/100g/min, which is in reasonable agreement with previous PET measurements of blood flow increase during visual stimulation [5].

The post-stimulation undershoot is stronger and longer after prolonged stimulations, and is almost nonexistent after stimulation periods shorter than 8 s. This behavior is not observed in the averaged CBF-weighted data. Contrary to previous reports [10] our measured onset lag between BOLD and CBF is negligible. In the recovery phase, the BOLD signal and CBF signal return to baseline together, but then BOLD continues to decline below the baseline level for more than 10 s, before returning even more gradually to baseline, whereas CBF signal change stops at the baseline level.
Figure 2.3: Average BOLD and CBF responses to stimuli of varying length. The post-stimulation undershoot, exclusive to BOLD, is most prominent following extended stimuli. Linear baseline drift was removed (0.2%/min), and an 8 s symmetric finite impulse response smoothing kernel was applied to both curves; this gives the false impression of noncausal behavior which must be discounted.

The peak CBF and BOLD changes from each stimulus in Figure 2.3 are plotted in Figure 2.4. There is a gentle loss of slope in the curve as CBF increases, indicating incrementally decreased relative sensitivity of BOLD to ever higher changes in CBF. This finding is consistent with the model prediction of a maximum possible BOLD signal for a given isolated CBF increase, assuming no other changes in physiological state. A physical explanation lies in the washout of deoxyhemoglobin from tissue. At high flow, nearly all of the deoxyhemoglobin is washed out, leaving relatively little residual intrinsic contrast agent to report subsequent increased blood flow.
Figure 2.4: Changes in BOLD accompanying changes in CBF, from peak changes in Figure 2.3, show saturation effect. The BOLD increase begins to taper as CBF increases, consistent with our model’s prediction of a maximal BOLD effect due to gradually more complete tissue washout of deoxyhemoglobin.

**BOLD undershoot:** Pixel-wise comparisons of post-stimulation undershoot to BOLD signal activation magnitude in activating occipital cortex shows an inverse correlation of BOLD magnitude to post-stimulation undershoot magnitude. Matched data from four subjects are shown in Figure 2.5 during gradient echo (sequence C) susceptibility weighted imaging during and after a one minute visual stimulation. The activation magnitude and undershoot magnitude were obtained per pixel from maps such as the example maps of Figure 2.6(A) and (B). The resulting plot shows that the post-stimulation undershoot emphatically does not scale with the BOLD activation signal. In fact, the pixels of highest BOLD signal have very low post-stimulation undershoot, and the pixels with highest relative undershoot have very low BOLD signal; this last statement is simply an artifact of the calculation method: lack of activation causes large variance in undershoot ratio, due to noisy near-zero values in the denominator.
**Figure 2.5:** Comparison of BOLD activation magnitude to post-stimulus undershoot. Scatter plot of four subjects (circle, square, triangle, diamond) shows undershoot versus BOLD response magnitude for each activating pixel separately. The vertical axis is scaled to BOLD to discount partial volume effects. Note the inverse relationship between BOLD signal magnitude and undershoot magnitude: pixels with large BOLD signal have no post-stimulation undershoot, and pixels with large post-stimulation undershoot have relatively small BOLD signal.

**Heterogeneity of BOLD and CBF:** Figure 2.6 shows an example map of the response to 60 s checkerboard stimulus followed by 60 s rest. Correlation maps for the onset of stimulus response (threshold at $r > 0.5$ for BOLD activation, $r > 0.3$ for CBF, using 60 sequential images each, TR=3 s) show similar, but slightly different areas of activation throughout peri-calcarine cortex. Correlation maps made by discarding the activation period, and considering only pre- and post-stimulation BOLD behavior show resemblance to the CBF-weighted more than the BOLD-weighted activation map. Difference maps between BOLD activation and BOLD undershoot show that non-undershooting areas are in extrastriate visual cortex. These extrastriate areas also show less intense perfusion activation. If BOLD activation magnitude is related to the degree of perfusion increase (see Chapter 4), then extrastriate cortex has higher BOLD activation for per perfusion increase than does striate cortex. Increased BOLD sensitivity in the region where
there is no post-stimulation undershoot suggests that the process causing post-stimulation undershoot also causes reduced BOLD effect for a given perfusion change.

Figure 2.6: Activation, post-stimulus undershoot, and perfusion activation maps during response to a 60 s flashing checkerboard stimulus. Color correlation maps are shown overlaid on source echo planar images. (A) The first map is for BOLD response at stimulus onset, calculated by the iterative method described above, using only the onset time points (shaded bar). (B) The undershoot period was mapped by considering only points beginning 8 s after the cessation of stimulus (shaded bar). (C) Perfusion map during stimulus onset.

2.5 Computer Simulations of Physiology and BOLD contrast
2.5.1 Dynamic modeling of oxygen transport

Having established a link between NMR parameters and static physiological states above, we model oxygen transport dynamics in order to arrive at an estimate of BOLD timecourse for transient changes in blood flow, blood volume, and oxygen utilization. In particular, we are interested in whether the recent observation of uncoupling between CBF and cerebral blood volume (CBV) [36], or oxygen transport and metabolic oxygen demand changes [37] (Chapter 4) more consistently explain empirical data.
Unlike the CBF contrast case, observations of tissue oxygen dynamics are not limited by relaxation within the slice. Oxygen tension varies dramatically between arterial and venous circulations, so transit time becomes important. Also, transient disequilibria between compartments may take place. Therefore, a minimally-distributed, multi-compartment model is required in order to describe the expected dynamic effects of oxygen transport on BOLD signal.

![Figure 2.7: Three compartments for oxygen: capillary, tissues, and veins within an imaging volume. The capillary compartment is distributed through its length as it interacts with the tissue compartment through symmetric diffusive exchange. Oxygen entering via arterial oxyhemoglobin is either metabolized within tissues or exits via veins.](image)

Because of the importance of capillary transit to deoxygenation of blood, we treat the capillaries as a distributed compartment (subscript \( c \) in Eqs. (2.7-2.12)), interacting with a well mixed tissue compartment (subscript \( t \)) and a single venous outflow compartment (subscript \( v \)). Only half of the oxygen content is hemoglobin-bound; the rest is dissolved in the much larger tissue volume. The presence of a tissue oxygen pool allows the possibility of transient disequilibrium between tissue oxygen and the end-capillary oxygen tension.

The above model excludes possible shunting of oxyhemoglobin around the capillary network and into veins. While counter-current vascular systems have been shown to cause large shunts of oxygen from artery to vein bypassing the capillary bed [38] which may
effect deoxyhemoglobin responsiveness to blood flow [39], the vascular arrangement in
the brain renders this less likely: the shortest path through tissue is likely through the
microvascular bed [40, 38].

<table>
<thead>
<tr>
<th>Dynamic Mass Conservation</th>
<th>Steady State Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c \frac{dc_c}{dt} = -F \frac{dc_c}{dx} + \frac{k}{\alpha}(c_c - \alpha c_v)$</td>
<td>$c_c(x) = c_a - \frac{R}{FE} \left(1 - e^{-\frac{k}{\alpha F} x}\right)$ (2.7)</td>
</tr>
<tr>
<td>$V_r \frac{dc_r}{dt} = -k(c_r - \tilde{c}_r/\alpha) - R$</td>
<td>$\alpha c_r = c_a - \frac{R}{FE}$ (2.10)</td>
</tr>
<tr>
<td>$V_v \frac{dc_v}{dt} = -F(c_v - c_c)</td>
<td>_{x=1}$</td>
</tr>
</tbody>
</table>

Table 2.1: State equations and steady state solutions for the 3-compartment model with a
distributed capillary compartment.

Three first-order linear state equations (Eqs. 2.7, 2.8, 2.9) and their steady-state solu-
tions (Eqs. 2.10, 2.11, 2.12) describe the system. Here $F$ is blood flow, $V_c$, $V_v$, $V_r$ are the
compartmental volumes, $c_c$, $c_v$, $c_r$ are compartmental oxygen concentrations, $k$ is resis-
tance to diffusion of oxygen from capillaries to tissue, $\alpha \approx 50$ is the ratio of oxygen solu-
bility between whole blood and tissue (the large value of $\alpha$ accounts for hemoglobin
oxygen affinity), and $R$ is the cerebral metabolic rate for oxygen ($CMRO_2$). The dynamic
equations allow for time-varying volumes, flow, and oxygen utilization. Numerical solu-
tions of these coupled partial differential equations generated the simulation data shown
below.

This model distinguishes between the unidirectional extraction coefficient ($E$) from the
total oxygen extraction fraction ($OEF$) (Eq (2.13)) [6, 41, 3]. This is the result of the
assumption that oxygen enters and leaves the tissue with the same hindrance in both direc-
tions ($k1 = k2$ in Eq Figure 2.7) and is metabolized at rate $k3$. These assumptions conform
to those of a modified Kety model [42], assuming a single lumped average transit time
represents all vessels.
\[ OEF = \frac{c_a - c_v}{c_a}; \quad E = 1 - e^{-\frac{k}{\alpha F}} = \frac{c_a - c_v}{c_a - \alpha c_v} \] (2.13)

The steady state solutions show that \( E \) limits the maximum metabolic delivery of oxygen: \( R_{\text{max}} = \lim_{F \to \infty} E F c_a = k/\alpha \). Since \( E \) decreases with increasing blood flow due to decreasing transit time for oxygen, the required flow increases to meet oxidative metabolism demand can be significantly greater than a simple proportionality. If the desired metabolic rate \( R \) is nearly the same as the diffusion limitation constant \( k/\alpha \), then \( F \) must be very high to allow near maximal oxygen diffusion to tissue. This is the mathematical basis for the apparent uncoupling of blood flow from oxygen demand due to diffusion limitation for oxygen delivery [9, 7]. At low diffusibility \( k \) (and thus low unidirectional extraction \( E \)), the tissue oxygen pool is separated from the blood oxygen by a diffusion barrier. This barrier increases the delay between changes in tissue oxygenation and blood oxygenation. The

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_t/k )</td>
<td>3 s</td>
<td>Equilibrium time constant for tissue oxygen due to diffusion from blood.</td>
</tr>
<tr>
<td>( \alpha V_c/k )</td>
<td>3 s</td>
<td>Equilibrium time constant for capillary oxygen due to diffusion into tissue</td>
</tr>
<tr>
<td>( V_c/F )</td>
<td>2 s</td>
<td>Blood flow transport through capillary.</td>
</tr>
<tr>
<td>( V_v/F )</td>
<td>2 s</td>
<td>Blood flow transport through vein.</td>
</tr>
<tr>
<td>( \alpha F/k )</td>
<td>0.70</td>
<td>Spatial exponential constant for oxygen distribution in unit length capillary.</td>
</tr>
</tbody>
</table>

Table 2.2: Exponential constants for coupled transport equations.

High inflow rate of hemoglobin-bound oxygen (\( \alpha = 50 \)) leads to a relatively diffusion-limited regime for the transport of oxygen from capillary to tissue, even though \( k \) itself is quite high. In fact, at rest the unidirectional extraction (Eq (2.13)) has been estimated at \( E \approx 0.5 \) [6, 43].
Insight to the time varying nature of variables \((F, R, c_i)\) can be gleaned by examining time constants in the coupled system (Table 2.2). The barrier to oxygen between blood and tissue is embodied in the time constants \(\alpha V_c/k\) for blood oxygen concentration and \(V_r/k\) for tissue oxygenation. Because the affinity of hemoglobin for oxygen offsets the much larger tissue volume almost exactly, the wash-in and washout constants are relatively symmetric, both 3 s for our simulation. The perfusion residence times in capillary and vein are given roughly by the time constants \(V/F\) for each compartment. For both capillary and vein, \(V/F = 2\) s in our simulations. The final constant in Table 2.2 describes the longitudinal decay of diffusion along the capillary, an exponential distribution determined by inflow of fresh oxyhemoglobin and longitudinal diffusion of oxygen to tissue. The spatial equilibration constant in unit-length capillary \(\alpha F/k\) corresponds to \(E = 0.5\) (Eq (2.13)).

2.5.2 Simulation Methods

We calculated time course of deoxyhemoglobin concentration in response to changes in CBF, CBV, and metabolic oxygen uptake (CMRO2), utilizing the Lax method for finite difference representation of partial differential equations [44], with 500 capillary bins of length \(\Delta x\) and time steps \(\Delta t\) such that \(\frac{F \Delta t}{V_r \Delta x} \approx 0.9\) to maintain the Courant condition of minimal added diffusion to maintain numerical stability [44]. We then converted deoxyhemoglobin concentrations in the capillary and venous compartments, along with time varying volume fractions, into MRI signal through numerical look-up to curves generated using Monte Carlo simulation for the susceptibility contrast model [26]. This model predicts changes in signal attenuation for changes in magnetic susceptibility \(\Delta \chi\) for capillary and venous blood vessel geometries at 1.5T. Simulations were performed for a range of starting conditions and models of oxidative and volume coupling to blood flow, in order to assess the sensitivity to parameter changes and to test certain hypotheses detailed below.
Initial values for $c_i, V_i, F, \alpha$, and $\text{CMR}O_2$ (variable $R$ in equations) from the literature are shown in Table 2.3. Brain activation was simulated by ramp changes in $F$, accompanied by changes in $V$ varying as the 0.4 power of $F$. Finally, temporal decoupling of $V$ from $F$ and of CMRO2 from $F$ is simulated and resulting post-stimulation undershoot evaluated.

<table>
<thead>
<tr>
<th>Name</th>
<th>Variable definition</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Volume</td>
<td>$V_v = 2%, V_c = 2%$</td>
<td>[1, 2]</td>
</tr>
<tr>
<td>Blood Flow</td>
<td>$F = 56 \text{ ml/100 g/min}$</td>
<td>[3]</td>
</tr>
<tr>
<td>Blood Oxygenation</td>
<td>$c_a = 0.2 \text{ ml O}_2 / \text{ml blood}$</td>
<td>[4, 3]</td>
</tr>
<tr>
<td>O$_2$ Metabolism</td>
<td>$R = 4.2 \text{ ml O}_2/100g/min$; $R/F = OEF = 0.4$</td>
<td>[5, 3]</td>
</tr>
<tr>
<td>O$_2$ Extraction</td>
<td>$E = 0.5$, results in transport $k = 1950 \text{ ml/100 g tissue/min}$</td>
<td>[6, 7]</td>
</tr>
<tr>
<td>Hemoglobin O$_2$ Affinity</td>
<td>$\alpha = 50$ (whole blood vs. tissue O$_2$ solubility)</td>
<td>[6, 4]</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>Capillary Hct $= 30%$; Venous Hct $= 40%$</td>
<td>[8]</td>
</tr>
</tbody>
</table>

Table 2.3: Oxygen transport simulation parameters.
2.5.3 Simulation Results

Each figure shows idealized time courses used in the simulations in its upper portion, and the simulated result in the lower portion. Except where otherwise noted, values from Table 2.3 were used in the simulations.

**Sensitivity to baseline conditions:** Several of the baseline parameters (Table 2.3), including arterial oxygen saturation, hemoglobin oxygen affinity, and microvascular hematocrit, are well known constants. A few of the constants may vary by region but may not vary through time. The three most physiologically valuable parameters, however, vary both in their resting state and through time: blood flow, blood volume, and metabolic rate for oxygen. Ratios of these are important determiners of MRI signal through their effects on deoxyhemoglobin (Eqs 2.10-2.12). We first explore the parameter space by varying these parameters separately for the resting state, and show how baseline conditions impact the BOLD signal changes relative to a fixed percentage blood flow increase.
A. CBV Dependence.

**Figure 2.8:** Simulations from idealized input variables. A. Dependence on baseline CBV fraction. Change in CBF and CBV only, starting at baseline conditions in Table 1. CBF is increased by 70%, CBV by 30%. Baseline CBV simulated at 1% through 3% of tissue volume. B. Effect of resting OEF on activation. Baseline R (CMRO2) is varied from 2.1 to 6.3 ml O2/min/100 g tissue, which changes the available headroom for BOLD contrast response.

**Resting blood volume:** Since the BOLD effect is dependent on tissue deoxyhemoglobin concentration, blood volume has a direct, nearly proportional effect on the magnitude of signal changes. Microvascular blood volumes have been measured between 2% and 4% of tissue volume; within this range, the signal dynamics are similar, but there is a noticeable effect on maximum signal change. Figure 2.8A shows this proportional effect.

**Venous oxygenation: Oxygen Extraction Fraction:** Figure 2.8B shows the dependence of the expected fMRI response on the initial blood oxygenation state. Larger resting extraction provides more “headroom” for functional activation. Because there is more signal attenuation with larger resting oxygen extraction, there is more signal increase for a given flow increase. Thus, the resting ratio of oxygen uptake to oxygen delivery is crucial.
for setting the percent signal change. Fortunately for interpretability, there is a fairly narrow range of accepted venous oxygen saturations. Jugular venous saturation below 50%, for example, is correlated with brain tissue hypoxia. Because the gray matter has a higher metabolic rate than most of the tissue draining to the jugular veins, one can infer that venous oxygen saturation is 60% or higher in the resting state.

**Coupling of Oxygen Extraction to Flow:** Theoretically, the extraction parameter $k$, which determines the oxygen extraction coefficient, $E$ (Eq (2.13)), is proportional to diffusion capacity between capillary and mitochondrion. Capillary geometry sets a limit on the upper bound of $E$ for a given microvascular network.

Because the temporal relationships between changes in blood flow, blood volume, and metabolic rate are uncertain, simulations were performed to separate the effects.

Figure 2.9A shows the substantial reduction of signal response which can occur when metabolic rate is increased quickly by activation. Note that even for equal fractional changes in $R$ and $F$ there is still a positive signal change due to flow, because $E$, and thus capillary deoxyhemoglobin, decreases as flow as flow increases (see Eq (2.7)). This may be offset, however, by the increase in venous volume. For large increases in metabolic rate, there is a signal dip below baseline both before and after the stimulus period, which reflects disequilibrium in the capillary during a single transit time for a rapid change in metabolic rate.

Figure 2.9B shows the effect of different values for $E$. The value has important implications for blood-tissue oxygen dynamics [43]. At low $E$, the tissue is starved of oxygen relative to the capillaries, causing a the small post-stimulation undershoot seen in these simulations. At high $E$, on the other hand, the transient mismatches between oxygen supply and demand are accentuated (the early dip and late overshoot in the simulations) which are not found in experimental data. A moderately low value of $E$ is chosen for the
other simulations, in accordance with the theory of diffusion-limited supply of oxygen to brain [6].

Figure 2.9: Coupling activation to hemodynamics and metabolism. A. When R (CMRO2) increases during activation, the oxygen extraction increases. This reduces the BOLD signal by increasing the production of deoxyhemoglobin. When the CBF and CMRO2 increase the same amount (AR=AF), almost all of the BOLD effect is removed. B. The dynamics of oxygen delivery are affected by the extraction coefficient E. For high extraction coefficient, there is a small transport induced early dip as metabolism quickly increases deoxyhemoglobin before it can be washed out by increased blood flow. For low extraction, there is a small post-stimulation undershoot as tissue oxygen supplies are gradually restored after depletion during the stimulation interval.
A. Lag in blood volume.   B. Lag in metabolic rate.

**Figure 2.10:** Temporal decoupling as a source of transient fMRI signal rebound. A. Volume changes are delayed with respect to flow changes, either as a change in blood volume response time constant (middle curve), or as a simple delay, which gives the largest post-stimulation undershoot. Change in the coupling time constant provides insufficient rebound to explain the measured undershoot. B. CMRO2 return to baseline is delayed by 5 and 10 seconds. The temporal behavior is governed by tissue oxygenation kinetics. The time constants resemble closely those measured for post-stimulation undershoot in BOLD fMRI.

If the onset time for oxidative metabolic change is slow, the effect will be visible both during and after activation [45]. While the signal reduction during activation is smaller than the post-stimulation undershoot in signal, there is a substantial effect during the activation period.

**Comparison of model to experiment:** Using physiological values measured by PET [3], the simulations are similar in both magnitude and temporal behavior to empirical data.
Figure 2.10 shows a comparison to primary visual cortex (V1), imaged with ASE EPI, TE=70 ms, TR=2 s before, during, and after 32 s stimulation with 8 Hz flickering checkerboard. The early dip seen as a tiny deflection in the simulation signal, and reported elsewhere [46, 47] with higher magnitude, is not observed in our fMRI data. The simulation is the expected signal resulting from square on-off activation induced increase in R by 20%, accompanied by flow increase of 50% and capillary and venous volume increase of 17% (0.4 power of flow [1]). A simulation in which the on-time of R was extended by 15 s to mimic oxygen debt matches the post-stimulation behavior remarkably well (Figure 2.10).

While it is possible for low E to deplete tissue oxygen, and can result in a small post-stimulation undershoot, the system cannot generate a large post-stimulation undershoot unless R or CBV is changing over the post-stimulation interval out of step with CBF.

**Figure 2.11:** Comparison with experiment: 10 s delay in R metabolic rate return to baseline after causes a post-stimulation undershoot that matches experimental data.
2.6 Discussion

The central thesis of this chapter is that blood flow-weighted and blood oxygenation-weighted MRI dynamics are closely coupled via a mechanism which is directly related to the coupling between perfusion and metabolism. Where differences between these measurements exist, such as in the example of the striate cortex of the visual system, there must be some physiological variation to account for the difference. Because of the short time scale of these noninvasive measurements, this is uncharted territory: future applications may provide detailed analysis of dynamic metabolic or perfusion state between processing strategies, for instance between mitochondria rich processing streams in the visual system (for example Figure 3.8, Chapter 3).

Theory, simulation, and experimental findings together elucidate mechanisms and highlight unresolved issues regarding the origins of transient BOLD signal phenomena. These are discussed below, organized according to the signal epochs defined in Figure 2.1 of the Introduction.

2.6.1 Baseline

In this chapter, we have assumed that changes in blood flow, blood volume, and oxygen utilization follow an orderly progression locked to the stimulus. Variations in the baseline levels of these variables would cause variations in the activation-induced BOLD signals, as we have demonstrated through simulation. Experimentally, this assertion is not verified. Deliberate modifications of the baseline state, for instance by super-activation on top of a graded minimally-to-moderately activating stimulus used as a baseline would offer an experimental approach to this question.

Within the physiologically relevant range, our simulations show baseline variations to affect only the proportionality constants between physiological measurements and MRI, rather than the gross behavior of the model. Because most of the unknown parameters are
fixed, a relative dynamic analysis between MRI parameters is still possible, the results of which place further limits on the possible parameter values.

The widely varying BOLD signal sensitivity of experimental subjects raises the question of which baseline parameters are more conserved. Teleologically, it would seem that brain metabolism and blood flow would be relatively consistent across subjects, and has been measured as such [5, 48, 49], whereas the baseline concentration of deoxyhemoglobin, which is dependent on venous volume and oxygen extraction fraction, would seem to be a physiologically irrelevant quantity, and thus may be less well conserved. If true, finding wide variation in deoxyhemoglobin would explain the variability between subjects. This question is addressed in Chapter 4.

2.6.2 The early dip

In our simulations, the early dip is typically 7% of activation signal magnitude. Comparing with our experimental BOLD signal standard error twice that, we must conclude that search for the early dip must be performed using higher signal-to-noise methods. In addition, such an order of magnitude decrease in expected feature magnitude would be a departure from the current set of assumptions. In particular, the assumption of a steady baseline may be violated at that level of significance. Intrinsic rhythms (detailed in the baseline section of the introduction) certainly may be entrained and cause small stimulus-locked fluctuations in signal which may be falsely reported as physiologically linked with neuronal activation. For instance, this observer has seen subjects involuntarily inhale on stimulus presentation; such a rapid, short decrease in intrathoracic pressure could manifest itself as a small decrease in cerebral venous pressure, and a sudden, short, elastic, cerebral venous volume dip. In spite of reports to the contrary [11, 17], functional MRI and other macroscopic techniques may thus be ineffective tools in the pursuit of explanations or regional specificity of tiny short-term fluctuations such as the early dip.
2.6.3 Rise to peak and plateau

Our observations and simulations both show rapid response to changes in neuronal activity, given rapid blood flow changes. With notable exception of some examples presented in Chapter 3, most functional MRI time-course measurements show a steady plateau phase without significant early loss of BOLD signal. Our data reported here also conforms to this generality. Our simulations, however, do not: in fact, when excessive stimulus locked metabolic change (Figure 2.9A) or time lags for metabolism or blood volume (Figure 2.10A) are introduced into the simulation, there is an early loss of BOLD signal during activation which is mirrored by the post-stimulation undershoot. Only when the on-times of physiological parameters are synchronized and the off-times are not is a post-stimulation undershoot produced without a mirrored early plateau drop (Figure 2.10B, Figure 2.11).

Others have reported early loss of plateau signal in the first minutes of stimulation [50], and one group reports that this is accompanied by transient increase in brain lactate [45]. Our contradictory evidence is presented in Chapter 3, where we measure plateau signal time course for a variety of stimuli and pulse sequences.

Loss of plateau signal can be due to one of several physiological processes. Loss of uncoupling between blood flow and metabolism, in other words, substantial increase in oxidative metabolism, would cause the steady-state activation signal to drop. However, because there is a tissue oxygen pool of substantial size and a diffusion gradient from the capillary, there would be an initial rise in signal until tissue oxygen is depleted (Figure 2.9A). The governing deoxyhemoglobin concentration time constant for this situation is $\alpha V_c/k$ (Table 2.2). For $E = 0.5$, this time constant is 3 s; 96% of equilibrium would be reached within 10 seconds.
Uncoupling of timing between blood flow increase and oxygen demand increase can also cause fluctuations in plateau signal. If the cerebral metabolic rate for oxygen increases 16% with activation, but is delayed in doing so, then the BOLD signal would lose 40% of its original value sometime during the stimulus. Such a finding has not been observed, but is postulated as a possible cause of the post-stimulation undershoot.

Uncoupling of timing between blood flow and blood volume would likewise cause BOLD activation fluctuation. If blood volume change of magnitude predicted by prior steady state measurements [1] is depicted changing at a different rate than blood flow, then the activation period signal could drop as much as 30% from its original value (Figure 2.10). Although such a decoupling has not been postulated or measured previously, recent work in our laboratory (Mandeville, personal communication) suggests a 15 s time constant for changes in blood volume in response to changes in blood flow. As seen in the figure, however, such an exponential delay would have minimal effect on the BOLD signal, as compared with an outright delay.

The magnitude of the BOLD signal in response to CBF changes is a matter of interest: because, as shown in Chapter 4, understanding the quantification of BOLD magnitude would allow dynamic measurement of oxidative metabolism. One aspect of BOLD magnitude predicted by our models is that of a saturation effect. This saturation effect is inherent in the design of BOLD effect models, as they are predicated on deoxyhemoglobin washout as the only important contrast mechanism. Increase of CBF decreases the remaining tissue deoxyhemoglobin available; this, in turn, decreases additional available BOLD signal. Figure 2.4 shows how BOLD signal becomes less sensitive to CBF as change in CBF increases. This is experimental verification of the threshold effect.

In fact, nonlinear fitting of the data in Figure 2.4 using our model \( c_v = c_0 - k/(\alpha F) \) as a starting point (fitting \( y = B_{max}\left(1 - \frac{k}{S_{CBF} + F_0}\right) \) by assuming no metabolic rate change
between data points) gives a maximum BOLD signal of 10.3%, and maximum CBF increase of 71%, which are within reasonable range of our measured maximum BOLD signal and flow in Chapter 4, albeit uncalibrated. Additional measurements of graded responses for BOLD and CBF would provide valuable new inputs to the model, and would help to establish additional findings regarding physiological contributions to functional MRI.

2.6.4 Decay, undershoot, and recovery

To date, the only major poorly explained feature in BOLD signals is the post-stimulation undershoot and recovery. Our experiments show the post-stimulation undershoot to occur in overlapping but distinct regions from maximum BOLD activation changes, to be absent from CBF activation studies, and to inversely correlate in magnitude with BOLD. Singularly interesting is the observation that the largest BOLD signal changes to not show post-stimulation undershoot, whereas small to moderate changes in BOLD show an undershoot which is relatively independent in magnitude from the activation, as evidenced by a linear cutoff in data points in the inset of Figure 2.5. The clear implication is that the pixels with large activations do not have the ability for post-stimulation undershoot. A corollary is that the process responsible for post-stimulation undershoot robs the activation signal of some of its magnitude, and thus is a phenomenon which takes place during the entire stimulation period. This clue to the physiology will be fleshed out in the comparison with our models.

The modeling of this chapter takes two steps toward understanding the phenomenon: 1) it establishes hypothetical physiological mechanisms for BOLD undershoot, and 2) it describes linkage between these mechanisms and mirrored findings which would be expected under these hypotheses. The first hypothesis is that BOLD undershoot is due exclusively to oxygen transport phenomena rather than metabolic changes or temporal
uncoupling. The second is that it is due exclusively to delay in CBV change after CBF change. The third hypothesis is that it is due to persistently increased metabolic rate for oxygen after stimulation ceases.

**Case 1: Undershoot caused by oxygen transport dynamics:** The impact on oxygen transport between capillary and tissue is noticeable in the simulations, but it is insufficient both in magnitude and in temporal extent to explain the post-stimulation undershoot. Tissue transport is most in play during stimulations which cause large increases in metabolic rate for oxygen, such as in Figure 2.9A. For the curve corresponding to 50% increase in metabolism, note the transient rise in BOLD early in the plateau phase, and the corresponding mirrored post-stimulation dip below baseline. These features have a 3 s time constant. One can imagine voxels with high blood volume and high oxygen extraction in which the residual BOLD effect is still respectable, and which shows mirrored peaks in early activation signal and post-stimulation undershoot. (For an example of an experiment matching this description, see Figure 3.8.) For the current experiment which has large post-stimulation undershoot and no early stimulation peak, there is no mechanism whereby simple compartmental sequestration of oxygen can explain the data.

Might the post-stimulation undershoot be attributable to the Bohr effect acting within capillaries to desaturate hemoglobin? Activation-induced tissue acidification may indeed increase tissue pO2 substantially by locally shifting the hemoglobin dissociation curve. However, after the tissue pO2 rises to a new equilibrium, there would not be a sustained drop in venous oxygen saturation, because the oxygen has to have somewhere to go. The oxygen saturation must necessarily follow Eq (2.12) after capillary-tissue equilibrium is achieved. Since this capillary to tissue rate constant $k/\alpha V \approx (3\ s)^{-1}$ is small, the Bohr effect should not have an effect on the MRI signal lasting more than a few seconds.
Before discarding this notion, we consider the possibility of oxygen shunting around the capillary bed as a confounding influence [39, 40]. Unlike other organs such as the heart, the arterial and venous structures in the brain do not run in a counter-current parallel arrangement. Thus there is little ability for oxygen to diffuse directly from oxyhemoglobin in the terminal arteriole to deoxyhemoglobin in the draining veins. While small gradients in pO2 have been observed along brain veins [38], the corresponding oxygen saturation changes along the veins are not significant. Therefore, we believe capillary deoxyhemoglobin to reflect oxygen transport rather than pH.

**Case 2: Undershoot caused by blood volume changes:** Blood volume changes are known to accompany blood flow changes, but until now it was assumed that blood volume matched blood flow in temporal detail. One might hypothesize instead that blood flow increases are caused by vasodilation, and that blood volume changes should therefore precede blood flow. On the other hand, since our BOLD measurement is confined to the high-compliance venous bed, one might consider that venous volume expands as the pressure in the bed increases, a supposition which would imply a delayed increase in blood volume changes with respect to blood flow. Filling time for veins \(V/F = 2\) s indicates that only a short time should be required for redistribution of blood volume after a flow change, if indeed the volume changes are dependent only on intravascular pressure and elastic vessel walls. Therefore, if blood volume changes out of synchrony with blood flow, the reasons must lie in details of vascular control in the arborized network of vessels, beyond the scope of the current model. Indeed, recent measurements in the anesthetized rat indicate a 15 s lag in blood volume change compared to blood flow change [36]. This motivated our use of a 15 s time constant model for temporal uncoupling of CBF from CBV in the simulations.
As with blood volume changes during the signal plateau discussed above, a simple 15 s time constant exponential sluggishness in blood volume does not produce sufficient timely BOLD signal change to explain the post-stimulation undershoot. Nonlinearities, such as those proposed in a “balloon model” [51], or alternatively a simple asymmetric delay in the blood volume response, can modulate the BOLD signal easily in a variety of ways (Figure 2.10). Therefore, care must be taken in interpreting dynamic changes in BOLD as changes in metabolic rate when the dynamics of blood volume have not been measured. Future experiments to establish blood volume dynamics during functional stimulation, using intravascular extrinsic contrast agents, will be crucial to a detailed knowledge of transient hemodynamics and metabolism.

**Case 3: Undershoot caused by metabolic rate changes:** An assumption that oxidative metabolism increases during functional activation anticipates the measurement of metabolic changes described in Chapter 4. Also shown in that chapter is that the measured metabolic rate increase for oxygen of 16% produces sufficient deoxyhemoglobin to reduce the BOLD signal by more than 30%. It stands to reason that such a metabolic rate increase, if sustained after blood flow returned to normal, would cause a rebound in signal with magnitude on the same order as the (metabolically reduced) activation signal itself. Simulation results in Figures 2.10 and 2.11 demonstrate this finding. In addition, step changes in metabolic rate give rise to relatively slowly-varying changes in BOLD due to the slow reestablishment of tissue oxygen in the face of metabolic changes. Figure 2.11 demonstrates similarities between an example experimental BOLD time course and simulated undershoot, which match well. Because of the possibility of CBV modulation of BOLD, however, metabolic rate change is not established as the sole originator of the undershoot.
Experimental evidence: does it break the deadlock? We have established that either CBV dynamics or oxygen metabolism dynamics are likely responsible for the post-stimulation undershoot in BOLD signal. We now examine the likelihood that either of these would be expected to behave according to details of our experimental findings. These details are lack of undershoot in very large BOLD changes (Figure 2.5), constant undershoot for moderate BOLD changes (Figure 2.5), and regionally specific high undershoot in regions of higher CBF signal (Figure 2.6).

The first observation is that there is no undershoot for pixels with BOLD activation greater than 5%. Conventional wisdom is that these regions are affected by large veins, or are dominated by high blood volume, in order to have such seemingly supra-physiological BOLD activation signals. One would expect relatively low metabolic rate sensitivity in such a vein-dominated region which may be draining much more than a small active cortical area. In contrast, one might expect blood volume effects to be larger, given the larger baseline blood volume. Thus, the absence of undershoot in high BOLD regions points towards metabolic rate undershoot as more consistent with the data.

The other observations are that undershoot magnitude seems uncorrelated to BOLD activation magnitude, when the activation is less than 5%, and also, that undershoot mimics CBF better than it mimics BOLD activation in its regional specificity. A muted implication here is that the undershoot and CBF somehow may change together to give constant activation with widely varying CBF and undershoot. Here the story is balanced. If either CBV or metabolic rate increases with CBF mitigate against BOLD increases, then BOLD will be more consistent between pixels than either CBF or CBV or metabolism changes. If the increase (either CBV or metabolism) is also causing the undershoot, then the undershoot would be expected to be large where CBF is large, and small where CBF is small. Either CBV or metabolism could equally explain the finding that undershoot mimics CBF
change better than BOLD activation mimics CBF. Which variable, CBV or metabolism, would be most likely to be decoupled from CBF? This is the remaining question. Its answer will provide the solution for measuring transient changes in oxidative metabolism using functional MRI.

2.7 Conclusion
The findings presented in this chapter establish the phenomenology of BOLD signals in visual cortex, and provide a well-grounded physical model for their production. Based on similarity of predicted to observed activation signal magnitudes, absence of observable early dip, and physiological candidates to explain the post-stimulation undershoot, we show that our simulations are capable of describing the phenomena of interest in sufficient detail to mimic all of observed findings. In addition, the simulations make predictions and raise physiological questions which point toward additional experiments. Tentative labelling of the post-stimulation undershoot as possibly representative of oxidative metabolism change provides a tantalizing potential for measuring oxidative metabolism dynamics.
References


Chapter 2


Chapter 3

Hemodynamics and metabolism during prolonged brain activation

Life is short
and the art long
the occasion instant
experiment perilous
decision difficult

– inscription outside Harvard Medical School Quadrangle
3.1 Introduction

One avenue for the study of possible effects of oxidative metabolic processing during task activation is the observation of long-duration stimuli. The functional MRI signal during prolonged steady state extended duration stimulation has been studied by several groups [1, 2, 3, 4] with widely varying results.

Using a blood oxygen level dependent (BOLD) MRI contrast based FLASH technique, Hathout [4] reported an MRI signal decrease in visual cortex to baseline after 15 minutes of continuous visual stimulation. In a similar FLASH experiment Frahm [3] observed a return of oxygenation sensitive MR signal to baseline after 1 to 2 minutes of sustained activation. This group also reported a transient increase and decrease in lactate signal during the first 3 minutes, with sustained blood flow during the entire stimulation duration including the time during which the BOLD signal disappeared. This set of observations suggested a transition from anaerobic to aerobic metabolism after 2 minutes of sustained stimulation and a corresponding initial uncoupling, followed by recoupling of oxygen delivery to metabolic demand after quasi-steady state is achieved. A prolonged post-stimulation undershoot lasting 2 to 3 minutes was observed, much longer than the typical 15 to 60 s undershoot that we reported previously [5].

In contrast, preliminary results from our work [1] have shown a sustained elevation in both BOLD contrast based signal and cerebral blood flow (CBF), consistent with no return to a resting state coupling. These results have recently been corroborated by others who reported similar sustained activation BOLD signal behavior findings using FLASH and echo planar imaging (EPI) [6, 7].

Controversy regarding the signal change dynamics may exist partially because the BOLD signal depends on physiological and physical variables that are not easily separated from one another. As described using biophysical models [8, 9, 10, 11], the activation -
induced BOLD signal change is affected by blood volume and blood oxygenation changes, among other factors. Blood oxygenation is strongly affected by the interplay between blood flow and oxygen consumption. It follows that the MRI signal change dynamics will be strongly affected by the relative dynamics of flow changes, blood volume changes, oxygen consumption changes, and subsequent blood oxygenation changes. The relative effects of different stimuli on the dynamics, locations, and magnitudes of these changes and also of these changes on MRI signal have yet to be determined completely. The work in this chapter was directed at the goal of clarifying some of these issues in the context of extended stimulation.

In the first section, sensitization to oxygenation is obtained using echo planar (EPI) gradient-recalled echo imaging, with confirmatory comparison to long-TR BOLD contrast to eliminate any possible flow weighting, and comparison to interleaved spiral scanning, to compare effects of non-single-shot sequences [12, 13]. Signal sensitive only to blood flow is obtained using inversion recovery within the imaging slab [14, 15].

The second section consists of two parts which include: 2a) the use of different types of visual stimuli known to selectively activate regions in the visual cortex with different concentrations of mitochondria (“blobs” and “interblobs” [16]), and 2b) the modulation of brain activation stimuli which correspond to different neuronal habituation effects (i.e. decreased rate of neuronal firing over time).

Overall, we were able to observe both extended duration flow effects and extended duration oxygenation effects, and were able to characterize neuronal habituation effects, differentiating them from possible slow increases in oxidative metabolic rate.
3.2 Methods

Functional MRI using EPI was performed at 1.5 Tesla using a GE Signa/ANMR resonant gradient echo-planar imaging scanner. Multi shot spiral scanning was also performed in one study. A single plane was obtained and in-plane motion correction was performed. Studies which demonstrated excessive motion as determined by a motion detection algorithm were not included in the results.

3.2.1 Selective Physiological MRI

Part 1A. Selective oxygenation and flow sensitization: These studies established a preliminary assessment of the behavior of flow and oxygenation in the visual cortex during extended duration stimulation periods. Flow sensitive (inversion recovery EPI: TI = 1000 ms, TR = 3000 ms, TE = 20 ms: spin-echo) and blood oxygenation sensitive (gradient echo EPI: TR = 3000 ms, TE = 40 ms) images were obtained in four subjects. The voxel volume was 3.1 mm x 3.1 mm x 10 to 15 mm. The stimulus was 10 Hz full field black and white alternating checkerboard visual stimulation. Timing was: 1 min. off, 1 min. on, 1 min. off, 20 min. on, 1 min. off, 1 min. on, 1 min. off. Plots of signal vs. time were obtained from regions of interest created by voxels that demonstrated a correlation coefficient above 0.5 to a boxcar function for both the initial and final off - on - off periods, shown as time periods “A” and “B” in Figure 3.1.
Figure 3.1: The timing was: 1 min. off, 1 min. on, 1 min. off, 20 min. on, 1 min. off, 1 min. on, 1 min. off. The first and last 3 minute periods (epochs A and B) were used as control stimuli for assessment of gross motion or baseline drift. The regions of interest used for time course analysis were chosen from voxels which had a response that exceeded a correlation coefficient of 0.5 with a box car function for both epochs A and B.

Part 1B. Selective oxygenation sensitization: A time course series of blood oxygenation sensitive images (gradient echo EPI: TE = 40 ms) with even longer TR (TR = 10 sec) were used. Voxel volume was 3.1 mm x 3.1 mm x 10 mm. The stimulus was 10 Hz full field black and white alternating checkerboard. Timing was: 1 min. off, 1 min. on, 1 min. off, 20 min. on, 1 min. off, 1 min. on, 1 min. off. (again illustrated in Figure 3.1). A plot of signal vs. time was obtained in the same manner as in Part 1A. The extended stimulation effects were further characterized by calculating the slope of a linear fit to the MR signal in each voxel in the active region during the entire 20 minute stimulation period. From the calculated slopes of these linear fits, the percent signal decrease after 20 minutes, relative to the initial increase, was calculated.

Part 1C. Multishot spiral scan vs. EPI comparison: To begin determining if some of the discrepancies with other findings might be based on differences in MRI scanning techniques, this study used a multishot spiral imaging instead of EPI. The pulse sequence parameters (TR / TE / θ = 500 ms / 30 ms / 30°), were chosen to minimize inflow effects. Images having a resolution of 1.5 mm x 1.5 mm x 10 mm were obtained with 10 inter-
leaves. Instead of using standard multishot FLASH techniques for the comparison, spiral scanning was performed due to its high stability and greater insensitivity to pulsatile motion effects. The stimulus was a full field 10 Hz alternating checkerboard for 6 minute durations separated by 2 minutes of darkness.

3.2.2 Neuronal and Physiologic Parameter Modulation

Part 2A. Selective “blob” vs “interblob” analysis: Specific tissues are known to have different concentrations of cytochrome oxidase, indicating differences in mitochondrial concentration and therefore oxidative metabolic rate. The effects of these differences on the long term behavior of the BOLD signal were tested by using visual stimuli thought to selectively stimulate “blobs” (regions of high mitochondria concentration) vs. “interblobs” (lower mitochondria concentration) [17]. The stimuli used to activate blobs were low spatial frequency color stimuli; those used for activating interblob regions were high spatial frequency black and white stimuli. BOLD contrast was used. (gradient-echo EPI, TE = 40 ms). The timing of the blob - only stimulation was 2 min. off, 8 min. on, 2 min. off, 8 min. on, 2 min. off. The stimulation timing for the direct blob vs. interblob comparison was: 2 min. off, 6 min. blob stimulation, 2 min. off, 6 min. interblob stimulation, 2 min. off, 6 min. blob stimulation, 2 min. off, 6 min. interblob stimulation, 2 min. off.

Part 2B. Habituation effect analysis: Towards the goal of characterizing neuronal habituation effects (i.e. decreased neuronal firing over time) on blood flow and oxygenation, stimuli thought to cause different degrees of neuronal habituation (reduction in neuronal firing) were used. BOLD contrast fMRI (gradient-echo EPI, TE = 40 ms) was compared to PET with matched paradigm. The stimuli that were used included a flashing diffuse red, flashing red LED goggles (Grass, Inc.), flashing white alternating projected checkerboard,
and continuous “on” featureless white background. The stimuli durations ranged from 6 minutes to 50 minutes.

These measurements were compared with previously unpublished PET results, courtesy of Dr. Peter Fox. Cerebral blood flow was measured 40 seconds after bolus intravenous injections of $^{15}$O-labeled water at 10 minute intervals [18] during 50 minutes of either continuous 8 Hz full field alternating red and black checkerboard stimulation or vibrotactile stimulation.

3.3 Results

3.3.1 MRI Parameter Modulation

**Part 1A. Selective oxygenation and flow sensitization:** The voxels that demonstrated a correlation coefficient greater than 0.5 for both the first and last 3 minute epochs (1 min “off”, 1 min “on”, 1 min “off”) are shown in Figure 3.2 for all four subjects. The signal from these voxels were averaged over space, normalized, then averaged across the four subjects to create the flow - weighted time course series and the oxygenation - weighted time course series shown in Figure 3.3. For each subject, flow and oxygenation remained elevated for at least the first 10 minutes of stimulation. A small and variable decrease in both flow and BOLD signal occurred in two subjects after about 7 to 10 minutes. An undershoot was observed following all stimulation periods for BOLD weighted sequences but not for flow weighted sequences, as observed previously [14, 5].
Figure 3.2: Activation images obtained from the four subjects in the study in part one. BOLD contrast were achieved using T2* - weighted EPI: TR = 3000 ms, TE = 40 ms, and the flow contrast was achieved using inversion recovery EPI: TI = 1000 ms, TR = 3000 ms, TE = 20 ms: spin-echo. Voxels demonstrating a correlation coefficient greater than 0.5 with a box car function for both epochs A and B are shown in white. The signals from these voxels were averaged to create the time course series shown in Figure 3.3.
Figure 3.3: Averaged (across four subjects) (a) BOLD contrast signal and (b) flow contrast signal from the visual cortex during extended duration stimulation. Both blood oxygenation and flow remain elevated during the entire 20 minute stimulation duration.
Part 1B. Selective oxygenation sensitization: Figure 3.4 is a plot from the voxels that were chosen in the same manner as in Part 1. The BOLD signal, now having contrast completely independent of flow effects, due to the use of a TR of 10 sec, remained elevated for the entirety of the stimulation duration. A simple display of a spatially averaged time course is potentially misleading if significant spatial heterogeneity in the signal response behavior exists. Figure 3.5 addresses this issue. The average fractional signal change, relative to baseline, during this time period was $1.75 \pm 0.11\%$. The maximum fractional signal change was 4.9 percent. Figure 3.5C shows the calculated percent change after 20 minutes relative to Figure 3.5B, the initial fractional signal change. The use of a voxel-wise linear regression calculation and subsequent fractional signal decrease calculation allowed the entire time course to be used in the analysis. The average signal change after 20 minutes was $-0.5\% \pm 0.46\%$. The changes ranged from +0.78\% to −3.89\%.

**Figure 3.4:** Time course series obtained using a similarly T2*-weighted sequence as in Part 1a, but with a TR of 10 sec to further reduce inflow sensitivity. The signal, exclusively sensitive to oxygenation changes, remains elevated for the entire 20 minute 10 Hz alternating checkerboard stimulation duration.
Figure 3.5: Assessment of the spatial heterogeneity of the extended duration signal changes. **A.** Anatomical image. **B.** Percent change image: first two minutes of the extended duration stimulation relative to the one minute baseline (rest state) preceding it. The average fractional signal change, relative to baseline, during this time period was 1.75 ± 0.11%. The maximum percent change was 4.9%. **C.** Calculated percent change, relative to the first 2 minutes of activation, at the end of 20 minutes stimulation. The calculated percent change, after 20 minutes, relative to the first 2 minutes, ranged from 0.8% to -3.9%. The average change was -0.5% ± 0.46%. To calculate the maps, a voxel-wise linear fit to the MRI signal during the 20 minute stimulation period was first performed. Second, based on the slope of the fitted line in each voxel, the fractional signal change at the end of 20 minutes was calculated. This allowed the use of the entire time series, rather than only a few images at the beginning and the end of stimulation, to spatially map long term effects.

**Part 1C. Multishot spiral scan vs. EPI comparison:** Figure 3.6 shows a time course MRI signal obtained from the visual cortex obtained using inflow-insensitive spiral scanning (TR = 500 ms, TE = 30 ms, and θ = 30°). The signal remains elevated during the entire 6 minute stimulus duration. A post-stimulus undershoot, lasting approximately one minute, was also observed.
Figure 3.6: Blood oxygenation sensitive (TE = 30 ms, TR = 500 ms, \( \theta = 30^\circ \)) signal from the visual cortex during 10 Hz alternating checkerboard stimulation obtained using multi shot spiral scanning. The signal remains elevated during the entire stimulation duration, and shows an undershoot lasting approximately one minute following the stimulation.

3.3.2 Physiologic Parameter Modulation

Part 2A. Selective “blob” vs. “interblob” analysis: Figure 3.7 shows the time course signal from the visual cortex during “blob” stimulation lasting 8 minutes. Two on-off cycles were averaged over time to create the time series display. The BOLD signal remained elevated and did not show a clear undershoot following stimulation. Figure 3.8 shows a time course comparison in which a diffuse red stimulation (“blob” stimulation) was alternated with a high spatial frequency black and white alternating pinwheel stimulation (“interblob” stimulation). The two minute control periods consisted of darkness. Two on-off cycles were averaged over time to create the time series display. The BOLD signal remained elevated for the entire 6 minute blob and interblob stimulation durations.
Figure 3.7: Blood oxygenation sensitive (TE = 40 ms, TR = 4 sec, \( \theta = 90^\circ \)) signal from the visual cortex during eight minutes of “blob” stimulation. Even though the stimulus selectively activates areas of high mitochondria concentration, the extended duration signal behavior appears to remain unaffected, although the data are noisy. Interestingly, an undershoot is not apparent following the cessation of the stimulus.

Lastly, it is interesting to note that, while blob and interblob stimuli gave similar BOLD signal increases, the post stimulation undershoot was significantly more apparent for the interblob stimulus than for the blob stimulus. Also apparent in Figure 3.8 is that the interblob stimulus elicited a larger initial signal increase before reaching a steady state elevated level after about one minute.
**Figure 3.8:** Blood oxygenation sensitive (TE = 40 ms, TR = 3 sec, θ = 90°) signal from the visual cortex during blob stimulation (8 Hz flashing diffuse red) and interblob stimulation (8 Hz alternating high spatial frequency black and white pinwheel). The signal remains elevated for the entire 6 minute stimulation durations. Again, post-stimulation undershoot is seen following “interblob” stimulation and is absent following the “blob” stimulation.

**Part 2B. Habituation effect analysis:** In this section, neuronal habituation effects are demonstrated. Comparison studies using PET are first shown. Figure 3.9 shows averaged PET measurements, across 7 subjects for the vibrotactile somatosensory stimulation and 9 subjects for 10 Hz alternating red and black checkerboard visual stimulation. The visual stimulation results demonstrated significantly less decreases in flow, and therefore less neuronal habituation, than did the vibrotactile stimulation.
Figure 3.9: Flow change characteristics, measured in V1 and S1 in 16 subjects using PET. Flow changes in V1 and S1 were obtained using $^{15}$O-labeled water injections at 10 minute intervals during 50 minutes continuous visual and somatosensory stimulation respectively. A significant flow decrease is observed in S1 but not in V1, demonstrating that extended duration flow responses can vary, due to habituation effects, depending on the stimuli used and cortical regions activated.

Figure 3.10 shows the MRI signal intensity from the visual cortex during continuous stimulation with 8 Hz flashing red LED goggles. The stimulation timing and the method by which the region of interest for the time course was chosen were similar to that in Part 1. The signal appears to slowly decrease, after 7 minutes, to just above half its initial amplitude by 20 minutes. Given the previous results, it is thought that this signal behavior is due to greater neuronal habituation effects with flashing red stimulation than with alternating black and white stimulation. Behavioral controls would aid in interpretability of this result.
Figure 3.10: Blood oxygenation sensitive (TE = 40 ms, TR = 3 sec, θ = 90°) signal from the visual cortex during 8 Hz flashing diffuse red LED stimuli using Grass™ goggles. The signal appears to decrease slightly over time, suggesting neuronal habituation.

A comparison of the effects of a flashing white stimuli with that of a constant (non-flashing) featureless white stimuli, with a baseline of darkness, clearly demonstrates relative neuronal habituation effects. Figure 3.11 shows the difference in the two effects. Each stimulus was applied for 6 minutes, with an interval of 2 minutes of darkness. The flashing white stimulus elicited a sustained response, and the constant (0 Hz) stimulus elicited an initial signal increase followed by a decrease, within 1 minute, to baseline. At the transition from darkness to brightness, V1 becomes activated, then returns to baseline after about 1 minute if the stimulus does not change.
Figure 3.11: Blood oxygenation sensitive (TE = 40 ms, TR = 4 sec, θ = 90°) signal from the visual cortex during either 10 Hz flashing white and during non-flashing white light stimulation. The signal remained elevated during the entire six minute flashing white light stimulation duration. During the non-flashing white light stimulation, the signal returned to baseline within about 1 minute after stimulation onset. The control condition was darkness. This transient effect is a clear demonstration of neuronal habituation.

Figure 3.12 shows differences across cortical regions in the dynamic effects of the constant featureless white stimuli. On the transition from brightness to darkness, several regions in the anterior (peripheral) V1 show a very brief MRI signal increase that seems to correspond to a brief burst of activity during the transition. This is a clear demonstration of a transient off - response to visual stimulation cessation.
Figure 3.12: Blood oxygenation sensitive (TE = 40 ms, TR = 2 sec, θ = 90°) signal from the visual cortex during steady (non-flashing) white light. The time course of the signal from region a (black voxels) and region b (white voxels) are shown. The horizontal bars indicate the time at which the stimulus was on. The signal returns to baseline after less than 1 minute of the stimulation. The signal from region a shows a short (10 sec.) burst of activity during the transition from an on to an off state. Signal from region b shows less of a signal change during this transition from brightness to darkness.

3.3.3 Summary of long-duration responses

Table 3.1 summarizes the essential results. As can be seen, the only stimulus that resulted in a consistent decrease in signal after 1 minute was the 0 Hz (constant) solid white stimulus. This decrease in the BOLD signal is hypothesized to be caused by decreased neuronal firing (i.e. neuronal habituation) and not by an uncoupling of flow with oxygen consumption. All other stimuli demonstrated sustained oxygenation and flow for at least seven minutes following the onset of stimulation.
### Table 3.1: Long-term BOLD and CBF responses from various stimuli.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>n</th>
<th>Contrast</th>
<th>Method</th>
<th>Stimulus Duration</th>
<th>Signal Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Hz alternating checkboard</td>
<td>4</td>
<td>BOLD</td>
<td>GE-EPI</td>
<td>20 min.</td>
<td>0-10% attenuation starting after 7 min.</td>
</tr>
<tr>
<td>4 Flow</td>
<td></td>
<td>Flow</td>
<td>GE-EPI</td>
<td>20 min.</td>
<td>0-10% attenuation starting after 7 min.</td>
</tr>
<tr>
<td>1 BOLD</td>
<td>1</td>
<td>BOLD</td>
<td>GE-Spiral Scan</td>
<td>6 min.</td>
<td>0% attenuation</td>
</tr>
<tr>
<td>1 BOLD</td>
<td>1</td>
<td>BOLD</td>
<td>long TR GE-EPI</td>
<td>20 min.</td>
<td>0% attenuation</td>
</tr>
<tr>
<td>8 Hz alternating checkerboard</td>
<td>9</td>
<td>Flow</td>
<td>O-15 PET</td>
<td>50 min.</td>
<td>0-10% attenuation by 50 min.</td>
</tr>
<tr>
<td>8 Hz vibrotactile</td>
<td>7</td>
<td>Flow</td>
<td>O-15 PET</td>
<td>50 min.</td>
<td>50% attenuation by 20 min.</td>
</tr>
<tr>
<td>8 Hz &quot;blob&quot;</td>
<td>2</td>
<td>BOLD</td>
<td>GE-EPI</td>
<td>8 min.</td>
<td>0% attenuation</td>
</tr>
<tr>
<td>8 Hz &quot;interblob&quot;</td>
<td>2</td>
<td>BOLD</td>
<td>GE-EPI</td>
<td>8 min.</td>
<td>0% attenuation</td>
</tr>
<tr>
<td>8 Hz flashing red LED goggles</td>
<td>2</td>
<td>BOLD</td>
<td>GE-EPI</td>
<td>20 min.</td>
<td>20-50% attenuation starting after 10 min.</td>
</tr>
<tr>
<td>8 Hz flashing solid white</td>
<td>2</td>
<td>BOLD</td>
<td>GE-EPI</td>
<td>6 min.</td>
<td>0% attenuation</td>
</tr>
<tr>
<td>0 Hz (constant) solid white</td>
<td>2</td>
<td>BOLD</td>
<td>GE-EPI</td>
<td>6 min.</td>
<td>100% attenuation by 1 min.</td>
</tr>
</tbody>
</table>

**3.4 Discussion**

**Part 1: MRI Parameter Modulation:** The data consistently demonstrated that both blood oxygenation and flow remained constant and fully elevated for at least 7 minutes of continuous stimulation. It is hypothesized that the decrease in signal occasionally observed in both oxygenation and flow weighted sequences after about 7 to 10 minutes of continuous stimulation is due to a small amount of neuronal habituation (i.e. decreased neuronal firing causing decreased flow and venous oxygenation) since both BOLD and flow signal decreased in relatively similar amounts and at the same time.
Part 2: Neuronal and Physiologic Parameter Modulation: The fact that the BOLD signal remained elevated for both "blob" and "interblob" stimuli suggests that baseline and/or activation-related differences in metabolic rate do not have a strong effect on the degree MR signal change or its extended duration behavior. Also, the similar steady-state BOLD signal increases, combined with "interblob" stimulus showing a larger post stimulation undershoot and a larger initial signal increase before reaching a steady state suggest a difference, at the beginning and cessation of activation, in the time for the metabolic rate to respectively increase and decrease relative to flow changes. It may also suggest a stimulus related difference in blood volume change dynamics relative to flow and oxygenation changes [19], or may be a result of luminance differences in the stimuli.

It is clear from the PET studies and luminance change fMRI studies that the degree of neuronal habituation, indicated by flow decreases over time, can vary widely depending on the type of stimulus used. This type of effect should be kept in mind when interpreting extended duration stimulation fMRI results.

Although no physiologic calibration was done, the results clearly suggest that the factors modulating BOLD signal amplitude in visual cortex are neuronal habituation (i.e. decreased neuronal firing) and not changes in oxidative metabolic coupling to perfusion.

Two other preliminary studies [6, 7] have demonstrated results similar to those presented here. Although our results differ radically from the elegant and coherent story told by Frahm et al [3], our overall difference with Hathout [4] is mitigated, in that several of our experimental results (although not our conclusions) are similar. This suggests the possibility that their observations might have been due to neuronal habituation effects.

More perplexing are the results of Frahm et al., which show a decrease in BOLD signal almost to baseline level after 2 minutes and then a subsequent undershoot after extended stimulation lasting at least 2 minutes. Possible explanations may include differ-
ences in pulse sequence sensitivities between FLASH and EPI, differences in the stimuli, or differences in the resting state conditions.

A simple biomechanical model (see Chapter 2) reproduces the results of Frahm et al. by allowing that inflow and outflow not necessarily equal each other at all points in time. The model can predict both the results of Frahm et al and the results presented here by varying the relative time constant of blood volume changes, blood flow changes, and blood oxygenation changes during the onset of activation and the return to baseline. Furthermore, these transient effects can be consistent with a continuous tight coupling between flow and oxidative metabolism [19]. Recent measurements of dynamic blood volume changes in response to sensory stimulation in the rat [20] suggest, in fact, a delay in blood volume change compared with blood flow change; the timing of such dynamic uncoupling has not been measured in humans.

PET studies that seem to agree with the results presented in this paper were recently published [21]: using the Kety-Schmidt technique, arterial-venous differences in oxygenation were shown to remain steadily decreased during an activation task involving larger cortical regions (Wisconsin card sort task). Chapter 4 describes additional MRI methods which show oxidative metabolism increasing up to 20% rapidly following visual stimulation, with no detectable loss over 5 minutes, consistent with results from the current chapter. Taken together, these results are most consistent with a model for metabolism in which there is no adaptation for metabolism separate from neuronal activation, and a tight temporal coupling between neuronal activation and the onset of metabolic oxygen demand.

3.5 Conclusions
An array of studies have demonstrated that both flow and oxygenation remained elevated for the entire stimulation durations, except when stimuli known to cause neuronal
habituation (decreased neuronal firing) were used. Pulse sequence strategies allowing exclusive flow and oxygenation sensitivity were used to confirm that flow and oxygenation remain elevated during prolonged activation. Also, multi-shot minimally flow sensitive $T2^*$-weighted spiral scanning was performed during extended duration stimulation. Oxygenation remained elevated during the entire stimulus duration.

Stimuli known to activate tissue with different mitochondria concentrations elicited no differences in extended duration behavior of blood oxygenation, although a post-stimulation undershoot was more apparent following the stimulus designed to excite inter-blobs (high spatial frequency, no color) rather than stimulus designed to excite blobs (color, low spatial frequency).

The effects of extended stimulation known to have different neuronal habituation effects were tested. Neuronal habituation effects were observed in V1 after about seven minutes using 8 Hz flashing red LED goggles. Clear neuronal habituation effects were demonstrated in V1 using a featureless and steady state white visual stimulation. With this stimulus, the BOLD signal returned to baseline after about 1 minute. Some regions showed a brief signal increase on returning to the off state.

These studies strongly suggest that flow and oxygenation, as measured on this spatial scale (approximately 0.9 ul) remain elevated and constant for the entire duration that neurons are firing at a constant rate. The observed decreases in both oxygenation and flow over time are attributed to neuronal habituation, which corresponds to a decrease in neuronal firing rate with specific types of stimuli.

Future efforts at elucidation of these changes include more precise hemodynamic sensitization (i.e. capillary perfusion, venous perfusion, blood volume) by MRI, higher resolution MRI techniques, more carefully matched experiments across imaging platforms and laboratories. Although visual stimuli are known to be less sensitive to attentional modula-
tion than other somatosensory stimuli, behavioral controls would aid in the interpretability of long-duration responses. We assume for the purposes of our analysis that subjects performed the requested function throughout the experiment: attending as best they were able to the fixation dot at the center of the visual stimulus, or in the case of stimuli without fixation dot, to keep their eyes open and looking straight ahead.

Taken together with the dynamic models of Chapter 2 and the calibration method of Chapter 4, these empirical observations build a consistent picture of oxidative metabolism which is rapidly responsive to task activation, does not increase or decrease without accompanying perfusion change, and for which differential coupling of flow to BOLD and BOLD increase to BOLD post-stimulation undershoot are seen with different types of visual stimuli. It remains to quantify the coupling of blood flow to BOLD signal, in order to estimate changes in oxidative metabolism which may be responsible for differences in temporal characteristics between the methods. We take up this topic in Chapter 4.
References


Chapter 4

Calibration of functional MRI signals:

Mapping and dynamics of oxidative metabolism

The good, if brief, twice good;
the bad, if brief, less bad!

– Baltasar Graciàn
4.1 Introduction

The measurement of cerebral metabolic rate for oxygen (\(CMR_{O2}\)) remains controversial. While some reports show little or no task-induced increase in metabolic oxygen demand with positron emission tomography (PET) [1, 2, 3], others have shown varying degrees of coupling of oxidative metabolism to glucose consumption (\(CMR_{glc}\)) and cerebral blood flow (\(CBF\)) [4, 5, 6], both of which increase dramatically with task activation [2, 3, 7]. The relatively large increase in \(CBF\) seen with task activation may be necessary to enhance the diffusion-limited delivery of oxygen [8, 9]; this results in increased total tissue oxygen content [10]. Current functional magnetic resonance imaging (fMRI) methods rely on this focal apparent physiological uncoupling of \(CBF\) from \(CMR_{O2}\) for the blood oxygen level dependent (BOLD) functional MRI signal in areas of activation-induced perfusion increase [11, 12]. While BOLD has been assumed a reasonable marker for \(CBF\) based on PET experiments showing tight coupling of \(CBF\) to \(CMR_{O2}\) at baseline throughout the brain, heterogeneous decrease in magnitude of BOLD by increased oxidative metabolism remains a plausible confounding effect.

This chapter challenges the current interpretation of fMRI by describing a way to use this confounding effect to measure changes in \(CMR_{O2}\): BOLD is calibrated against \(CBF\) MRI [11, 13] using carbon dioxide breathing as a physiological standard [14, 15]. The goals of the study were to map the baseline metabolic state as reported in the deoxygenated hemoglobin calibration parameter, to utilize calibration maps to generate relatively assumption-free measurements of \(CMR_{O2}\) resolved both temporally and spatially, and to compare these measurements between regions and between subjects. The new results are discussed as they pertain to the physiology of brain activation and to the interpretability of BOLD-based brain mapping techniques.
The wide variability in the BOLD response in brain parenchyma, both between subjects and between differently activated brain regions, remains poorly understood. Does it reflect variability in neuronal or hemodynamic activation levels? Attributes of the baseline state, rather than the activation response, may account for variation in BOLD between subjects and between regions.

4.2 Theory
The BOLD effect is interpreted as a change in observed NMR transverse relaxation rate, \( \Delta R_2^* \), which is linearly dependent on the blood volume fraction \( f_v \) [16, 17, 18] and dependent on magnetic susceptibility difference between blood and tissue, \( \Delta \chi \), raised to a power \( \beta \). The susceptibility difference, which is proportional to blood deoxyhemoglobin \( (dHb) \), has a supralinear effect: a linear large vessel component is combined with small vessel contributions, which tend towards a quadratic effect on relaxivity according to the Luz-Meiboom model for diffusion-mediated exchange on the capillary scale [16, 17, 18, 19]. Comparing to prior Monte Carlo NMR simulations [17] using 2% venous and 2% capillary volume fractions, varying venous oxygen saturation from 60% to 95%, we fit the relaxivity profile over these values to a model \( \Delta R_2^* \propto (\Delta \chi)^\beta \); for these values and our imaging parameters, the fit found \( \beta = 1.5 \), corresponding to the expected balance between capillary and venous contributions.

Fick’s law describes conservation of oxygen delivery and oxygen uptake, stating the mass-balance principle that oxygen delivery is proportional both to blood flow \( (CBF) \) and to the arteriovenous oxygen difference [20]. Because the arteriovenous difference is proportional to deoxygenated hemoglobin production, Fick’s law can be written in terms of deoxyhemoglobin: \( dHb \propto CMR_{O2}/CBF \). In terms of physiological variables, \( \Delta R_2^* \) between time 0 and \( t \) is then
\[ \Delta R^*_2(t) \approx f_i(t) \left( \frac{CMR_{O_2}(t)}{CBF(t)} \right)^\beta - f_i(0) \left( \frac{CMR_{O_2}(0)}{CBF(0)} \right)^\beta. \]  

(4.1)

We define \( B_i, F_i, \) and \( V_i \) as the BOLD signal, CBF signal, and blood volume at time \( t \) normalized by ratio to a baseline period in the experimental paradigm. \( rCMR_{O_2} \) is the cerebral metabolic rate for oxygen relative to baseline. For small change in relaxivity \( (TE\Delta R^*_2 \ll 1) \) the BOLD signal is \( B_t \equiv 1 - TE\Delta R^*_2(t) \). Substituting the above normalized variables into Eq (4.1) and extracting the baseline values, we have

\[ B_t - 1 \approx f_i(0) \left( \frac{CMR_{O_2}(0)}{CBF(0)} \right)^\beta \left[ 1 - V_i \left( \frac{rCMR_{O_2}(t)}{F_i} \right)^\beta \right]. \]

(4.2)

CBF is directly measurable by arterial spin tagging perfusion MRI; we selected the FAIR method (flow sensitive alternating inversion recovery) for this report [11, 13]. We express blood volume changes in terms of CBF: \( V = F^\alpha \) where \( \alpha = 0.38 \) is taken from a previous study [21] and verified using MRI contrast agents by Mandeville, Weisskoff, and Rosen (data not shown). We aggregate the baseline values from Eq (4.2) and the proportionality constant relating BOLD signal change to deoxyhemoglobin into a calibration parameter: \( M \ll f_i(0)(CMR_{O_2}(0)/CBF(0))^\beta \). For convenience, this relation is written as a proportionality; the addition of constants for magnetic susceptibility of deoxyhemoglobin, susceptibility effects on transverse relaxation, and echo time dependence would complete the equality.

\( M \) is dependent on baseline tissue deoxyhemoglobin content; it thus may vary between trials and between brain regions. \( M \) can be thought of as the baseline deoxyhemoglobin concentration, scaled in terms of BOLD signal. Because BOLD signal is dependent on washout of this deoxyhemoglobin, \( M \) is the BOLD signal headroom for blood flow-induced signal changes, assuming no concomitant metabolic change.

To estimate \( M \), we calibrate against the physiological challenge of hypercapnia (subscript \( H \)), assuming there is no increase in metabolic rate over baseline during hypercapnia.
We then manipulate Eq (4.2) to determine its implicit proportionality constant including baseline values:

\[
M = \frac{B_H - 1}{1 - F_H^{-\alpha/\beta}}
\]  

(4.3)

where \( B_H \) and \( F_H \) are BOLD and CBF ratios for CO\(_2\) breathing versus baseline. Because hypercapnia causes dramatic increase in blood flow which washes out deoxyhemoglobin, both \( B_H \) and \( F_H \) are expected to be greater than one, and since the exponent on \( F_H \) is negative (-1.12 in our model), \( M \) must be positive. Because \( M \) is also the maximum BOLD signal change for the current baseline state, we also have \( M > B(t) - 1 \) for any activating stimulus.

With the fMRI experiment calibrated by determination of \( M \), the normalized time courses of perfusion \( F(t) \) and BOLD fMRI \( B(t) \) can be used to compute dynamic oxidative metabolism maps \( rCMR_{O_2}(t) \) relative to baseline.

\[
rCMR_{O_2}(t) = F(t)^{1-\alpha/\beta} \left( 1 - \frac{B(t) - 1}{M} \right)^{1/\beta}
\]  

(4.4)

Application of Eqs. (4.3) and (4.4) provides the desired measurement of BOLD sensitivity to \( CBF \), and dynamics of the relative metabolic rate for oxygen.
Figure 4.1: Model dependency on design parameters. Ranges of calculated $rCMR_{O2}$ were obtained by varying $\alpha$ and $\beta$ through plausible values. The blood volume coupling exponent $\alpha$ has been measured at 0.38 [21]; Monte Carlo simulations show $\beta = 1.5$ for our experiment. We used these values (arrows) for our analyses.

We finally consider the sensitivity of our results to the details of our model. We computed our average result as a function of the only undetermined parameters of the model, $\alpha$ and $\beta$, and plotted results in Figure 4.1. If blood volume does not change with blood flow, then our $rCMR_{O2}$ estimates are at most 6% low. If the susceptibility effect is linear ($\beta = 1$), then our $rCMR_{O2}$ estimates are at most 4% high. In the small vessel limit of quadratic dependence, our estimates would be 2% low. Sensitivity of our model to these parameters is therefore small, on the order of our standard error of measurement for a single subject (see Table 4.1).

4.3 Methods
Volunteers\(^1\) breathed gases delivered through a non-rebreathing face mask with bag (Baxter #1203) at 15 liters/min, attending to a projected image. This was accomplished by using pre-mixed compressed gasses (Air Products, Inc.) with a standard medical regulator.

\(^1\)Written informed consent was obtained according to protocol approved by the Massachusetts General Hospital subcommittee on human subjects.
delivering a continuous stream of gases at 15 L/min through extended tubing entering the magnet room through a wave interrupting port in the patch panel from the machine room. The face mask, a standard disposable high-delivery model, was modified by adding a second one-way valve gasket to prevent inspiration of room air, and by removing the metallic nose piece. This necessitated taping the mask around the nose and mouth to prevent air leakage. Excess gas delivery rate inflated the attached high-flow inspiration reservoir, and spilled out of the breathing circuit through two one-way valves in the mask body.

During eight paired trials in five subjects, periods of baseline stimulus were interleaved with images of a radial pinwheel alternating at 12 Hz, as described in Chapter 2. After 5 one-minute periods of photic stimulation alternating with baseline, inhalation gas was changed manually from compressed air to a mixture of 5:21:74 percent CO₂:O₂:N₂ for 5 minutes. During the final 5 minutes, the subjects again breathed air and repeated the same stimulus as during the first 5 minutes. To test the response to longer duration stimuli, additional experiments were performed in two subjects with 5 minutes of visual stimulation and 4 minutes of CO₂ breathing separated by 2 minutes of baseline condition. Physiological monitors recorded heart rate, respiratory rate, oxygen saturation, and end-tidal CO₂.

After identifying calcarine cortex and aligning the imaging plane to incorporate the calcarine sulcus along its greatest linear stretch, echo planar MRI images (Advanced NMR/General Electric Signa 1.5 T) were collected using 7 mm slice thickness and 3.1 mm square voxels from a 32 ms readout window as described in Chapter 2. A data matrix was collected of 128x64 voxels representing 40cmx20cm field of view. The following two pulse sequences were used:

(A) BOLD asymmetric spin echo, TR=2 s, TE=70 ms, refocusing pulse advanced 25 ms from TE/2; this gives T2* BOLD weighting through a virtual gradient echo of 50 ms,
but matches the FAIR spin echo portion to equally discount large flowing vessels. Development of this pulse sequence is described in Chapter 2.

(B) FAIR inversion recovery spin echo, TR=3 s, TI=1 s, TE=45 ms. Subtraction of nonselective from selective echoes produced the FAIR signal, proportional to perfusion. As reported by Kwong [11] and others and commonly used in our laboratory, the slice-selective initial inversion pulse thickness was doubled to 14 mm to ensure flat excitation across the spin echo 90 degree slice profile. The selective pulse inversion recovery alternated temporally with an almost identical but nonselectively inverting pulse sequence; the RF pulses were identical between the two, but the nonselective pulse slice selection gradient is zeroed for the duration of the RF pulse. The difference in signal between the two pulse sequences then lies only in differences in spin turnover between the nonselectively inverted spins outside the slice, and the selectively inverted spins outside the slice. Because of the necessity to excite all of the potential in-slice blood one second before the imaging pulse (TI=1 s), the body RF coil was used for excitation, and a 5 inch surface coil was used for receiver coupling.

The order of experiments (A) and (B) was randomized between trials. As a subtraction of two signals, FAIR has \( \sqrt{2} \) more noise per signal than the inversion recovery sequences used in Chapter 3. However, its utility lies in the fact that the FAIR signal is proportional to blood flow. In other words, zero flow corresponds with zero signal with FAIR. This feature is a necessary part of the method.

Using image display and analysis suite xds developed by the author, regions of interest within primary visual cortex were selected to include areas responsive both to hypercapnia and to photic stimulation for both FAIR and BOLD experiments. Pixels with large baseline variances or large calculated variance in \( M \) were excluded by automatic threshold removal. Accounting for inter-experiment motion, the regions were then matched between
experiments (A) and (B), and were identical for visual stimulation, hypercapnia, and baseline periods within each experiment. Temporal windows were chosen for summary analysis to include only time points after the stabilization of signal, typically from 10 s after stimulus change until the next stimulus change, and after 15 s in the post-stimulation baseline state to minimize the effect of transient signal undershoot. For the visual stimulus analysis, the first five minutes of alternating stimulus and baseline was utilized; for the hypercapnia statistics, baseline points were taken between visual stimuli both before and after the CO₂ breathing portion of the experiment.

Data for each subject were pooled into baseline, stimulated, and hypercapnia epochs for both pulse sequences. After removing drift in the BOLD signal with a quadratic fit to baseline points, ratios were computed for hypercapnia to baseline ($CBF_{F_H}$ and BOLD $B_{H}$) to calculate $M$ using Eq (4.3). Least squares estimates of average stimulation and time courses of $CMR_{O2}$ were computed using Eq (4.4), including second order statistics for noise estimation (see Appendix). These were compared with observed inter-trial variation to assess individual variation in BOLD sensitivity and metabolic and hemodynamic reactivity.

### 4.4 Results

Breathing carbon dioxide enriched air resulted in $5±1$ mmHg increases in end-tidal CO₂ in all subjects, along with a small increase in respiratory rate but not heart rate or oxygen saturation. This level of hypercapnia elicited $18±3\%$ average ± s.e. increase in $CBF$, with concomitant BOLD signal increases of $1.8±0.2\%$ (Table 4.1). The average $M$, calculated separately for each trial, was $7.9±0.7\%$. $CMR_{O2}$ increases averaged $16±1\%$ during photic stimulation, calculated separately for each trial from average $45±4\%$ increase in $CBF$, and BOLD increase $1.7±0.5\%$. $CMR_{O2}$ increased much less than $CBF$ in all subjects; however, this moderate increase in $CMR_{O2}$ limited the BOLD effect on average to $68\%$ of its maxi-
mal value for the given perfusion increase.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Voxels</th>
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<th>Photic Stimulation</th>
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<tr>
<td></td>
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<tr>
<td>1A</td>
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<td>±0.001</td>
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<td></td>
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<td>±0.008</td>
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<tr>
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<td>1.27</td>
</tr>
<tr>
<td></td>
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<td>±0.05</td>
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<tr>
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<td>1.26</td>
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<td>1.21</td>
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<tr>
<td></td>
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<td>±0.01</td>
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<tr>
<td>4A</td>
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<td>1.11</td>
</tr>
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<td>±0.002</td>
<td>±0.02</td>
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<td>±0.02</td>
</tr>
<tr>
<td>5B</td>
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<td>1.017</td>
<td>1.29</td>
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<tr>
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<tr>
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<td>±0.002</td>
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<tr>
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<td></td>
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<td>57</td>
</tr>
</tbody>
</table>

**Table 4.1:** Oxidative metabolism averaged over regions of interest during 10 trials.

Least squares estimates ± standard errors in primary visual cortex. $B_H$ and $F_H$, BOLD and CBF hypercapnia divided by baseline; $B_S$ and $F_S$, BOLD and CBF photic stimulation divided by baseline; $M$, BOLD sensitivity calibration parameter; $rCMR_{O2}$, change in cerebral metabolic rate for oxygen.

a. Long duration (5 minute) stimulus paradigm; not averaged into Figure 4.2.

b. $rCMR_{O2}$ is the only estimate without measurable inter-trial variance compared with Monte Carlo simulated measurement noise, SD(sim) ($\alpha = 0.7$). The remainder have significant variance over measurement noise ($P < 0.001$; $F = 10$ at $\alpha=0.001, n_1=9, n_2=9$).
Cross-subject time course averaging for eight trials (Figure 4.2) revealed BOLD and CBF signal responses which track the stimulation paradigm to within two image collection frames (12 s). The dynamic response of $CMR_{O2}$ calculated from this average shows prompt tracking of metabolism to stimulus, increasing transiently by up to 20%, and prompt recovery during rest periods. Only the first five minutes was used for the photic stimulation averages shown in Table 4.1 to minimize the effects of subject motion in later time points. Additional experiments with five minute stimulation periods (Trials 3B and 4B in Table 4.1) showed no significant differences from those with short periods: there was no habituation effect or loss of oxidative metabolism over time.
Figure 4.2: BOLD and CBF time courses normalized to the hypercapnia signal change, averaged across 8 paired trials. Compared to hypercapnia-induced signal change, the CBF signal outstrips the BOLD signal changes during photic stimulation. rCMR(O2) time course (below) calculated from the same data shows metabolic response within seconds of photic stimulation onset. No temporal smoothing was done: all time points (6 s resolution) were collected and calculated independently. Note that by analytic design the average rCMR(O2) during the hypercapnia period and baseline periods are both set to 1.

Maps of photic stimulation and hypercapnia normalized to baseline (Figure 4.3) show general cortical signal increase during hypercapnia, and localized primary visual cortex signal increase during photic stimulation using both BOLD and FAIR methods. Positive signal change is required during the hypercapnia stimulus for valid voxel estimate of \( M \) to be calculated (nonzero denominator in Eq (4.3)). Comparison of valid \( M \) estimates in visual to peripheral cortex shows increased \( M \) in visual cortex, implying increased BOLD signal sensitivity. Maps of \( rCMR(O2) \) also show focally increased metabolic uptake in primary visual cortex.
Figure 4.3: Maps from trial (A) of each subject from Table 4.1 show regional variations. The first column shows regions of interest in yellow superimposed on an anatomical image weighted for slow flow to highlight veins: note prominent sagittal sinuses. No venous structures were seen within the regions of interest. The second and third columns show BOLD hypercapnia and task activation responses as color overlays: colors represent signal increases from 1% (red) to 3% (yellow). The fourth and fifth columns show CBF hypercapnia and task activation, increasing 20% to 80%. In the next column, the calibration parameter M calculated from hypercapnia data alone is shown in color from 1.5% to 20%. The rightmost column shows $rCMR_O2$ for each subject, from 3% to 30%, calculated from M and task activation images. All subjects show a confluent patch of increased $rCMR_O2$ averaging from 13% to 19% within visual cortex. Some peaks reach up to 30% increase in metabolism, corresponding to peaks of blood flow up to 70%.

Individual responses are tabulated in Table 4.1 with standard error estimates calculated from second order statistics in the source images. Large variance is seen between trials in the BOLD responses and in the M calibration parameter. This discrepancy between expected and measured inter-trial variance is eliminated for $rCMR_O2$, indicating much less variability for metabolic changes than for BOLD fMRI sensitivity and responsiveness.

Simulations were performed to determine the effect of noise on model-based estimates. Figure 4.4 shows how variance in the estimates is dramatically improved by a simple 3x3 voxel smoothing step at the beginning of analysis. We also see the effect of noise...
on bias in the estimation of $M$, due to the largest noise source occupying the denominator of $M$, which self-corrects for $rCMR_{O2}$. 


Figure 4.4: Noise propagation simulation results, from Monte Carlo simulation with means and second order noise characteristics taken to match single voxel or nine voxel averages from the primary data. (BOLD and FAIR baseline SNR 100:1, FAIR perfusion signal component 4%). While the maximum likelihood for the $M$ estimate is skewed towards lower values, the bias is self-correcting in the estimate for $rCMRO_2$, which shows no noise bias.

4.5 Discussion

The calibration of functional MRI represents an extension of commonly used brain mapping techniques, allowing interpretation of activation signals in terms of the underlying physiology without requiring assumptions about the baseline metabolic state. Other work has previously developed theoretical relationships between perfusion and BOLD signal [11, 18, 22], but interpretation in terms of metabolism required the equivalent of estimating $M$ from literature values.

Calibration robustness: The two design parameters for the model, relating blood flow to estimated blood volume changes, and relating deoxyhemoglobin to transverse relaxation, are shown to have little effect on the results. This insensitivity is due to the nature of the calibration step, which defines a base operating point around which only the small perturbations in physiology are detected. Because of this feature, the method is robust to inaccuracies in the design parameters, which may also vary spatially. We determine $rCMRO_2$
independent from baseline physiology, enabling the noninvasive investigation of metabolic reactivity in diseased tissues and altered physiological states.

Of note, there are no \textit{a priori} assumptions regarding blood volume fraction, resting capillary or venous oxygen saturation, blood flow, or metabolic rate for oxygen, as these are all accounted for in the single calibration step. The presence of large draining veins in an imaging voxel contributes excess noise in that voxel which may require its exclusion from analysis, but would not bias the metabolism measurement according to our model.

The assumption of no metabolic change for mild hypercapnia is based on global measurements in animals. In fact, a higher degree of hypercapnia is associated with modest increase in \( CMR_{O2} \), possibly due to catecholamine release, blood brain barrier breakdown and repair mechanism activation, or other concomitant homeostatic response to hypercarbia or acidosis. Any increase in metabolic rate during hypercapnia would cause our method to underestimate task activated increases in metabolic rate. Any other control stimulus could also be used which generates a known metabolic response while also causing a large blood flow change.

We assume that the extracted oxygen is metabolized, rather than being transported out of the tissue. An alternative hypothesis has been advanced, of oxygen shunting due to counter-current exchange between arteries and nearby veins. This may be significant in muscle and skin [23], and may be important for deoxyhemoglobin-dependent nitric oxide release in arterioles [24]. However, countercurrent oxygen exchange has been shown to have a negligible effect on brain hemoglobin saturation [25], perhaps partially due to reduced counter-current vasculature arrangement compared with other tissues. Thus, direct coupling of brain capillary deoxyhemoglobin to metabolism remains a reasonable assumption for our model.
Concern for flow effects on BOLD images is minimized by long repetition interval and use of the asymmetric spin echo pulse sequence. Pulsatile flow may shield some intravascular spins from NMR visibility; however, our use of similar spin echo based pulse sequences between BOLD and CBF eliminates mismatches between visible spin populations.

**Magnitude of metabolism changes:** Our observations are consistent with those advocating a partial coupling of blood flow to oxygen demand [26, 4, 8, 5], and difficult to reconcile against those declaring little or no oxidative metabolism change during task activation [1, 2]. Diffusional limitation on oxygen delivery to brain has been proposed, requiring large CBF increases to support smaller CMRO2 increases [8, 9]. While not confirmatory, our results are consistent with this theory. Our results also differ from those claiming slow conversion to aerobic metabolism in the brain [27, 6, 28], but are consistent with MRI results showing no reduction in BOLD over time [29].

Interestingly, we show larger increases both in hemodynamic response (CBF increases 22% to 79%) and in metabolic response (CMRO2 increases 11% to 23%) than comparable PET experiments. This may be secondary to partial volume effects in the PET experiments; combined fMRI and PET experiments may help to sort out these technique-dependent magnitude differences.

**Dynamics of metabolism changes:** Our method allows noninvasive, dynamic, continuous monitoring of oxidative metabolism changes with temporal resolution of several seconds. The oxidative metabolic changes are rapid and tightly coupled to stimulus onset and cessation (Figure 4.2), concordant with optical spectroscopic measurements in animals [30, 31], which show highly localized relative deoxygenation in active columns beginning within hundreds of milliseconds. This early deoxygenation has been implicated as a mech-
anism of an early dip in BOLD signal which precedes the hemodynamic alterations in the first two seconds of stimulation [32].

Care must be taken in examination of transient changes in oxidative metabolism using our methods. The model as described assumes tight coupling of perfusion to blood volume, based on prior work performed at steady state. However, recent experiments in rats show that blood volume changes may lag BOLD effect changes with a 14 s time constant [33]. Unless blood volume is measured separately, time course estimates which rely on tight flow-to-volume coupling may underestimate $CMR_{O2}$ during the up-regulation and overestimate $CMR_{O2}$ during down-regulation of $CBF$, such as during post-stimulation undershoot of BOLD. When less than 14 s temporal resolution is needed, the model for blood volume change should include a delay component, easily implemented by filtering the $CBF$ data once the human response is known. Alternatively, blood volume time course may be collected in a separate experiment using an intravascular contrast agent [33].

**Interpreting the calibration parameter:** In addition to $CMR_{O2}$, we have measured the local sensitivity of BOLD to changes in $CBF$. The sensitivity parameter $M$ — precisely the needed calibration parameter for $CMR_{O2}$ estimation — is interpretable in its own right. Variations in $M$ bear directly on variations in observed BOLD signal magnitude. These variations may be due to differences in blood flow, venous volume, metabolic rate, or a combination of these.

BOLD sensitivity may be quite variable, both regionally and between subjects. In voxels dominated by veins, the baseline blood volume contribution to $M$ is quite large; a threshold cutoff for $M$ provides a ready method for exclusion of large vessels from regions of interest. Conversely, voxels with small $M$ or poorly defined $M$ ($F_{H} = 1$, Eq (4.3)) are rendered insensitive to BOLD because they have little reactive deoxyhemoglobin-containing blood volume. Maps of the $M$ parameter may be used to divide tissue into three
groups: regions with large blood volume, expected to contain large veins and excludable from analysis; regions with reactive parenchyma sensitive to BOLD imaging and directly interpretable; and those which are insensitive to BOLD, and must not be interpreted either as active or inactive.

Mapping $M$ throughout the brain using a global stimulus such as hypercapnia will allow quantitative estimation of sensitivity to BOLD between physiologically disparate brain regions, such as primary somatosensory cortex, association cortex, higher level centers, deep gray matter and brainstem, which have widely varying microvascular anatomy. Calibration will be useful in comparing magnitudes between subjects and between physiological states, and is necessary to disambiguate cerebrovascular from cerebrometabolic reactivity. Apart from task-activation experiments, calibration of BOLD adds to our knowledge of physiology underlying fMRI, and may allow extension of brain mapping to comparison of activation magnitudes between brain regions which would otherwise be difficult to interpret.

**Implications of the calibration parameter:** The observation of high $M$ in primary visual cortex raises issues of magnitude and variability of BOLD signals. Primary visual cortex is known to be reliably responsive to task activation, with larger BOLD responses in brain parenchyma than any other known task activation experiment. Previously, we had attributed the robustness of visual activation to the large area subserving primary visual sensory input, the relatively small attentional modulation of visual activation, and the ease of repeatable stimulus presentation. Our data suggest another possibility: baseline BOLD headroom. In our single slice experiments, the largest values for $M$ (up to 16%) were found in visual cortex, with smaller $M$ (3-5%) in surrounding parietal and occipital cortex (see Figure 4.3). Why should visual cortex be unusually sensitive to BOLD? Likely this is
attributable to the microvascular anatomy of this region, which is known to contain a disproportionate concentration of venules.

Between-subject differences in signal change have been puzzling to the fMRI community, with some subjects showing 1% BOLD signal changes and others repeatedly demonstrating 5% or greater signal changes within brain parenchyma (data not shown). Why are some subjects strong activators? An explanation is provided by variability of $M$ out of proportion to noise and much larger than variability in $\text{CBF}$ or $\text{CMR}_{O2}$. It is the baseline tissue deoxyhemoglobin concentration which determines the sensitivity of BOLD contrast; the changes in blood flow and oxidative metabolism between subjects may be, in fact, relatively invariant.

**Limitations and future work:** The chief limitation of this technique is methodological: subjects are required to wear a mask and additional minutes of imaging are required to obtain the calibration data, placing limitations on study design. If subject physiological state (e.g. caffeine and dietary intake) is regulated and volumetric image re-registration can be done, then calibration may be possible during a separate session. However, the requirement for collecting perfusion and BOLD-weighted data persists. We have implemented a hybrid pulse sequence, combining the elements of our methods (A) and (B) which has the potential of measuring $\text{CMR}_{O2}$ in a single trial. Calibrated fMRI will be most useful when physiological interpretation is desired, such as during assessment of drug responses, and for evaluating response in tissues which are physiologically distinct, such as midbrain and brainstem.

Improved signal to noise ratio in perfusion images would aid $\text{CMR}_{O2}$ measurement. Our current method estimates $\text{CMR}_{O2}$ changes in 0.6 cc volumes (similar to PET resolution) averaged over several time points or several subjects. Possible improvements include
continuous inversion arterial spin tagging, increased field strength, increased imaging
time, and improved receiver coils.

We quantify relative rather than absolute changes in metabolism. Absolute quantification
would require knowledge of baseline metabolism, whereas calibration of \( M \) determines
only the baseline MRI effect of deoxyhemoglobin; percent saturation is not
obtained because oxyhemoglobin is not visible. Administration of an intravascular con-
trast agent along with arterial sampling has the potential of fixing the constant terms of \( M \)
separately from the blood volume, blood flow, and metabolic rate, and may allow more
complete quantification of metabolic changes.

Our approach promises to provide new insights to understanding activity-linked
metabolism in the human brain. Additionally, the methods provide a bridge between previ-
ous steady-state physiological measurements using PET and dynamic measurements of
transient hemodynamic-linked phenomena observed with fMRI. The interpretation of
dynamic physiology provided by application of this calibration technique is essential to
the application of fMRI to diseased tissues, for which the physiological baseline is
unknown and may vary through the course of an experiment.

Future application to delineate metabolic behavior unique to specific processing
streams such as magnocellular versus parvocellular visual cortex, higher order centers,
deep gray matter structures, and regions of reported negative activation, all will provide
new noninvasive physiological windows on the relationship of metabolism to brain func-
tion.

4.6 Appendix: Error Analysis

Noise in the input ratio images for hypercapnia normalization (BOLD \( B_H \) and CBF
\( F_H \)) and time courses (BOLD \( B_t \) and CBF \( F_t \)) produces uncertainty in the estimates of \( M \)
and CMRO_2. We show the effect of noise for the case when relative blood volume is approximated as _F_i^alpha_, and the additive source image noise is wide-sense stationary. Covariance in the data arises from reuse of the baseline points between B_H and B_i, and between F_H and F_i, and in the case of a combined FAIR/BOLD pulse sequence (see Discussion), in the BOLD data which form half of the FAIR image pairs.

In the case of our experiment, with identical baseline points between F_H and F_i, we define h, t, b as independent samples with variances σ_h^2, σ_t^2, σ_b^2, such that F_H = h/b and F_i = t/b. The covariances then are σ^2_{F_H} = (h^2σ_h^2 + h^2σ_t^2)/b^4, σ^2_{F_i} = (t^2σ_t^2 + t^2σ_b^2)/b^4, and σ^2_{F_HF_i} = σ_b^2ht/b^4. The variance in the estimate for M is then

\[ \sigma^2_M = \left( \frac{\partial M}{\partial B_H} \right)^2 \sigma_{B_H}^2 + \left( \frac{\partial M}{\partial F_H} \right)^2 \sigma_{F_H}^2 + \left( \frac{\partial M}{\partial B_I} \right) \left( \frac{\partial M}{\partial B_H} \right) \sigma_{B_HF_H}^2 \]

(4.5)

where the partial derivatives are \( \frac{\partial M}{\partial B_H} = \frac{M}{B_H - 1} \) and \( \frac{\partial M}{\partial F_H} = \frac{(\beta - \alpha)}{F_H^\beta (B_H - 1)} \).

Let R stand for rCMRO_2; its expected variance is

\[ \sigma^2_R = \left( \frac{\partial R}{\partial B_I} \right)^2 \sigma_{B_I}^2 + \left( \frac{\partial R}{\partial F_I} \right)^2 \sigma_{F_I}^2 + \left( \frac{\partial R}{\partial M} \right)^2 \sigma_M^2 \]

(4.6)

where \( \frac{\partial R}{\partial B_I} = -\frac{R}{\beta (M - (B_I - 1))^\beta} \), \( \frac{\partial R}{\partial F_I} = \frac{R (\beta - \alpha) B_I}{F_I} \), and \( \frac{\partial R}{\partial M} = \frac{-R}{\beta M (B_I - 1)^{\beta-1}} \).

The above statistics \( \sigma_M \) and \( \sigma_R \) allow estimation of variance for a given experimental design.
References


Chapter 5

Physiological implications and the future of functional MRI

The things that will destroy us:

politics without principle
pleasure without conscience
wealth without work
knowledge without character
business without morality
science without humanity
worship without sacrifice

— Mahatma Ghandi
5.1 The current state of functional MRI

The goal of this thesis was to provide a missing link of physiological interpretation between cellular activity in the brain and functional MRI phenomenology. With a focus on hemodynamics and oxidative metabolism in the brain, it has succeeded in harnessing the unprecedented spatio-temporal resolution of MRI for interpretable physiological measurements, most notably, the detection of substantial increases in oxidative metabolism following task activation in visual cortex.

Possible increases and decreases of oxidative metabolism in activating brain have remained controversial, in part because of signal to noise issues and baseline physiology stability issues associated with the gold standard: serial PET measurements. The new MRI method reported here, which allows blood oxygen dependent (BOLD) MRI interpretation in terms of blood flow, blood volume, and oxidative metabolism, has resolved two controversies in the field: the question of modulation of oxidative brain metabolism, and the question of flow-metabolism coupling during long duration stimuli.

Using our novel noninvasive dynamic method localized oxidative metabolism increases of 16±1%, averaged over visual cortex during simulation, corresponded to blood flow increases of 45±4% and BOLD functional MRI signal increase 1.7±0.5%. This substantial rapid increase in oxidative metabolism (Chapter 4), while not yet replicated by others, is the most striking evidence yet of task-induced oxidative metabolism increases in active brain. Prior PET measurements of metabolic rate for oxygen, developed originally by Mintun et al. [1], relied on the separate estimation of unidirectional oxygen extraction and blood flow to derive the estimate of oxygen metabolic rate individually for both active and inactive states. As can be seen by this brief description, the PET method involved more experimental steps and utilized a model of similar complexity to the one reported here.
The MRI method for metabolism measurements reported here offers advantages over the Mintun PET method. The PET method for mapping changes required the subtraction of two noisy estimates of total metabolic rate, in an attempt to detect a difference. Instead, our method detects the difference directly, based on a calibration at the current physiological state. Differences are more readily measured, with smaller error bars, by our method than with PET. Unfortunately, the baseline metabolic rate is missing with the MRI measurement: this is the unavoidable penalty of operating-point detection techniques. The noninvasive aspect allows multiple repeated measurements; with the concomitant reduction in noise, superlative spatial resolution will be possible. While it is not necessary to perform this experiment with echo-planar or rapid scanning as done here, additional methods for physiological sensitization of conventional pulse sequences will be useful for extension to high-resolution conventional MRI.

An active controversy regarding functional MRI has to do with the consistency of fMRI responses during long-duration stimuli. Frahm et al. [2], building on observations by Hathout et al. [3], developed an elegant and coherent model of activation-induced hemodynamics and metabolism which fit their multi-modality data and which supported a surprising new hypothesis: that, like muscle, aerobic metabolism in the brain takes minutes of activity to elicit, and that when turned on, reestablishes tight blood flow-metabolism coupling and thus eliminates the BOLD effect. Supporting evidence came from in-vivo spectroscopy, which showed a lactate buildup during the first several minutes of activation, followed by reduction in lactate concomitant with total loss of BOLD signal over several minutes. A major portion of Chapter 3 was expended in experimentally disproving their flawed hypothesis. Not only is the brain aerobic at rest, but near instantaneous deoxygenation of blood in active columns has been reported by the numerous workers using optical
spectroscopy in animals [4, 5], supporting our underlying belief that rapid oxidative processing is what the brain does best. More simply said, the brain is not a muscle.

Of note, our investigations have not addressed a related issue. The metabolic rate for glucose increases more than the metabolic rate for oxygen according to PET measurements, indicating that at least a portion of the increase in metabolism from task activation is non-oxidative [6]. Fox’s original data showed 50% increase in both CBF and glucose consumption. After excluding an outlier which Fox did not exclude (the outlier showed 12% metabolic rate decrease during activation), the remaining average of 4 subjects in his study showed 9±5% increases in oxygen metabolism. While less than we report, this level of aerobic increase would still provide a substantial percentage of ATP production increase through aerobic metabolism. The buildup of lactate and temporary oxygen debt remains a plausible scenario for post-stimulation maintenance of oxygen metabolism as a source of the post-stimulation undershoot.

The original experiments for this thesis were directed at interpreting the post-stimulation undershoot seen in BOLD data (Chapter 2). Ironically, while important advances were made in the physiological calibration of functional MRI, several of the original questions directed at short term activation induced changes remain open. The confounding effect of possible uncoupled blood volume from blood flow throws in doubt attempts to measure transient changes in oxygen metabolism based on changes in BOLD and CBF alone. In order to better define the problem, an accounting of short-term determinants of BOLD signal was made, simulations performed, and the stage set for more complete analysis in terms of physiology once methods to measure blood volume dynamics become perfected. The addition of a contrast agent-based dynamic blood volume functional MRI method will complete the package, and allow extension of the methods from Chapter 4 to be applied to the data in Chapter 2.
5.2 Novel methodologies
Numerous technical problems were surmounted in the development of this dissertation, and noted in the individual chapters. Theoretical developments included the development and application of appropriate hemodynamic and oxygen transport models, the development of models of dynamic MRI contrast mechanisms, the sensitization of MRI to physiology, and simulations, analytical methods, and visualization techniques for functional experiments.


The chief experimental developments were:

1) experimental methods to compare CBF with BOLD reliably and with minimal artifact, including pulse sequence development; 2) methods for manipulating CBF independent of metabolism, development of MRI gas inhalation methods; 3) methods for robust stimulus presentation and timing, including triggering the stimulus by the scanner to minimize jitter in stimulus presentation.

5.3 Future work and potential applications
Calibrated functional MRI is a brand new tool which shows promise for placing fMRI brain mapping techniques on a solid physiological basis. However, before it is used in an application setting, additional validation steps should be undertaken.

The first major question is whether blood volume behaves as the model assumes. Because blood volume changes affect BOLD signal the same way that oxygen metabolism changes would, it is crucial that blood volume changes accompanying activation be understood. Methods are under development which would allow dynamic blood volume func-
tional MRI [7]; combination with the calibrated fMRI methods utilizing CBF and BOLD would remove the only major remaining assumption, and also allow extension of the method to short term dynamic changes. Once the CBF to CBV dynamic coupling is characterized for normal humans, and tested for variation in abnormals, it will become clear whether or not our initial assumption of tightly-coupled CBV is appropriate.

Accurate measurement of the M parameter throughout the brain for multiple subjects will quantify the extent of the BOLD “mislabling” problem: borders of BOLD sensitivity may be mistaken as borders of functional activity. It may well be that brain regions of interest have similar M, or that M is a function of cytoarchitectonics and such can be estimated based on brain region without measuring it in each subject. Such findings would be a boon to the brain mapping community, as the alternative, resorting to lower signal-to-noise methods such as perfusion imaging, would significantly hamper progress in the field. Validation of the BOLD methodology for brain mapping is important, in order to avoid placing the thousands of already published studies in doubt.

It may be possible to detect differences in metabolism for different processing streams in a region of interest. Such is the case for “blob” versus “interblob” stimuli, which vary their onset and offset behavior within the same region (see Chapter 3). Teasing apart of cerebrometabolic activity from cerebrovascular responses will allow a more complete description of the variations in physiology underlying functional activation.

Ultimately, both establishment of the M parameter and measurements of metabolic changes may have clinical utility. One immediate possibility is in the assessment of brain tissue with tenuous blood supply. Studying potential carotid endarterectomy patients would allow comparison of the M parameter to PET OEF, which has already been shown to be a useful predictor of stroke risk in this population. The higher signal to noise and
spatial sensitivity of fMRI may make calibrated fMRI, or at least the calibration step itself, a more well suited tool for this application.

5.4 Implications for physiology and functional imaging
The experimental and theoretical work presented here provides a basis for physiological interpretation of functional MRI.

The finding of 16% increase in oxidative metabolism during functional activation is a dramatic departure from conventional thought. 95% of the brain’s metabolism at rest is aerobic. Given the diffusion limitation on oxygen delivery and low baseline tissue oxygen partial pressures, it is surprising that oxygen utilization is able to increase as much as our measurement indicates. One possibility is that oxidative metabolism in the active brain is absolute: all of the available tissue oxygen may be consumed, and the lactate buildup observed during stimulation [8] may be due to oxygen starvation. Such a situation incidentally would argue for metabolic origin of the post-stimulation undershoot.

It remains to be determined whether BOLD signal modulation by oxygen metabolism significantly alters activation maps. Our preliminary report indicates that BOLD activation is at least twice as sensitive in striate cortex as it is in extrastriate cortex (Chapter 4); this would lead to the conclusion that the borders, at least of striate cortex, are demarcated by BOLD sensitivity rather than by neuronal activation. In practice, this potential mislabeling problem has not yet become an issue; possibly, this is because there are no gold standard high resolution regional activation comparisons handy. Validation study of the BOLD contrast brain mapping technique is therefore necessary, and may be attacked along two parallel fronts: 1) BOLD sensitivity measurements can be made throughout the brain with higher spatial resolution and higher signal to noise, by concentrating on the hypercapnia response to CBF and BOLD sensitive sequences exclusively. 2) Alternative brain mapping
strategies, such as perfusion-sensitive fMRI, optical window methods in animals, and high resolution PET should be compared in detail to discern differences between brain activations detected by BOLD and those detected by other strategies which are not affected by variation in baseline deoxyhemoglobin concentration.

5.5 Ethical considerations

As physicians and scientists, we are trusted with lives and resources which are precious and deserve thoughtful stewardship. When planning and reevaluating research initiatives, one must be ever mindful of potential applications, of alternatives to resource consumption, and ultimately, the overall potential or lack of potential of our current line of work to the betterment of mankind. This assessment must be an active, conscious part of the research endeavor; otherwise, personal priorities and daily demands will drown out the voice of reason. Hence this meager tribute to ethics.

As a field, brain mapping belongs to the basic sciences: adding tools to the tool box so that when an important question comes up, the answer will be ready and waiting. While I believe in basic science as an investment in the future, I remain unconvinced that the majority of functional brain imaging studies are worthwhile. The high cost of imaging time underscores the need for frugal experimentation, and for thoughtful choice of experiments and of experimental design.

It is easy to overemphasize utility for the work of this dissertation: while calibrated functional MRI has the potential to allow measurements of tissue metabolic state, it is unlikely to save lives in the near term; there is a big difference between a research tool and a clinical tool. With patience, the techniques presented here may unfold into a general method for assessing metabolic responsiveness. There may be applications in assessing
treatment for Alzheimer’s and other dementias, for predicting surgical outcomes, or for diagnosing disease.

Even if these techniques do not see clinical application, I believe the current work still to have been worthwhile. Addressing questions in this thesis has taught several people the art of doing science, and has brought answers to some questions about metabolic regulation in the brain.

Cautious but rapid forward progress today is the best hope we have for the future. I look forward to the challenge ahead.
References


