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2005. In "International Review of Neurobiology: Neuroimaging" (M. Glabus, ed.).
Elsevier, New York.
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The imaging revolution in cognitive neuroscience began with a quantifiable, stable measure of cerebral physiology. Positron emission tomography (PET) was applied to the measure of cerebral blood flow (CBF) in the 1980s (Raichle et al., 1983). Using injected, radiolabeled \(^{15}\)O water, and tracer kinetic theory, it was possible to create images that measured the perfusion of brain tissue in physiologic units (cc of blood / 100 g of tissue / minute). Exploiting the neuro-vascular coupling that links local changes in neural activity to local changes in blood flow, PET was subsequently used to study the relationship between cognition and neural activity (Petersen et al., 1988). PET functional imaging had a far-reaching impact, although a consistent limitation of the method was its relatively poor temporal resolution (on the order of a minute) which often stood in contrast to the rapidity of the mental operations under study.

In the 1990s, blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) was introduced. Unlike PET images, and as will be discussed in greater detail below, the BOLD fMRI signal has no stable, absolute interpretation and tends to slowly drift up and down over time. Despite this limitation, BOLD fMRI quickly supplanted PET for cognitive neuroscience applications. In part, the success of BOLD fMRI lay in the ubiquity of the necessary hardware and its noninvasive nature. Also critical, however, was the greatly improved temporal resolution the method could provide (on the order of seconds). Technical refinements of BOLD fMRI have brought further improvement in temporal resolution. Through careful experimental design BOLD fMRI is able to distinguish randomly ordered neural events that occur several times a second (Dale and Buckner, 1997) and to detect neural onset asynchronies on the order of 100 ms (Menon et al., 1998). Efforts to combine simultaneous electroencephalogram (EEG) and fMRI measurements evidence a continuing need for more precise measurements of neural events in time (see chapter 10, this volume).

There are some cognitive processes, however, that evolve over much longer time-scales. Many forms of perceptual or motor learning, for example, are manifest as enduring changes in performance that accumulate over minutes to hours. Emotional behavior, another example, is marked by gradual change from one mood state to another. These sorts of mental operations are imperfectly studied using BOLD fMRI. The limitation is the aforementioned instability of the BOLD signal at long time scales, which makes it difficult to distinguish interesting changes in neural activity from noisy fluctuation of the BOLD signal. Ideally, an imaging method would be available that provides the stable signal of PET imaging and the high temporal resolution and noninvasiveness of BOLD.

A relatively new imaging method, perfusion fMRI, provides this methodological synthesis. Arterial spin labeling (ASL) perfusion fMRI permits the non-invasive quantification of regional brain tissue perfusion using labeled, inflowing arterial protons as an endogenous tracer (Detre and Alsop, 1999). To date, ASL imaging has been used primarily as a clinical tool to measure resting cerebral blood flow in pathological states (e.g., (Alsop et al., 2000)). Now, after a series of
technical improvements and methodological developments, perfusion fMRI is poised for wide-spread application to cognitive neuroscience hypotheses. In this chapter we consider perfusion fMRI for functional neuroimaging. We will first discuss the basic physics and properties of the method. Next, we will compare different techniques for generating ASL images and consider the consequences of those techniques for the signal and noise properties of ASL. These properties of ASL will be explicitly contrasted with those of BOLD fMRI. We will consider the implications of the stability of the ASL signal for data analysis and experimental design. Finally, we will highlight some limitations of ASL methods, and describe current efforts to overcome these restrictions.

**Acquisition of perfusion MRI data**

ASL imaging operates in a manner rather analogous to $^{15}$O PET. The basic scheme is to label water in arterial blood as it flows through the neck into the brain. The label is generally provided by radio-frequency (RF) pulses that alter the magnetization of protons in the arterial water (referred to as inversion). The labeled protons act as a diffusible tracer, and once they reach the capillaries pass into the brain tissue. There, they affect the total magnetization present, and thus alter a subsequently acquired MRI image of the brain (Figure 1). Indeed, virtually any pulse sequence (gradient echo, spin echo, spiral) can be used to “read out” the magnetic effects of the label. The ‘magnetic’ tracer has a decay rate of T1 (about 1-2 seconds), which is sufficiently long to allow perfusion of the microvasculature and tissue to be detected but short enough to allow dynamic changes to be monitored.

There are several possible approaches to performing the magnetic labeling, and these can be grouped into continuous and pulsed techniques (Wong et al., 1998) (Figure 2). In continuous ASL (CASL), flow-driven adiabatic inversion is used to label arterial blood water flowing through the inversion plane during a time window of about a few seconds (Williams et al., 1992). Pulsed ASL (PASL) uses nearly instantaneous inversion of spins in a slab proximal to the imaged tissue (Kim and Tsekos, 1997). In general, CASL techniques provide greater perfusion contrast than PASL methods, but are technically more difficult to implement. Continuous irradiation can also deposit significant RF power into the subject, which may be limiting at higher magnetic fields. It has been argued that the efficiencies of the two labeling schemes are comparable, as PASL
is generally sampled at a higher rate although SNR is lower than CASL (Wong 1998). We observed that CASL perfusion image series are more stable in time than PASL, probably because long labeling pulses in CASL average the effects of flow variability from systolic to diastolic cycle. Finally, the image coverage is generally larger in CASL than PASL which has to make room for a relatively thick inversion slab for labeling.

A further refinement is that a post-labeling delay can be introduced prior to the slice acquisitions. Generally, longer delays optimize accurate quantification of regional CBF, as the delay reduces the effect of different arterial transit times to different brain regions (Alsop and Detre, 1996). This improvement is balanced by a reduction in the magnitude of perfusion functional activation, as longer delays give more of an opportunity for the spins to relax (Gonzalez-At et al., 2000). In practice, once the labeling period, post-label delay, and image acquisition times are considered, each labeled image requires 2-3 seconds to acquire.

The effects of the label are quantified by a pair-wise comparison of the label images with separate images acquired with control labeling. The control condition attempts to replicate the frequency-dependent and off-resonance effects of the labeling without producing significant arterial spin labeling. There are several approaches to accomplish this, including positioning the labeling pulses outside the brain volume, or performing a “double inversion” of the spins (Detre and Alsop, 1999). For our purposes here it is sufficient to note that most functional MRI applications of ASL involve the interleaved acquisition of label and control images. The comparison of these temporally adjacent label and control images reveals the perfusion effect, and is also the source of the distinct noise properties of ASL as compared to BOLD fMRI that we will discuss later. Each perfusion image represents a 4-6 second interval of time, as the image is the difference between adjacent label and control images that themselves took 2-3 seconds to acquire.

Several methods are then available for the conversion of the raw, differenced

![Figure 2. Comparison of ASL techniques. Yellow indicates the labeling plane and gray the site of control labeling. (A) In continuous adiabatic labeling, a long duration, spatially narrow RF pulse is used to tag protons as they flow through the imaging plane. The control image is produced by either applying the label distal to the imaged slice or (in multi-slice CASL) performing a “double inversion” proximal to the imaged slice. Quantification is based upon the known label efficiency. (B&C) Pulsed ASL using either (B) selective slab or (B) selective/non-selective inversion. A larger volume of blood is labeled using a very brief pulse. PASL images can be acquired more rapidly and quantification is based upon the label duration. The control inversion is applied distal to the imaging plane in selective inversion, and to the imaging slice itself in selective/non-selective inversion.](image)
image into a quantitative measure of cerebral blood flow (e.g., (Wang et al., 2002)). CBF measurements using CASL perfusion MRI has been compared to $^{15}$O-PET CBF in the same subjects and an excellent correlation was observed between these modalities both at rest (Ye et al., 2000a) and with functional activation (Feng et al., 2004). Although it is possible to analyze perfusion fMRI data without performing the conversion to CBF values, we will argue below that quantification of the perfusion effect improves across-subject and session statistical power. Figure 3 shows an example of the resulting, quantified perfusion image.

As mentioned, the effects of ASL are independent of the pulse sequence used to obtain images and sample the resulting changes in magnetization. This offers the potential of acquiring images with little susceptibility weighting, such as spin-echo or spiral, while maintaining the same sensitivity to the perfusion effect. This is an advantage for examining brain regions with high susceptibility gradients such as the inferior frontal and temporal lobes (Figure 4) (Wang et al., 2004). However, to maximize slice coverage and minimize acquisition times, many ASL implementations use gradient-echo echoplanar images for measuring
magnetization. Importantly, the raw image data in this case also contains BOLD contrast. This allows BOLD and perfusion effects to be compared within the same data set (Wong et al., 1997).

**Noise and signal properties of perfusion fMRI**

The most important properties of perfusion fMRI for functional neuroimaging result from the altered temporal noise of perfusion data as compared to BOLD fMRI. These noise properties in turn are a consequence of the manner in which the perfusion signal is generated as the difference between successive images. In this section, we will discuss: 1) techniques for obtaining the perfusion signal from the raw, label and control images, 2) the noise properties of perfusion fMRI data, 3) temporal and 4) spatial resolution.

**Differencing methods:** In most cases, perfusion fMRI data are acquired as interleaved label and control images, although the label acquisitions can be used independently to detect perfusion changes (Duyn et al., 2001). The simplest way to obtain the perfusion signal is to perform a pair-wise subtraction of adjacent label and control images (Figure 5). In the ideal case, label and control images would be acquired at precisely the same time, so that the only difference between the two images would be attributable to the effect of the label. Because of practical limitations of the method, such as the basic requirements of the transit time of blood from the neck to the distal cortex, at least two seconds elapse between the acquisition of a labeled image and the next control. During this time fluctuations in the signal value can create changes between the label and the control that are not the result of the effect of label. For example, when gradient-echo images are used to measure tissue magnetization, and the subject is engaged in a task that has a periodic structure, then BOLD signal changes can contaminate the difference between label and control images. As a result, other methods have been used to derive the perfusion signal, including “surround subtraction”, in which the label is subtracted from the average of the two adjacent control images (Wong et al., 1997), and “sinc” subtraction in which sinc-interpolated label and control images are subtracted (Aguirre et al., 2002). Either approach is suitable for analysis of blocked or other slowly-changing patterns of neural activity, which is likely to be the most common type of experimental design studied with perfusion fMRI.

![Figure 5. Differencing methods available to generate perfusion data from label and control images. The source ASL data are composed of interleaved label (L) and control (C) images. The perfusion (P) images are generated by taking the difference between label and control. “Adjacent” subtraction is the simple difference between adjacent image pairs. “Surround” subtraction averages pairs of label images. “Sinc” subtraction uses sinc interpolation to obtain an estimate of what the label time series would have been had it been acquired one TR earlier. “Inter-trial” subtraction pairs label and control images from identical points in time with respect to the onset of experimental trials, which occur every x TRs.](image-url)
A final option, "intertrial subtraction" (Yang et al., 2000), forgoes the attempt to place label and control images in absolute temporal register, and instead pairs label and control images by their timing with relation to experimental events. A simple example of an experiment that is appropriately analyzed with intertrial subtraction is a sparse event-related design in which an identical stimulus is presented briefly every 18 seconds, while images are acquired at a TR of 2 seconds. The derivation of the perfusion signal then involves identifying pairs of label and control images that are acquired at equal points in time in relation to the onset of stimuli. By design, these label and control images will be drawn from separate trials. An advantage of the intertrial subtraction technique is that it effectively removes any artifact in the measured perfusion signal introduced by BOLD effect and therefore most accurately recovers the true shape of the perfusion hemodynamic response. Recently, Liu and Wong (Liu and Wong, 2005) provided a unified model within which the properties of these differencing methods might be considered as specific cases of modulator functions.

Perfusion fMRI temporal noise properties: The character of perfusion fMRI data is best understood first in contrast to the properties of BOLD fMRI. BOLD fMRI data collected from human subjects in the absence of any experimental task or time varying stimuli (i.e., under the null-hypothesis) demonstrate greater power at some frequencies as compared to others. Specifically, there is increasing power at low frequencies, a distribution of power that is well characterized by a 1/frequency (1/f) function (Zarahn et al., 1997), as well as other models (e.g., autoregressive model; (Purdon and Weisskoff, 1998)). There are two important consequences of this low frequency noise. First, the uneven distribution of noise means that BOLD data are not temporally independent under the null-hypothesis. As a consequence, standard parametric (Aguirre et al., 1997; Zarahn et al., 1997) and non-parametric (Aguirre et al., 1998a) statistical tests are invalid for the analysis of BOLD data (requiring instead the use of modified general linear models such as are implemented in SPM). Second, the greater noise at low frequencies will cause a relative reduction in sensitivity for some experimental designs. Specifically, experiments with fundamental frequencies in the lower range (e.g., a boxcar design with 60 second epochs) will have reduced sensitivity, due to the presence of greater noise at these lower frequencies.

It is to be expected, however, that the temporal autocorrelation of ASL perfusion data will differ from that of BOLD. Specifically, the subtraction methods that derive the perfusion time series from adjacent, temporally interleaved images will dampen long-time-scale autocorrelation present in the source noise. In both simulations and empirical data (Aguirre et al., 2002; Wang et al., 2003a) we have demonstrated that the power spectrum of perfusion data is essentially flat (Figure 6).

Figure 6. Perfusion fMRI data are independent under the null hypothesis. The power spectrum typically observed in BOLD fMRI data (dashed line) has ever increasing power at lower frequencies. In contrast, the power spectrum of perfusion data (solid line) is flat, indicating that the observations are independent in time under the null-hypothesis.
The uniformity of the noise is best assured by sinc subtraction, although the difference between the differencing methods is slight (Aguirre et al., 2002; Liu and Wong, 2005; Wang et al., 2003a). A further verification of the independence of perfusion data is provided by the Durbin-Watson statistic, which tests for autocorrelation against a first order, auto-regressive model (Figure 7). Perfusion fMRI data are independent under the null hypothesis. The power spectrum typically observed in BOLD fMRI data (dashed line) has ever increasing power at lower frequencies. In contrast, the power spectrum of perfusion data (solid line) is flat, indicating that the observations are independent in time under the null-hypothesis. Thus, perfusion data are nearly independent under the null-hypothesis. The temporal independence of perfusion data has implications for data analysis and experimental design, both of which are discussed in the following sections.

Hemodynamic response and temporal resolution: Because each perfusion image is derived from two time points, the temporal sampling of perfusion data is twice as coarse as the source images. In practice, each perfusion image reflects events over a 4-6 second period. This relatively poor temporal resolution would seem to preclude using perfusion fMRI methods to accurately measure the shape of the perfusion hemodynamic response that is evoked by changes in neural activity. In fact, it is possible to do so by systematically adjusting the timing of the stimulus presentation with respect to the image acquisition (Josephs et al., 1997), allowing one to sample the shape of the evoked response at arbitrarily high temporal resolution (although at a corresponding cost in the accuracy of the estimate of the response at each time point). Using this approach with the intertrial subtraction method, Yang and colleagues measured the shape of the ASL hemodynamic response (Yang et al., 2000). They found that the evoked perfusion was quicker to begin and narrower in its duration compared to the BOLD response. Others have obtained similar results (Aguirre et al., 2002; Liu and Gao, 1999) (Figure 8). This is to be expected as ASL is primarily sensitive to signal changes.
at capillary sites, and therefore does not reflect the more sluggish venous signal that dominates the BOLD response. High-field studies of perfusion activation using ASL contrast further indicate that the perfusion signal begins to increase as early as 600 ms after the onset of functional stimulation (Silva et al., 2000), earlier than the ‘initial dip’ in BOLD contrast.

**Spatial resolution:** Because BOLD contrast is primarily due to changes in intravascular deoxyhemoglobin concentration, functional activation may be observed overlying venous structures draining the activated region. In some studies BOLD activation has been seen in direct correspondence with macroscopic vessels, with susceptibility effects extending into adjacent cortex (Hoogenraad et al., 2001). In contrast to BOLD fMRI, perfusion fMRI uses a diffusible tracer (magnetically labeled arterial water) which can exchange with tissue water. Because the decay time of this tracer is quite brief (on the order of 1 second), there is minimal accumulation of the tracer in venous structures. As a result, signal changes in perfusion fMRI are not observed over veins, resulting in better localization of signal changes over activated cortex (Silva et al., 1999). It has further been demonstrated that ASL perfusion fMRI provides equivalent spatial resolution to the ‘initial dip’ in BOLD, which has been demonstrated to have superior spatial precision than the aftergoing response (Duong et al., 2000). The point-spread function of BOLD fMRI at FWHM been measured at 3.5 mm in visual cortex (Engel et al., 1997). It is unclear to what extent this resolution is dictated by the hemodynamic point-spread, or whether lateral connections between adjacent neural areas produces spreading neural activity. Perfusion fMRI might be demonstrated to have a smaller point-spread function, although this is technically difficult to assess given limitations in signal-to-noise (SNR) that then mandate the acquisition of rather large voxels using multi-slice perfusion fMRI (discussed below). Finally, the limited responsiveness of perfusion methods to venous signals may in part explain why areas of activation consistently appear smaller in perfusion studies as compared to BOLD fMRI (for example, in figure 4). A relatively weaker signal change may also contribute to this common observation.

**Pre-processing and statistical analysis with perfusion fMRI**

The primary characteristic of data pre-processing and statistical analysis for perfusion fMRI is that many of the idiosyncratic properties of BOLD fMRI that dominate neuroimaging data analysis are not of concern in the analysis of ASL data. We will discuss how perfusion fMRI data: 1) may be analyzed with any parametric or non-parametric test, including permutation methods, 2) do not require, and are weakened by, the application of extrinsic temporal smoothing, 3) do not have temporally dependent spatial correlations, so they uniformly benefit from spatial smoothing, and 4) provide for improved population inferences.

**Permutation analysis for perfusion fMRI:** The absence of serial correlation of the error terms in perfusion fMRI renders unnecessary the use of the "modified" general linear model (Worsley and Friston, 1995) for the analysis of perfusion activation experiments. Indeed, perfusion fMRI data that has been appropriately differenced (as discussed above), can be analyzed using SPM software with the “PET” settings. Further, like PET data, it is possible to assess the significance of statistical results from within-subject perfusion fMRI studies using permutation methods (Nichols and Holmes, 2002), an approach that is not valid
for BOLD fMRI data. Working with Tom Nichols, we have shown that permutation methods control the map-wise false-positive rate and provide appropriately uniform distributions of p-values in null-hypothesis perfusion data. Additionally, when used to analyze experimental data, permutation approaches provide for lower map-wise significance thresholds than do traditional Gaussian Random Field approaches (Figure 9). The essential point is that statistical thresholds for detecting activation are lower when significance is assessed using permutation as compared to traditional GRF methods, and that perfusion fMRI data are eligible for this benefit whereas within-subject BOLD fMRI data are not.

**Temporal smoothing:** The presence of low-frequency temporal noise in BOLD fMRI data, and its variability from voxel-to-voxel, has led to the practice of high-pass filtering BOLD fMRI to minimize low-frequency noise, and low-pass smoothing to “condition” the autocorrelation structure and reduce bias (Friston et al., 2000). Because perfusion fMRI data do not possess temporal autocorrelation under the null-hypothesis, these measures may not be necessary for the analysis of ASL data. Indeed our evaluation of the receiver-operator characteristics of different analysis approaches (Wang et al., 2005a) confirmed that such temporal manipulation degrades the performance of perfusion fMRI. Thus, considered together with our previous point, no temporal autocorrelation need be assumed for perfusion fMRI data, nor does any need to be imposed.

**Spatial coherence and smoothing:** An additional issue in fMRI analysis concerns the “spatial coherence” of the voxel time series in imaging data (Zarahn et al., 1997). Spatial coherence is akin to “smoothness”, but also includes components of spatial correlation that cannot be captured by a continuously differentiable auto-covariance function (e.g., measurements of full-width at half-maximum smoothness). Perhaps surprisingly, spatial coherence can be assessed (and vary) at different temporal frequencies — it is in effect a measure of the degree to which power at a particular temporal frequency shares phase across space. In BOLD fMRI, spatial coherence has been found to vary systematically across temporal frequency, in that lower temporal frequencies tend to share phase to a greater extent across space than high frequencies (Zarahn et al., 1997). As a consequence, spatial smoothing of BOLD data acts to augment temporal noise in the low-frequency range, and can deleteriously impact experimental power (Aguirre et al., 1997).

Because perfusion fMRI data do not possess temporal autocorrelation in time under the null-hypothesis, we might predict that there will be less of an effect of temporal frequency upon spatial coherence. Indeed, this is what we have found (Wang et

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**Figure 9.** Mapwise thresholds using permutation for perfusion fMRI data. Shown are the probability density functions averaged across a group of 10 subjects performing a motor response task studied with PASL. The plots show the map-wise t-threshold as a function of α level for different methods of assessing significance. Permutation thresholds were lower than those obtained by Gaussian Random Field (GRF) theory (or Bonferroni correction) at an α of 0.05. (Aguirre et al., 2003).
al., 2003a) (Figure 10). Perfusion data derive a statistical power benefit from spatial smoothing that is greater than that seen for BOLD fMRI (Wang et al., 2005a). The consequence of this is that perfusion fMRI data will usually benefit from spatial smoothing during pre-processing, although the standard caveats regarding the optimal detection of activations of different scales still applies (Worsley et al., 1996).

Population inference: Neuroimaging questions are sometimes asked about groups of subjects, as opposed to results from a particular subject. For example, one might ask if the population from which a set of subjects are drawn possess a hypothesized effect, or if two different populations differ in the evocation of some effect. These types of hypotheses are appropriately tested within the context of a random-effects model (Friston et al., 1999), in which a single effect measurement is obtained from each subject. A “second level” statistical test is then performed upon this group of effect values. In practice, these effect measures are the scaling values calculated for parameters of interest that model each subject’s data.

Such designs appropriately account for variability in the magnitude of the effect across subjects.

In the ideal case, all variability present in across-subject data would be explained by variability in the magnitude of evoked neural activity. In reality, there are several other sources of variability in the BOLD fMRI effect size. For example, between subject differences in physiology likely produce variability in neuro-vascular coupling which, even in the presence of identical magnitudes of neural activity, would lead to different BOLD fMRI signal changes. These sources of between-subject variability in the BOLD effect act to reduce the statistical power of random effects analyses.

We might expect that between-subject variability in task effect sizes will be different when measured with perfusion fMRI as compared to BOLD fMRI, as perfusion is measuring a single, normalized physiologic parameter (as opposed to blood oxygenation, volume, and flow in BOLD). We have confirmed that between-subject variability in task effect sizes are smaller when measured with perfusion fMRI as compared to BOLD fMRI (Aguirre et al., 2002; Wang et al., 2003a), and that this beneficial effect is maximized when perfusion data are converted to absolute CBF values. Therefore, group analyses may be more powerful when conducted with perfusion imaging as compared to BOLD imaging, even if the magnitude of signal change (relative to within-subject noise) is lower for perfusion data than for BOLD.

**Experimental design with perfusion fMRI**

In the introduction to this chapter, we suggested that perfusion fMRI may be particularly well suited to the detection of slow changes in neural activity. We will
now consider this important aspect of perfusion fMRI experimental design. We will also describe the combined use of BOLD and perfusion contrast in a single experiment.

Detection of slow changes in neural activity: Consider an experiment that seeks to detect a slow, continuous change in neural activity over the course of several minutes. Such a neuronal profile might be produced by motor sequence learning, in which a subject learns a pattern of finger movements in response to visual cues over 20 minutes of continuous training. While performing this kind of task, subjects evidence faster reaction times to the stimuli, even if they are unaware that there is a pattern to the responses that they are producing (Nissen and Bullemer, 1987). Ideally, we would want to follow the profile of neural change, and relate that to cognitive states, such as awareness of the presence of a pattern, and behavioral measures, such as response times and error rates. Such a study could not be performed with BOLD fMRI, however. This is because the power of the experimental paradigm would lie within the greatly elevated noise range of the data, and it would not be possible to distinguish neuronal change from signal drift.

An alternative approach often employed in these circumstances is to interleave the learning task and control task during the scan session. This “chops” the behavior to a higher temporal frequency, placing it within the range that BOLD fMRI can detect. Changes in neural activity during the learning condition can then be compared to the presumably stable signal obtained during the control condition, and the effects of signal drift removed. While a profitable approach in a number of studies (Shadmehr and Holcomb, 1997), there are limitations to this behavioral manipulation. First, it prevents continuous performance. Typically, the learning and control conditions must be alternated every 30 seconds or so. It is possible that the mental operations that
support sequence learning are altered by frequent interruption. Second, it requires the assumption that the control condition does not change over time. It is possible that subjects engage rehearsal or preparatory strategies during the “control” task, in which case the baseline itself would be altered over time, rendering the comparison with the learning condition suspect.

Using perfusion fMRI, however, it is possible to directly study the time-course of a change in neural activity, even if it evolve over an hour or days. This is because the perfusion fMRI signal has uniform noise across temporal frequencies, and thus should have the same power to detect a given change in neural activity whether it evolves over 30 seconds or 30 minutes. We have recently studied slow motor learning using perfusion imaging (Olson et al., In Press). Ten subjects performed a serial response time task continuously for 20 minutes. For the first 15 minutes, there was a pattern in the sequence of stimuli to which they responded, although they were initially unaware that the stimuli had any particular order. During this training, reaction times fell linearly with time. After 15 minutes, the sequence changed without warning or comment to a new, random order of stimuli (termed the “transfer” sequence) for another 5 minutes. Figure 11 shows the time course of cerebral blood flow measured from the left ventral, premotor cortex during this period. A steady decline in CBF is clearly visible during the training, followed by a discontinuous rise in CBF in response to the switch to the “transfer” sequence. These data demonstrate that perfusion fMRI can be used to reliably detect even very gradual changes in neural activity, and provide us with the ability to relate dynamic changes in neural processing with behavior over long time scales.

The stability of the perfusion signal is such that the notion of “long time scale” can be pushed to the extreme. The perfusion signal is sufficiently stable that the CBF difference between rest and finger movements, for example, can be reliably measured even if these images are obtained on different days (Wang et al., 2003b) (Figure 12). This emphasizes that the quantified perfusion image has an absolute value—it is not required that the signal during one condition be compared to the signal obtained in a different condition during the same session. One might, for example, relate the absolute regional CBF during a particular behavioral state to a personality measure across subjects: perhaps CBF within the amygdala during viewing of unpleasant pictures might be found to correlate with a clinical depression rating scale.

Thus, perfusion fMRI provides an opportunity to study slowly evolving behaviors or perform longitudinal studies that would be awkward to conduct using BOLD fMRI. One can readily imagine other sorts of behaviors (e.g., emotional state) that would be greatly altered by rapidly switching between conditions or experimental effects with a time course that cannot be manipulated (e.g., pharmacokinetics of an administered drug). Studies of emotion, pharmacologic

![Figure 12. Perfusion fMRI activation for motor activity studied on two different days. This subject participated in a perfusion fMRI scan on one day during which he remained still. The next day, he performed a continuous motor task involving finger opposition. The quantified perfusion signal is sufficiently stable such that the difference in CBF in the motor cortex is revealed even when studied on different days (Wang et al., 2003b).](image)
treatment, rehabilitation, learning and sleep are all domains of cognition and clinical practice for which perfusion may be a relevant tool.

Theoretical (Aguirre et al., 2002) and empirical (Wang et al., 2003a) analyses have shown that, for behaviors that evolve over time-scales longer than 1 or 2 minutes, perfusion fMRI will provide superior, within-subject sensitivity to changes in neural activity than does BOLD fMRI. It is, of course, worth emphasizing that for changes in neural activity that proceed more rapidly, BOLD fMRI continues to provide markedly better within-subject power, in some cases by a factor of 2-3. This is particularly the case for event-related designs. Because BOLD fMRI continues to be superior to perfusion for many classes of experimental design, it may be advantageous in some circumstances to use both methods in concert.

Combined BOLD and perfusion studies: As was noted earlier, the perfusion effects of ASL are independent of the pulse sequence used to obtain the images after the label period. Thus, if echoplanar images are used, the raw image data also contain BOLD contrast, allowing BOLD and perfusion effects to be compared within the same data set. It is the case, however, that when optimized for ASL acquisition, the echoplanar sequence is no longer ideal for the BOLD effect. In particular, a shorter TE is desirable for ASL data collection, whereas a longer TE is preferable for BOLD SNR. Despite this, BOLD signal changes can readily be detected in the source label and control image data.

BOLD and ASL effects are best combined when they are used to seek different temporal structures of neural activity within the same dataset. In our sequence learning example, our main focus was upon the slow changes in neural activity that accompany learning. It was the case, however, that subjects occasionally pressed the wrong button of their response pad while performing the task. Would it be possible for us to detect neural profiles associated with error responses? These errors were sporadic and brief, having a temporal structure similar in many ways to a “sparse” event related design. Such changes in neural activity would be difficult to detect using perfusion, but are within the sensitivity range for BOLD fMRI. We were able to identify regional changes in BOLD signal in the unsmoothed, label and control images associated with error trials (Olson et al., Submitted).

It appears that the voxel-wise BOLD and perfusion time series data are uncorrelated under the null-hypothesis (GKA, unpublished observations). As a result, with clever experimental design, one may use the BOLD signal effect to define regions of interest, and then within those regions probe for more subtle, slow changes in the perfusion signal. These approaches can combine the best of these two different imaging modalities.

Limitations and developing areas

The potential benefits that perfusion fMRI can provide are balanced by several significant limitations. Principal among these is the smaller SNR for ASL imaging as compared to BOLD. The change in raw image intensity between the control and labeled images is about 1%, as compared to 2-4% in BOLD. A practical consequence of this limited SNR is that ASL images are typically acquired with relatively large voxels or thick slices in an effort to improve sensitivity. A second limitation is the speed of image acquisition. Because perfusion volumes are calculated using pairs of images, the temporal sampling of the
resulting data is half of the source data. Moreover, the time to acquire a single image is lengthy: roughly a second each for label, delay, and image acquisition. A corollary problem is that the need to reduce image acquisition time prompts the use of 2-dimensional echoplanar imaging (EPI) for read-out. EPI images can be acquired quickly, but are distorted and severely degraded in the presence of static susceptibility gradients, such as are found near the frontal sinuses and auditory canals, causing loss of signal from orbitofrontal and temporal cortex.

There are, however, several ways in which these limitations might be ameliorated. For example, improved ASL data can be obtained by scanning at higher field strengths. The stronger magnetic field has two beneficial effects. First, the raw SNR is improved, as it is proportional to the main field strength. Second, the T1 relaxation time of the label is prolonged at higher field strength. As a result, the loss of spin labeling while blood is traveling from the labeling plane to the cerebral tissue is much less than that at standard field, producing greater perfusion signal in brain tissue while reducing arterial transit related artifacts and quantification errors. As was mentioned, application of CASL at high field strengths can be difficult, as the deposition of radio-frequency energy could potentially exceed acceptable levels. There are ways, however, to finesse these limitations and obtain CASL images with acceptable energy levels (Wang et al., 2005b) (Garraux et al., 2005) . More efficient labeling approaches are also being developed to improve SNR of perfusion signal while reducing RF power at high magnetic field. For activation studies at high field, the use of optimal image acquisition schemes, particularly shorter TE, has been proven crucial to yield improved functional sensitivity(Wang et al., 2002) . This is because higher blood flow accompanying neuronal activation may lead to direct outflow of portions of labeled arterial spins into veins, which have very low signal or are even invisible due to strong susceptibility effects at high field.

Additional improvement can be provided by phased array coils, which combine a set of smaller receive coils to cover a larger image volume. When placed in the proximity of head, an array coil with 8 channels is able to yield more than 4 fold SNR gain in the cortex at 1.5 Tesla (de Zwart et al., 2002). While surface coil methods can introduce undesirable inhomogeneity into the image (i.e., the center of the image is “darker” compared to the periphery), these effects are minimized in quantitative ASL perfusion images due to calibration using the control images. Phase array coils can also be used to perform “parallel” imaging, to obtain data with shorter echo times and thus reduced susceptibility artifact (Wang et al., Submitted). Finally, perfusion SNR in time can be improved by performing background suppression, in which the magnetization of the static brain tissue is reduced, thereby increasing the dynamic range for the detection of the ASL approach (Ye et al., 2000b). This method requires, however, the use of a 3-dimensional method for image readout as background suppression can only be accomplished at a single acquisition time (which is not provided, for example, by multislice EPI).

As mentioned, methods other than EPI can be used to obtain the images that are sensitive to the perfusion effect. Spin-echo imaging is one example that has decreased susceptibility artifact (Figure 4), but still suffers from field distortion. Additionally, spin-echo is also a 2-dimensional method that does not allow the use of background suppression. Ideally, perfusion images
would be acquired using a 3-dimensional sequence that is not susceptibility sensitive and can be obtained rapidly. The rapidity is a challenge for 3-dimensional methods, but is essential if the data are to be used for fMRI in which multiple images must be acquired quickly to provide a time-series. Candidate, fast imaging methods include 3D fast spin-echo (FSE) and 3D GRASE (Feinberg et al., 1995; Fernandez-Seara et al., submitted). The combination of 3D algorithms with parallel imaging and array coils provides a promising approach towards whole brain perfusion imaging free of susceptibility effects.

By combining these refinements, it is possible that ASL based, perfusion fMRI will see an improvement in SNR of 4 fold or more. With improved sensitivity, it will be possible to obtain data with greater spatial resolution, taking full advantage of the selectivity of ASL methods to capillary signals. The promise of ASL therefore is a fully quantifiable, stable, physiologic measure with improved spatial resolution and minimal anatomical distortion. It should prove useful now and in the years ahead as a tool for the study of cognition and neural function.

References


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