

Functional MRI based on changes in Cerebral Blood Volume

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- **Introduction**

Brain function can be studied non-invasively using Blood-Oxygenation-Level-Dependent (BOLD) (1) contrast or Cerebral Blood Flow (CBF) based contrast using Arterial-Spin-Labeling (ASL) techniques (2). Neuronal activities also cause changes in Cerebral Blood Volume (CBV) and a variety of MRI approaches can be used to detect such effects. CBV-based fMRI has two potential advantages. First, since the vasodilatation/vasoconstriction during brain activation/inhibition mainly occurs in microvascular vessels (diameters $< 200\mu\text{m}$), the CBV fMRI is expected to show only microvascular activation regions, which is localized to the brain parenchyma (3). Therefore, CBV fMRI provides higher spatial specificity compared to the BOLD technique. Second, CBV fMRI can be combined with other fMRI methods to study different aspects of the hemodynamic responses during functional activation and/or physiological challenges. The spatial and temporal characteristics of these multi-modal fMRI responses can provide us with a tool for quantitative evaluation of brain physiology, such as oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen (CMRO₂), from which one can study how the brain regulates its blood supply and maintain homeostasis during normal state and in pathology.

- **Methodologies for CBV-based fMRI**

The key requirements for CBV-based fMRI are to 1) make the MR signal sensitive to CBV; 2) (ideally) exclusively sensitive to CBV. To meet the second requirement, the MR protocol has to be designed such that physiological changes (e.g. blood oxygenation, blood flow) other than CBV only have minimal effects on the MR signal. To achieve the sensitivity of the MR signal to CBV, one can utilize certain “unique” properties of the blood when considering the MR signal formation. The approaches to evaluate CBV changes can be generally separated into two categories (Table 1): methods that do not require the administration of exogenous contrast agent and use the intrinsic MR properties of the blood for CBV sensitivity; and methods that require the use of contrast agent and encode the CBV information in the MR signal by the vascular distribution of contrast agent. Notably, the first fMRI study ever reported was actually performed using bolus-tracking contrast agent-based CBV method (4). However, due to the need of contrast agent injection and limited time-window for data acquisition (one bolus only allows the acquisition of one time-point), that method is not widely used.

In this lecture, we will discuss five different methods based on the properties of blood related to T₁, T₂, T₂*, magnetization transfer, and contrast agent distribution. Depending on the particular method used, the MR signal reflects activation-related changes in total CBV, arterial CBV (CBV_a) or venous CBV (CBV_v). Table 1 summarizes the characteristics of different methods.

Method	Principle	Advantages	Pitfalls	CBV baseline value (ml blood/100ml brain)	CBV change (%)	
w/o contrast agent	VASO (measures total CBV changes)	Uses T1 property of blood to modulate blood signal	Allows dynamic imaging of CBV-related signal changes	Quantification of baseline CBV, requires contrast agent. Quantification of CBV change requires assumption of baseline CBV value.	5.5	15% at 4x4x10mm ³ voxel size; 46% at 2x2x5mm ³ voxel size
	MEGESE (measures venous CBV)	Uses extravascular BOLD T2* effect of the venous blood to modulate tissue signal	Allows quantification of CBV in physiologic units	Many assumptions and approximations in the theory remain to be validated; blood signal needs to be accounted for or removed	2.97 (venous CBV)	53% (venous CBV increase)
	VERVE (measures vCBV changes)	Uses intravascular BOLD T2 effect of the venous blood to modulate blood signal	Allows dynamic imaging of signal changes related to venous CBV changes	Tissue T2 may also be dependent on refocusing interval; does not allow quantification of absolute venous CBV	N/a	16% at 4x4x5mm ³ voxel size
	MOTIVE (measures arterial CBV)	Uses MT to modulate the tissue signal and uses ASL approach to modulate the blood signal	Allows quantification of arterial CBV in physiologic units	Venous blood signal was neglected and this assumption is not applicable at most fields for human use	1.06 (arterial CBV)	N/a

w/ contrast agent	Contrast agent- based CBV fMRI (total CBV changes)	Use the distribution property of the contrast agent (exclusively located in intravascular space) to modulate the tissue signal	Allows dynamics imaging of signal changes related to total CBV; high sensitivity	Require contrast agent administration; the exact relationship between CBV and MR signal is not established and is likely dependent on vascular geometry	N/a	24% at 0.15x0.15x0.1 5mm ³ in rats
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Table 1

❖ Vascular-Space-Occupancy (VASO) dependent fMRI

This approach is based on the distinctive T1 values (~1350ms at 1.5T and ~1624ms at 3.0T) of the blood to separate it from other tissue types (e.g. gray matter, white matter, CSF) (5). Since blood T1 is minimally dependent on the oxygenation status at typical clinical field strengths (4), it is assumed that the entire space occupied by the blood can be treated as a single compartment and, therefore, the VASO signal reflects changes in total CBV.

The pulse sequence for VASO fMRI is a non-slice-selective inversion recovery sequence. After the inversion of the starting longitudinal magnetization, $M(0)$, from parallel to the magnetic field to antiparallel, the parenchymal components relax back to equilibrium. Because T1 differs between blood and tissue, the point at which the magnetization crosses through zero differs. Longitudinal magnetization cannot be detected, but when, at a time $t=TI$, such an inversion experiment is followed by excitation of transverse magnetization (which is detectable), the effects of the inversion preparation determine the starting signal magnitude. This signal decays exponentially with the transverse relaxation time, which is described by T_2^* for gradient-echo imaging and T_2 for spin-echo imaging. When a gradient-echo pulse sequence is used for image acquisition with echo time TE , the signal dependence is:

$$S = M(TI) \cdot e^{-TE/T_2^*} = M_0 \cdot (1 - 2e^{-TI/T_1} + e^{-TR/T_1}) \cdot e^{-TE/T_2^*} \quad (1)$$

in which TR is the time needed for a single MRI acquisition. Thus, for VASO-fMRI to be successful, $M_{\text{blood}}(TI)$ must be zero, while $M_{\text{tissue}}(TI)$ should preferably be as large as possible. The method must be optimized to ensure that the blood signal nulling is achieved in a manner that is insensitive to changes in blood flow velocity (to avoid inflow artifacts) and blood oxygenation during brain activation. The technique was made insensitive to blood flow velocity changes by inverting magnetization in a spatially nonselective manner. Since T1 depends on the magnetic field, blood T1 for each particular field strength must be determined to optimize TI for nulling. In addition, to minimize residual BOLD effects, the time of detection of the maximum MRI signal after excitation (the TE) should be kept as short as possible.

One can show that the VASO signal change is related to CBV change by:

$$\frac{\Delta S}{S} = \frac{-\Delta CBV \cdot C_{\text{blood}}}{C_{\text{par}} - CBV_{\text{rest}} \cdot C_{\text{blood}}} \quad (2)$$

in which C_{par} and C_{blood} are the water densities of parenchymal tissue and blood, respectively, in ml water/ml substance. Typically, $C_{\text{par}}=0.89$ ml water/ml parenchyma and $C_{\text{blood}}=0.87$ ml water/ml blood, and they are not dependent on field strength.

VASO fMRI has been tested using flashing checkerboard visual stimulation, providing robust activation in the visual cortex. Note that activation causes a VASO signal reduction, corresponding to a CBV increase (see Eq. 2). This is different from BOLD or CBF methods, in which the MR signal increases upon stimulation. However, note that this is only because of the different mechanisms in MR signal formation, and the underlying physiologic parameters are consistent with each other.

In addition, the VASO approach has been applied to the application of retinotopic mapping in humans (6), yielding clear delineation of multiple representations of the visual field in the early visual areas. More recently, the VASO approach has been combined with the steady-state T1 effect of contrast agent (Gd-DTPA) for quantification of absolute CBV (7).

A pitfall of the VASO approach is that only the CBV-related MR signal change can be measured and the quantification of CBV and its percentage change require careful considerations. In particular, the flow effect may still be present in the VASO signal when using short TR, which could cause overestimation of the CBV changes. Other confounding factors of the VASO technique include the partial volume effect of CSF, potential possibility of tissue T1 change during activation, and incomplete nulling of blood signal related to variation in hematocrit in vessels of different size.

- ❖ Multi-echo gradient and spin echo (MEGESE) sequence for venous CBV (CBV_v) estimation based on T2* shortening effect of venous blood on extravascular tissue

Deoxyhemoglobin in the blood is paramagnetic and it can cause MR signal dephasing in the extravascular tissue. This transverse magnetization decay is in addition to the intrinsic spin-spin relaxation (T2) and is related to both the blood oxygenation saturation (Y) and CBV_v . Ignoring the T2 effect and field inhomogeneity, the signal decay can be approximated by two asymptotic forms, one short time scale expression and one long time scale expression (8):

$$S_{\text{short}} = S_0 \cdot e^{-0.3 \cdot vCBV \cdot (\delta\omega \cdot TE)^2} \quad \text{when } TE \cdot \delta\omega \leq 1.5 \quad (3)$$

$$S_{\text{long}} = S_0 \cdot e^{-R2' \cdot (TE - t_c)} \quad \text{when } TE \cdot \delta\omega > 1.5 \quad (4)$$

where S_0 is the MR signal in the absence of any deoxyhemoglobin, $\delta\omega$ is the deoxyhemoglobin induced frequency shift, which is proportional to $1-Y$, $R2' = R2^* - R2$, $t_c = 1/\delta\omega$.

An and Lin (9) utilized the fact that the two expressions are different in terms of the signal dependence on Y and CBV_v , and thereby can be fitted separately to obtain independent estimations of these two parameters. A multi-echo gradient and spin echo sequence (MEGESE) was used to acquire images with a number (typically 10) of gradient-echoes acquired before the spin-echo and a second set of gradient-echoes acquired after the spin-echo. The purpose of a MEGESE sequence rather than a conventional gradient-echo sequence is to obtain the estimation of R2 simultaneously with R2* in one experiment, thereby allowing the subtraction of R2 effect from the R2* and Eq. 3 and 4 can be used for quantification of Y and CBV_v . In their first paper, a CBV_v of 16% was measured in the brain parenchyma, which is larger than literature values and may be attributed to the fact that the measured R2' also include effects from macroscopic field inhomogeneity that is not related to venous blood. In a follow-up paper, these investigators acquired an additional field map and used it to estimate the true R2' related to deoxyhemoglobin, and a whole brain CBV_v of 2.98% was observed, which is closer to the expected value

(total_CBV x venous_fraction = 4.7% x 0.7 = 3.29%). The application of this method to evaluate CBV_v changes during hypercapnia has also been tested recently, giving 53% increase in CBV_v (10).

One drawback of the MEGESE approach is that the quantification of CBV is based on a simplified model of the vascular network (each microvessel is assumed to be an infinitely long cylinder) and, in reality, this assumption may not be applicable. Furthermore, the theory requires the measurement of $R2'$ related to deoxyhemoglobin only, and even though field map can be used to subtract out part of the field inhomogeneity, the intravoxel inhomogeneity will still be present and would require ultra-high-resolution field map to eliminate. In addition, the signal from the blood is neglected in the theory, which may cause error in the estimation. However, the authors have addressed this in a follow-up paper where the intravascular contribution was minimized through the application of magnetic field gradients (10).

Similar to using the extravascular BOLD effect to study CBV, the intravascular BOLD effect also depends on CBV and can be used to assess this parameter (11,12). This principle is also used in the VERVE technique.

❖ Venous Refocusing for Volume Estimation (VERVE) fMRI

The basic concept of VERVE fMRI (13) is the assumption that the transverse relaxation times of the tissue and fully-oxygenated blood are not dependent on the spacing of the refocusing pulses (termed CPMG τ_{180}), whereas the venous blood T2 is. Therefore, one can use this property of the venous blood to detect the changes in CBV_v . The authors interleave the EPI acquisitions with tightly-spaced refocusing pulses (64 refocusing pulses with $\tau_{180}=3.75\text{ms}$) and sparsely-spaced refocusing pulses (8 pulses with $\tau_{180}=30.0\text{ms}$), thereby maintaining a constant TE of 240ms. As a result, the tissue and arterial blood signals will be identical for the two cases, assuming their T2 is independent of the τ_{180} . On the other hand, the venous blood signal will be different for the two cases. It is further assumed that the venous blood T2 difference between the short and long τ_{180} is relatively insensitive to the Y within the range of interest (i.e. $R2_{3.75\text{ms}} - R2_{30\text{ms}}$ is independent of Y). Therefore, signal subtraction can be carried out to obtain CBV_v weighted signals, similar to the subtraction performed in ASL experiments. The VERVE method has been tested using flashing checkerboard visual stimulation. A $16\pm 2\%$ CBV_v increase is seen in the visual cortex.

One issue about the VERVE method is that the key assumption that the tissue T2 is independent of the refocusing interval may not be valid, especially for tissue around microvessels where the diffusion dephasing effect cannot be neglected. One can reduce the two τ_{180} values to ensure that, within that range, the tissue T2 is minimally affected. However, such choice will also reduce the range of venous T2 variation, thereby reducing the sensitivity of the technique. Another potential complication is that the quantification of CBV_v change requires an additional calibration step, which needs to be conducted for each field strength, hematocrit level and τ_{180} value. Furthermore, the assumption that the venous blood T2 difference between the short and long τ_{180} is independent of the Y is not strictly correct, which can cause error in the CBV_v estimation.

❖ Modulation of Tissue and Vessel (MOTIVE) approach for estimation of arterial CBV (CBV_a)

Contrast between tissue and blood can also be achieved using the magnetization transfer (MT) approaches. It has been shown that tissue suppression with MT enhances the BOLD effect

(14) and should be useful to determine CBV. Kim and Kim (15) proposed that the MT pulse can be used to selectively modulate the tissue signal, while only having negligible effect on the blood signal because of inflow of fresh spins as well as minimal macromolecular blood content. When combining this with arterial spin labeling (ASL), the ASL pulse can be used to selectively modulate the blood signal assuming that all spins in the arterial blood are effectively inverted, whereas the tissue is affected following a different equation related to perfusion. The signal from venous blood is neglected due to very short T2 at high field (5-7ms at 9.4T) (15). Based on these theory and assumptions, the CBV_a and CBF can be simultaneously estimated using different levels of MT saturation in combination with ASL pulses.

Using this method, the authors estimated a CBV_a of 1.0 ± 0.3 ml blood/100 g brain in rat cortex and in basal ganglia. Accounting for the density of brain (1.06g/ml), this corresponds to $1.06 \pm 0.32\%$ (i.e. ml blood/100ml brain) in terms of volume fraction, which is in reasonably agreement with expected value of $\text{total_CBV} \times \text{arterial_fraction} = 4.7\% \times 0.3 = 1.41\%$.

In addition, Petersen and colleagues have also proposed to use ASL technique to separate the CBV_a and CBF effects when combining with the crusher gradients (16).

Note that, in the MOTIVE approach, the venous blood signal is completely neglected in the calculation. This would require further validation at 9.4T and, for experiments at lower field strength, this assumption is invalid. In addition, the theory used in the MOTIVE approach involves many approximations to linearize the equations, the effect of which remains to be investigated.

❖ CBV fMRI using paramagnetic contrast agent

The previous techniques use specially designed MR pulse sequences to obtain CBV information and does not require the administration of contrast agent. On the other hand, contrast agents can also be used to achieve CBV weighting of the MR signal. This is based on the assumption that the contrast agent only occupies the intravascular space and does not penetrate the blood-brain-barrier.

Belliveau et al. have used Gd-DTPA first-pass curves to detect CBV changes during visual stimulation (17). More recently, Mandeville and colleagues have used long blood-half-life contrast agent, monocrystalline iron oxide nanoparticle (MION), for dynamic imaging of CBV changes (18). Robust activations were detected and the sensitivity using this technique was found to be higher than that of the non-contrast BOLD technique (19), providing a useful alternative for brain mapping in animals. In general, it is assumed that tissue $R2^*$ (or $R2$) change caused by the contrast agent is mainly extravascular and therefore approximately proportional to CBV. Even though there are some numerical simulation works to support such an assumption, the precise relationship between the transverse relaxation rates and CBV will be dependent on the vascular geometry and distribution of vessel diameters, similar to the $R2^*$ effect due to the deoxyhemoglobin. A big weakness of these approaches is that the intravascular effects are neglected, while large signal changes in the draining veins are obvious in both spin echo and gradient echo experiments when giving a contrast agent. Also, the situations around microvessels and large vessels will be different. Therefore, quantification of CBV change is not trivial. In addition, the use of contrast agent obviously increases the invasiveness of the technique, and in particular the MION has not been approved for human use in the US.

● **Conclusions**

Until this millennium, the only way to study CBV changes with fMRI was the use of contrast agents or the BOLD effect. In recent years methods have become available that can

measure CBV effects more specifically by modulating blood (using T1) or tissue (using MT) signal contributions. It is expected that these new methodologies will be further developed in the coming years and then may become a viable alternative for certain fMRI studies. They will definitely become important in the study of fMRI mechanisms.

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