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# Biophysics of BOLD fMRI investigated with animal models

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ABSTRACT

The widely-used BOLD fMRI signal depends on various anatomical, physiological, and imaging parameters. Thus, it is important to examine its biophysical and physiological source in order to optimize, model and accurately interpret fMRI. Animal models have been used to investigate these issues to take systematic measurements and combine with conventional invasive approaches. Here, we reviewed and discussed multiple issues, including the echo time-dependent intravascular contribution and extravascular contributions, gradient-echo vs. spin-echo fMRI, the physiological source of BOLD fMRI, arterial vs. venous cerebral blood volume change, cerebral oxygen consumption change, and arterial oxygen saturation change. We then discuss future directions of animal fMRI and translation to human fMRI. Systematic biophysical BOLD fMRI studies provide insight into the modeling and interpretation of BOLD fMRI in animals and humans.



## 1. Introduction

The blood oxygenation-level dependent (BOLD) fMRI technique [1,2] relies on changes in deoxyhemoglobin (dHb) content, which acts as an endogenous paramagnetic contrast agent [3]. Changes in local dHb content in the brain lead to alterations in signal intensity on magnetic resonance imaging (MRI) [1,2]. It is thought that neural activation leads to an increase in oxygen delivery without commensurate elevation in cerebral oxygen consumption [4,5], which results in a decrease in capillary and venous dHb content. Decreased paramagnetic dHb content should enhance the MRI signal in the venous vessels as well as in the surrounding tissue. Since successful application of BOLD contrast to human functional brain mapping was reported [6–8], BOLD fMRI has been a major tool for mapping brain function in humans.

As mentioned, BOLD fMRI is sensitive to changes in the susceptibility effect induced by dHb, which is dependent on oxygenation level and cerebral blood volume (CBV) [9]. Since various anatomical, physiological, and imaging parameters contribute to changes in BOLD signals [10], it is important to examine the source of such changes in order to optimize acquisition parameters, model BOLD contrast, and accurately interpret fMRI data. Investigations of biophysical and physiological BOLD sources can be performed in humans, but are often limited by experimental duration and lack of a gold standard. Thus, animal studies are needed to perform extensive averaging and systematic measurements with invasive approaches.

Since the BOLD fMRI field is now a quarter century old, many excellent review articles are available on human applications and neurophysiology (e.g. [11,12]). This article therefore focuses on findings based primarily on animal studies for investigations of biophysical sources of BOLD fMRI (see also [10,13]). Anatomical sources of gradient-echo and spin-echo BOLD signals are reviewed. Then, the quantification of BOLD signals are discussed, and its underlying assumptions are examined through experimental evidence. Personal perspectives on future animal fMRI for BOLD mechanism studies are mentioned, as well as translation to human fMRI.

## 2. Underlying biophysics of BOLD signals

In a given voxel, the MRI signal intensity with dephasing effects (i.e., frequency shifts) induced by numerous vessels is summed, resulting in a decrease in $T_2^*$ and a decrease in MRI signal [9]. The signal in the voxel can be described according to the equation

$$S(TE) = \sum_i S_{0i} e^{-TE/T_{2i}} e^{-i\omega_i TE} \quad (1)$$

where the summation is performed over the parameter *i*, which designates small-volume elements within the voxel. The $\omega_i TE$ indicates the phase shift of location *i* at echo time TE. $S_0$ represents the magnetization at the steady state condition, which is also related to

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inflow effect. Since the contribution of each parameter to fMRI was discussed previously [10], we only discuss the oxygenation-induced MRI signal. In the general BOLD model, it is assumed that there is (i) no inflow effect, (ii) no cerebrospinal fluid or white matter contribution to the imaging voxels, and (iii) no arterial blood contribution. Signal changes induced by neural activities are due to changes in $T_2$ (the first term) and the phase of the venous blood and extravascular gray matter tissue (the second term in Eq. (1)). Both intravascular (IV) and extravascular (EV) water protons contribute to BOLD signals.

### 2.1. Intravascular BOLD signals

Blood water $R_2$ and $R_2^*$ values are directly related to paramagnetic dHb content [14]. Water rapidly exchanges between dHb-containing red blood cells (RBC) and plasma, and also diffuses in the presence of the magnetic field gradients generated by the dHb inside RBCs. These exchange and diffusion processes result in a loss of phase coherence, called 'dynamic' (time irreversible) averaging. Blood $R_2$ can be written as $A_o + K(1 - Y)^2$, where Y is the oxygen saturation level, $A_o$ is a constant term, and K scales quadratically with the magnetic field and is also dependent on the echo time used in spin-echo measurement [15,16]. In addition to the dHb-content-related $R_2$ change, a frequency change is also observed, which is dependent on magnetic field, oxygen saturation level, and the angle between vessel direction and $B_0$ [9]. Because multiple vessels at different orientations typically exist within a given pixel, their multiple frequency shifts cause a phase dispersion (rather than a net phase shift) and a reduction in blood $T_2^*$.

Venous blood $T_2$ is shortened relative to tissue $T_2$ at high magnetic fields (see discussion in [10,17]). Thus, the IV contribution is reduced at higher fields when TE is set to tissue $T_2$, and can be investigated by flow-crushing bipolar gradients. The IV contribution is predominant at 1.5–3.0 T [18–21], <20% of the total BOLD signal at 7 T [21,22], and negligible at 9.4 T when TE > tissue $T_2$ [23,24]. Fig. 1A and B shows SE BOLD fMRI responses at various echo times obtained at 9.4 T during visual stimulation. Without the use of crushing gradients, nonlinear TE-dependent BOLD signals were observed, with more for surface pixels with larger vascular volume fractions (Fig. 1A). Note that similar nonlinear TE-dependent BOLD responses were also observed at 3 T [25]. When a *b*-value of 200 s/mm$^2$ was applied, the IV component was suppressed, leaving a linear TE-dependent EV BOLD signal (Fig. 1A). The IV component is about 50% of the total SE BOLD signal with a TE of 20 ms at 9.4 T, and is reduced when a longer TE is used (Fig. 1B). Overall, the IV BOLD contribution is less at higher magnetic field strength and longer TE [10,17,22].

### 2.2. Extravascular BOLD signals

The susceptibility effect generated by dHb affects tissue beyond the blood vessel with a magnitude related to $(r/a)^2$, where *r* is the distance from the vessel to the region of interest and *a* is the vessel radius [9]. This shows that the susceptibility effect induced by larger vessels extends further away to EV tissue and nearby cerebrospinal fluid (possibly neighboring voxels) (see a schematic in [13]). In a voxel with numerous microvessels, the susceptibility-induced relaxation rate of EV water spins is closely related to the amount of dHb [9,26–28]. Susceptibility effects will increase with (i) an increase in venous CBV and consequent increase in dHb content in the voxel, (ii) a decrease in venous oxygen saturation level, or (iii) an increase in magnetic field ($\omega_0$). The susceptibility-induced BOLD fMRI signal almost linearly increases with venous blood volume and magnetic field strength [9,17,26–28].

Although the gradient-echo (GE) BOLD contrast is predominantly used, it is still worthwhile to examine spin-echo (SE) contrast due to its improved specificity. For vessels larger than the tuned size of the maximal $R_2$ effect, the extravascular $R_2$ change is reduced, while $R_2^*$ change remains high [28]. The tuned vessel size (typically 3–10 μm in diameter at high fields) decreases with a longer echo time (i.e., longer diffusion distance) and a higher magnetic field (i.e., larger susceptibility gradient) [28]. Thus, SE BOLD signals predominantly originate from small vessels including capillaries. The field-dependency of extravascular $R_2$ change is linear to quadratic [9], and is expected to be linear at ultrahigh fields [17,29]. Furthermore, the SE BOLD response peaks at a magnetic field of 7–9.4 T according to simulations [17,29]. Experimentally, BOLD fMRI percent changes at the same echo time (16 ms for GE, and 25 ms for SE BOLD) were found to be similar at 7 T and 11.7 T [30]. Since higher magnetic fields induce higher EV and less IV BOLD signals, the similar BOLD response at the two magnetic fields can be explained by a match between an increase in the EV BOLD signal and a decrease in the IV signal at 11.7 T.

### 2.3. GE BOLD vs. SE BOLD fMRI at high resolution

The IV water proton spins of vessels of any size and EV spins of microvessels contribute to both GE and SE BOLD signals, while the EV spins around macrovessels contribute mostly to GE BOLD signals. Consequently, GE BOLD fMRI is always more sensitive than SE BOLD fMRI; thus, most fMRI studies utilize GE BOLD contrast. When the IV contribution of macrovessels is removed using high fields and/or bipolar gradients (see Fig. 1A and B), SE BOLD fMRI can improve the spatial specificity to microvessels and neighboring tissue, which should be close to active neuronal sites. However, its sensitivity is poor, especially for high-resolution SE BOLD fMRI, which requires high magnetic fields.

To test the specificity of fMRI, we obtained GE and SE BOLD fMRI (Fig. 1C and D) of cat brain responding to visual stimulation with 156 × 156 μm$^2$ in-plane resolution at 9.4 T [31]. The highest GE BOLD signals were observed at the surface of the cortex (yellow pixels in Fig. 1C), where many large draining veins exist. This large vessel problem is accentuated at high spatial resolutions because the distribution of venous vessels across voxels is highly uneven (e.g., >10% in the large vessel-containing vessels vs. 2–5% in cortical voxels) [32,33]. The BOLD signal in the cortical surface is reduced in SE BOLD fMRI (Fig. 1D), and the highest SE BOLD signals occur at the middle of the cortex where the highest neural activity is expected, demonstrating the improvement of spatial specificity in neural active sites.

One important question is whether we should use SE BOLD rather than GE BOLD fMRI at high fields of >7 T. There are multiple issues that need to be considered for the selection of SE BOLD over GE BOLD fMRI. (1) The GE BOLD contrast is more sensitive than SE BOLD contrast, regardless of field strength. In order to take an advantage of the high specificity of SE BOLD fMRI, high-resolution studies are essential, further reducing sensitivity. (2) At higher fields, the baseline GE MRI signal intensity in large vessel-containing voxels is reduced due to higher susceptibility effects. Consequently, even though the BOLD percent change is higher at higher $B_0$, the absolute signal change may be reduced due to reduced baseline intensity, resulting in a decrease in statistical values. (3) In GE BOLD studies, the susceptibility effect of pial venous vessels is larger at higher fields. Thus, the separation of BOLD signals originating from microvessels within the voxel and macrovessels from neighboring voxels is complicated in GE BOLD fMRI, as seen in Fig. 1C. The SE BOLD contrast is beneficial for layer-dependent high-resolution BOLD studies. (4) SE BOLD requires an additional 180° pulse, which can be a problem of specific absorption rate (SAR) at high fields, limiting the number of imaging slices. In general, SE BOLD fMRI is an alternative option for high-resolution fMRI studies with limited coverage (e.g., [34]).

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