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Cerebral Blood Volume MRI with Intravascular Superparamagnetic Iron Oxide Nanoparticles

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Abstract

Cerebral blood volume (CBV) is a crucial physiological indicator of tissue viability and vascular reactivity. Thus, non-invasive CBV mapping has been of great interest. For this, ultrasmall superparamagnetic iron oxide nanoparticles (USPIO) including monocrystalline iron oxide nanoparticles (MION) can be used as long half-life, intravascular susceptibility agents of CBV MRI measurements. Also, CBV-weighted fMRI with USPIO provides enhanced sensitivity, reduced large vessel contribution, and improved spatial specificity compared to conventional blood oxygenation-level dependent (BOLD) fMRI, and measures a single physiological parameter that is easily interpretable. We review physiochemical and magnetic properties as well as pharmacokinetics of USPIO in brief. We then extensively discuss quantifications of baseline CBV, vessel size index, and functional CBV change. We also provide reviews of dose-dependent sensitivity, vascular filter function, specificity, characteristics, and impulse response function of CBV fMRI. Examples of CBV fMRI specificity at the laminar and columnar resolution are provided. Finally, we briefly review application of CBV measurements to functional and pharmacological studies in animals. Overall, the use of USPIO can determine baseline CBV and its changes induced by functional activity and pharmacological interventions.

Keywords

cerebral blood volume; iron oxide; contrast agent; fMRI; pharmacological MRI; MION; cortical columns; ultrasmall superparamagnetic iron oxide nanoparticles (USPIO)

Introduction

Cerebral blood volume (CBV) is a crucial physiological indicator of tissue viability and vascular reactivity as CBV changes are directly linked to the regulation of blood flow under conditions of both normal and abnormal physiology. It is generally thought that dilation and constriction of blood vessels is the major mechanism that maintains cerebral blood flow (CBF) within an autoregulatory range, and adjusts blood flow to perturbations, such as those

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induced by CO₂ changes and neural stimulation. Thus, non-invasive measurement of neurovascular morphology and function is important.

In order to quantify CBV with magnetic resonance imaging (MRI), an intravascular contrast agent can be utilized. When MR signal intensities are compared before and after the intravascular contrast agent administration, the difference is related to blood volume (Johnston et al. 1987; Villringer et al. 1988; Belliveau et al. 1991; Rosen et al. 1991). Most common approaches for human studies are to inject Gd-DTPA into blood as a bolus (Villringer et al. 1988), and to image during the first pass of the contrast agent. The delivery of the agent into brain vasculature is closely related to blood flow and, therefore, the onset and peak times of MRI signal changes induced by the contrast agent varies depending on the blood flow characteristics of the tissue. However, a time-integral of the MRI time course during the first pass is an index of CBV (see the article in this issue by Calamante). Due to the relatively short blood half-life of Gd-DTPA, its use for dynamic CBV studies is quite limited (Belliveau et al. 1991). Alternatively, iron oxide nanoparticles can be used as a blood pool agent (with a longer half-life than Gd-DTPA) for CBV imaging, and are generally classified into superparamagnetic iron oxide (SPIO) particles with a mean particle diameter of >50 nm, and ultrasmall superparamagnetic iron oxide (USPIO) particles with a smaller hydrodynamic diameter (Corot et al. 2006). With the same coating material (e.g., dextran), the former has a much shorter blood half-life than the latter (Weissleder et al. 1990). In order to use these iron oxide nanoparticles for CBV studies, an agent with a relatively long half-life is preferred in order to maintain the same concentration during the experimental duration. Thus, USPIO is the choice for functional CBV studies at a steady state, while both USPIO and SPIO can be used for baseline CBV and angiographic imaging.

The relationship between the susceptibility effect and tissue relaxation rate changes can be approximately determined by analytical solutions (Yablonskiy and Haacke 1994; Kiselev and Posse 1999) and by Monte Carlo simulation (Ogawa et al. 1993; Weisskoff et al. 1994; Boxerman et al. 1995). Iron oxide particles can be used to determine baseline CBV distribution and provide insights into vessel size (Dennie et al. 1998; Tropres et al. 2001). In the late 1990s, long half-life USPIO particles were used for functional studies (Berry et al. 1996; Kennan et al. 1998; Mandeville et al. 1998; van Bruggen et al. 1998), while Joseph Mandeville and his colleagues later expanded the use of USPIO for functional and pharmacological MRI studies (Mandeville and Marota 1999; Mandeville et al. 2001; Mandeville et al. 2004; Mandeville 2012). This approach to use USPIO for functional studies is often referred to as “increased relaxation for optimized neuroimaging (IRON)” (Chen et al. 2001), monocrystalline iron oxide nanoparticle (MION) (Shen et al. 1993), CBV-weighted or contrast agent-enhanced fMRI.

In this review, we will describe characteristics of USPIO, baseline CBV and vessel size measurements, functional CBV changes and application of CBV measurements to animal research. However, it is not our intention to review all CBV articles using USPIO, but rather to focus on principles and critical issues related to the quantification of CBV and its change. A recent review article of USPIO applications in animal studies can be found by Wu et al. (Wu et al. 2004). It should be emphasized that USPIO distributes into blood plasma, thus all quantitative measurements of baseline CBV need to consider hematocrit levels, while it is generally assumed that the hematocrit level does not change during relative CBV measurements. All of the following figures were obtained from our laboratory in the cat cortex.

Physiochemical Properties and Pharmacokinetics of Iron Oxide Nanoparticles

Iron oxide nanoparticles contain an iron oxide crystal core with a biodegradable coating. The core size of USPIO is 4–10 nm, and its structure can be a single crystal form (monocrystalline iron oxide nanocompounds, MION) (Shen et al. 1993). A hydrophilic polymer coating surrounding the iron oxide core is necessary to provide stability of the iron oxide in aqueous solution, and its common materials are dextran or its derivatives (e.g., carboxymethyl dextran). In addition to the hydrodynamic diameter, coating material and surface charge will influence blood half-life and biodistribution. Thus, the blood half-life of laboratory-made USPIO can vary between different batches and laboratories. Also, blood half-life is dependent on species and dose. For example, commercially available Feraheme (ferumoxyl; AMAG Pharmaceuticals, Lexington, MA) with a 30 nm hydrodynamic diameter and a polyglucose sorbitol carboxymethylether coating has a blood half-life of ~15 hours in humans (Feraheme instruction sheet) and >2 hours in rats. In pigs, a blood half-life of dextran-coated MION is found to be ~50 min (Wagenseil et al. 1999). In our studies, we found that the blood half-life is shorter in cats than in rats. In order to increase blood half-life, we often infuse dextran solution to compete with dextran-coated USPIO. For most animal studies, a long half-life is preferred to maintain the same concentration during a long experimental time. Many review articles are available for the physiochemical properties, pharmacokinetics, and biosafety of USPIO (Weissleder et al. 1995; Bonnemain 1998; Corot et al. 2006).

If a sterile and isotonic USPIO vial is obtained from commercial vendors (e.g., AMAG Pharmaceuticals), it should be directly injectable into the blood stream. If USPIO is obtained from academic laboratories, its *in vivo* use may require further preparations by suspending it in phosphate buffered saline (PBS) and filtering through a sterile filter. If the USPIO batch is exposed to high temperatures, its particles may disintegrate and results in a shorter blood half-life.

USPIO nanoparticles are absorbed by the spleen, liver, bone marrow and lymph nodes (Weissleder et al. 1990), and are biodegradable. It is not assumed to have any long-term toxicity. Repeated i.v. injections of MION in monkeys (accumulated dose: 128 mg/kg) for over 1 year did not induce any noticeable side effects (Vanduffel et al. 2001; Leite et al. 2002). However, repeated injections can increase blood iron levels, which can be removed by an iron chelating agent, such as deferoxamine, that binds free iron in the bloodstream and enhances its elimination.

Magnetic Properties of Iron Oxide Nanoparticles

Superparamagnetic properties occur when the crystal size is smaller than the ferromagnetic domains (~30 nm) (Corot et al. 2006). Furthermore, USPIO possess magnetism only when an external magnetic field is applied and the induced magnetism is almost saturated at ~1 T (see Fig. 4 in (Shen et al. 1993)) for MION. The magnetism of USPIO is dependent on core sizes, so different production batches may have different core sizes as well as magnetisms. A change of proton relaxation rates (unit of s^{-1}) due to iron oxide particles can be described as $\Delta R_1 = r_1 \times C$ and $\Delta R_2 = r_2 \times C$ where r_1 and r_2 are the constant ($s^{-1}mM^{-1}$) and C is the iron concentration (mM). Note that 1 mg Fe/ml is equivalent to 17.9 mM Fe. Relaxivity constants can be different in different magnetic fields (B_0) (see Fig. 2 in (Corot et al. 2006)). r_1 is robustly determined by a linear fitting of R_1 vs. concentration, while R_2 vs. concentration is slightly non-linear at high iron concentrations (Wu et al. 2004). Thus, Fe concentration can be determined from ΔR_1 of USPIO solution and r_1 . Relaxivity constants of Feraheme are $r_1 = 38 s^{-1}mM^{-1}$ and $r_2 = 83 s^{-1}mM^{-1}$ at 20 MHz (0.47 T) (Li et al. 2005).

At 1.5 T, r_1 of MION is $13.9 \text{ s}^{-1}\text{mM}^{-1}$ (Bjornerud et al. 2002). In our 9.4-T measurements, r_1 of MION is $1\text{--}1.7 \text{ s}^{-1}\text{mM}^{-1}$ in saline solution and blood plasma and r_2 is $70\text{--}110 \text{ s}^{-1}\text{mM}^{-1}$ in saline solution (Zhao et al. 2003). r_1 decreases with B_0 , while r_2 is almost independent of B_0 . Note that in order to measure R_1 and R_2 of blood with USPIO, withdrawn blood can be centrifuged and only the plasma fraction retained in order to eliminate the precipitation of red blood cells during MR measurements.

In addition to changes in blood R_1 and R_2 , USPIO induces the susceptibility effect, which can be used to quantify blood volume (see below). The susceptibility effect is closely related to induced magnetism and Fe concentration. In order to determine the susceptibility effect of USPIO, two capillary tubes filled with saline (one oriented parallel to B_0 , while the other is perpendicular to B_0) are secured inside a cylinder containing a blood/USPIO mixture and the frequency difference between the peaks originating from each of the capillaries is measured (Chu et al. 1990; Spees et al. 2001; Tropres et al. 2001; Kim et al. 2007). The spectroscopic frequencies of water peaks originating from parallel and perpendicular-oriented capillaries ($\nu_{\parallel}$ and $\nu_{\perp}$, respectively) immersed in the withdrawn blood differ due to both hemoglobin in red blood cells and USPIO in plasma, and their separation can be expressed as $\nu_{\parallel} - \nu_{\perp} = 2\pi \cdot \Delta\chi_{\text{blood+agent}} \nu_0$ (Chu et al. 1990; Spees et al. 2001; Tropres et al. 2001), where $\Delta\chi_{\text{blood+agent}}$ is the susceptibility difference between the arterial blood containing USPIO and water, and ν_0 is the spectrometer resonance frequency. All susceptibility values are expressed in CGS units. Since the susceptibility of plasma is similar to that of water (Weisskoff et al. 1992), the susceptibility effect of the agent in plasma ($\Delta\chi_{\text{agent}}$) can then be determined for each animal by the relationship, $\Delta\chi_{\text{blood+agent}} = Hct[Y \cdot \Delta\chi_{\text{oxy}} + (1 - Y) \cdot \Delta\chi_{\text{deoxy}}] + (1 - Hct) \cdot \Delta\chi_{\text{agent}}$, where Hct is experimentally measured; Y is the oxygenation level of blood; $\Delta\chi_{\text{oxy}}$ and $\Delta\chi_{\text{deoxy}}$ are the susceptibility differences between fully oxygenated red blood cells and water and between fully deoxygenated red blood cells and water, respectively. Since arterial blood is fully oxygenated, the $\Delta\chi_{\text{deoxy}}$ term can be ignored and the $\Delta\chi_{\text{oxy}}$ value is set equal to -0.026 ppm (Weisskoff and Kiihne 1992) to -0.035 ppm (Spees et al., 2001). The difference between reported $\Delta\chi_{\text{oxy}}$ values does not greatly impact the $\Delta\chi_{\text{agent}}$ calculation (e.g., $\Delta\chi_{\text{oxy}}$ difference of 0.009 ppm , -0.026 vs. -0.035 ppm , is much smaller than $\Delta\chi_{\text{agent}}$ of $\sim 0.35 \text{ ppm}$). Note that the difference between $\Delta\chi_{\text{oxy}}$ and $\Delta\chi_{\text{deoxy}}$ reported in literature ranges between 0.18 ppm (Weisskoff and Kiihne 1992) and 0.27 ppm (Spees et al., 2001). Alternatively, in order to minimize the contribution of hemoglobin, only plasma can be used for susceptibility measurements. In our studies with 15 mg Fe/kg i.v. injection into rats, $\Delta\chi_{\text{agent}}$ of USPIO in plasma is 0.36 ppm (i.e., corresponding to the frequency shift of 904 Hz at 9.4 T) (Kim et al. 2007). Since the induced magnetism is saturated at $\sim 1 \text{ T}$, most animal studies performed between 4.7 T and 11.7 T have similar frequency shifts induced by USPIO. In our studies, the frequency shift induced by USPIO at 9.4 T was found to be 1.1 times that at 4.7 T (Zhao et al. 2003).

Quantification of Baseline Total Blood Volume

After USPIO is injected into the blood stream, a steady state condition is quickly achieved (Weissleder et al. 1990). Even when a small dose is used (e.g., $1 \text{ mg Fe/kg body weight}$), blood T_1 and T_2 are shortened significantly. In our 9.4-T rat studies (Kim and Kim 2005), $1 \text{ mg Fe/(kg body weight)}$ decreases arterial blood T_2 from 40 ms to 14 ms , and blood T_1 from 2.30 s to 1.14 s . The USPIO i.v. injection dose of 1 mg Fe/kg body distributes to $60 \text{ ml volume/kg body}$, resulting in the USPIO concentration of $\sim 0.017 \text{ mg Fe/ml blood}$ (e.g., with a hematocrit level of 40% , a plasma USPIO concentration of $0.028 \text{ mg/ml plasma} = 0.50 \text{ mM}$). Thus, when a highly T_1 -weighted pulse sequence with a short TE is used, especially at low B_0 , blood vessels can be visualized due to the shortened T_1 . This property has been exploited for angiography (Anzai et al. 1997; Li et al. 2005).

USPIO has been commonly used for CBV imaging in animals. The relationship between MION doses and R_2^* is important to determine the proper dose of USPIO. For this, cats were used for determining the relationship between tissue R_2^* change and USPIO dose at 9.4 T (Zhao et al. 2003). A 2 mg Fe/kg dose of USPIO was repeatedly injected into the cat femoral vein until a total dose reached 10 mg/kg. After every injection, T_2^* -weighted images with TEs of 6 – 40 ms were obtained at a steady state condition (> 2 min after the injection), and R_2^* was calculated. The relation between $\Delta R_{2,agent}^*$ of gray matter at 9.4 T and an USPIO injection dose was: $\Delta R_{2,agent}^* (s^{-1}) = 5.49K$, where K is the USPIO dose as a unit of mg Fe/kg body weight. This observation is consistent with results measured in the rat brain with USPIO, $\Delta R_{2,agent}^* = 5.80K$ at 4.7 T (van Bruggen et al. 1998), 4.25K at 3 T (Pathak et al. 2003), and 5.28K at 2.35 T (Tropres et al. 2001). This shows that $\Delta R_{2,agent}^*$ induced by USPIO is similar at different magnetic fields. It should also be noted that the relationship between the USPIO dose and blood $\Delta R_{2,agent}^*$ is nonlinear, unlike $\Delta R_{1,agent}$ (Bjornerud et al. 2002).

To measure CBV with USPIO, it is preferable to inject a 5–15 mg Fe/kg dose to create a large contrast between pre- and post-contrast images, and to have sufficient signal intensity of post-contrast T_2^* -weighted images. The signal change induced by USPIO is directly related to the contents of iron in a given pixel, and can be converted to $\Delta R_{2,agent}^* (s^{-1}) = \ln(S_{pre}/S_{post})/TE$, where S_{pre} and S_{post} is the signal intensity before and after USPIO. For example, FLASH images of cat brain with TE = 10 ms and pixel resolution = $78 \times 78 \times 2000 \mu m^3$ were acquired before and after a bolus injection of 12 mg Fe/kg MION (Fig. 1B and C). The calculated ΔR_2^* map induced by MION (Fig. 1D) shows exquisite intracortical vessel patterns running into the cortex (Bolan et al. 2006; Kim and Kim 2011), low intensity in white matter, and high intensity at the cortical surface. Interestingly, the middle part of the cortex has a slightly higher $\Delta R_{2,agent}^*$ band. This ΔR_2^* value can be converted into CBV under an assumption of the static dephasing domain as previously reported (Yablonskiy and Haacke 1994).

The static dephasing domain is commonly considered to be $1/(\text{the frequency shift induced by USPIO}) \ll R^2/D$, where R is the vessel radius, and $D = 0.8 \times 10^{-3} \text{ mm}^2/\text{s}$ (Tropres et al. 2001). Based on our studies, the frequency shift induced by a MION dose of 15 mg Fe/kg is ~900 Hz. The MION dose of 5 mg Fe/kg is expected to induce a frequency shift of ~300 Hz, equivalent to $(1/\text{frequency shift}) = 1/(2\pi \times 300) = 5 \times 10^{-4}$, which, for a $2 \mu m$ radius capillary, is much less than $R^2/D = (2 \times 10^{-3})^2/0.8 \times 10^{-3} = 5 \times 10^{-3}$. For the condition where static dephasing is dominant, the change in apparent transverse relaxation rate in tissue due to the contrast agent and in the absence of stimulation ($\Delta R_{2,agent}^*$) (Yablonskiy and Haacke 1994) can be described as:

$$\Delta R_{2,agent}^* = \frac{4}{3} \pi \cdot (1 - Hct) \cdot \nu_{CBV} \cdot \Delta \chi_{agent} \cdot \gamma B_0, \quad [1]$$

where ν_{CBV} is the whole blood volume fraction (ml blood/ ml brain), which includes both plasma and blood cells, and γ is the gyromagnetic ratio, which is $2.675 \cdot 10^8 \text{ rad}/(\text{s} \cdot \text{T})$. Baseline ν_{CBV} (ml/ml) can be determined with Eq. [1] from $\Delta R_{2,agent}^*$ and from Hct and $\Delta \chi_{agent}$ obtained from arterial blood. Assuming cortical Hct of 0.40 and $\Delta \chi_{agent}$ of 0.29 ppm (i.e., $0.36 \text{ ppm} \times (12/15)$), $\Delta R_{2,agent}^*$ of 70 s^{-1} in Figure 1D (9.4 T) corresponds to ν_{CBV} of 3.8 ml/100 ml. These ν_{CBV} values are then converted to baseline CBV_t (ml/ g) values by dividing by the blood density of 1.06 g/ ml (Herscovitch and Raichle 1985).

Generally, it is assumed that susceptibility-induced dephasing effects within a voxel are not influenced by neighboring voxels for CBV estimation. However, this assumption is not valid at a high spatial resolution (e.g., Fig. 1). Especially, baseline CBV determined from Eq. [1]

is over-estimated when large vessels in neighboring voxels induce field gradients (see region near cortical surface in Fig. 1 and Fig. 2E). An additional assumption is that the origin of relaxation change is primarily from the extravascular compartment (Yablonskiy and Haacke 1994). The USPIO-induced signal change is on the order of ~50% in the extravasculature, while the intravascular blood signal before the MION injection is only a few percent, which results in a very small error of CBV values.

A major advantage of USPIO over Gd-DTPA for CBV mapping is the longer blood half-life of USPIO. This allows acquisition of high-resolution images of entire brain and dynamic CBV measurements during interventions (see below) without concern for changes in contrast agent concentration. One major issue of USPIO is that it is not approved for human CBV studies, unlike Gd-DTPA. A detailed description of Gd-DTPA measurements can be found in Fernando Calamante's article of this issue. Compared to gadolinium chelates (about 1 nm size), USPIO is more likely to remain in the blood stream due to its larger hydrodynamic diameter and, thus, is a better choice for CBV mapping in animals.

Mapping Baseline Microvascular Volume and Vessel Size Index

The field gradient generated by blood iron oxides decreases by $(a/R)^2$, where a is the distance from vessel to the region of interest and R is the vessel radius. During the echo time typically chosen for MRI studies (e.g., ~ 50 ms), water molecules diffuse 10 – 20 micrometers (i.e., $(6 \times D \times TE)^{1/2} = (6 \times 0.8 \times 10^{-3} \times 0.05)^{1/2} = 0.015$ mm). Thus, phase coherence of water spins near capillaries will be dynamically averaged over the steep field gradients produced by blood USPIO during TE, which cannot be recovered by a 180° radiofrequency pulse, while the dephasing effect around large veins can be refocused. Thus, the signal change in T_2^* -weighted images is sensitive to all sized vessels, while that in T_2 -weighted images is most sensitive to small vessels, where the diffusion contribution is dominant. The vessel size tuning of $\Delta R_{2, \text{agent}}$ is sensitive to the USPIO dose ($\Delta \chi_{\text{agent}}$) and echo time; a higher dose and shorter TE results in tuning to a smaller vessel diameter (Weisskoff et al. 1994; Boxerman et al. 1995). According to Monte Carlo simulations, the tuned vessel diameter is ~4 μm for a frequency shift of 160 Hz and TE of 100 ms (corresponding to 900 Hz and 3.2 ms TE for the same diffusion distance over the same field difference) (see Fig. 2 in (Boxerman et al. 1995)). Both ΔR_2 and ΔR_2^* increase with vessel diameter up to the tuned vessel diameter, but ΔR_2^* is always larger than ΔR_2 . Beyond the tuned diameter, ΔR_2^* plateaus, while ΔR_2 decreases with vessel diameter (Fig. 1 in (Boxerman et al. 1995)). Thus, ΔR_2^* is sensitive to total CBV, while ΔR_2 is an index of microvascular CBV (Dennie et al. 1998; Tropres et al. 2001). Figure 2 shows ΔR_2^* and ΔR_2 maps of the cat brain induced by 10 mg Fe/kg MION, obtained using echo planar imaging techniques with the pixel resolution = 156 $\mu\text{m} \times 156 \mu\text{m} \times 2$ mm at 9.4 T (Zhao et al. 2006). As seen in Fig 1D, the surface of the cortex has very high $\Delta R_{2, \text{agent}}^*$ (Fig. 2B), but low $\Delta R_{2, \text{agent}}$ values (Fig. 2C). Cortical profiles (Fig. 2E) show the highest $\Delta R_{2, \text{agent}}^*$ at the cortical surface because of large pial vessel contributions, and the highest $\Delta R_{2, \text{agent}}$ in the middle of the cortex, which is consistent with previous findings that middle cortical layers have the highest microvascular density (Tieman et al. 2004). Interestingly, $\Delta R_{2, \text{agent}}^*$ decreases with cortical depth up to ~0.5 mm, which is likely due to the extended dephasing effect from large surface vessels and the partial volume fraction of surface vessels within the 2-mm slice thickness.

Baseline microvascular volume distribution can be obtained by $\Delta R_{2, \text{agent}}$ maps. Since angiogenesis is of great interest, baseline CBV studies have often been performed in tumor models. Le Duc et al. (Le Duc et al. 1999) obtained $\Delta R_{2, \text{agent}}$ maps in normal and glioma-bearing rats, and found a high vessel density in the peripheral area and a low vessel density in the central area of the tumor. Wu et al. (Wu et al. 2003) measured microvascular volume

distribution in mice with a 30 mg Fe/kg injection at 9.4 T, and found that CBV in the cerebral cortex is higher in transgenic APP mice than in wild-type controls. Dunn et al. (Dunn et al. 2004) used $\Delta R_{2,agent}$ in blood and tissue to determine microvascular volumes during angiogenesis, and compared those with in vitro vascular fluorescent microscopy. The microvascular CBV values of control and vasodilated chronic hypoxic rats correlate well with those obtained from microvascular morphology. These indicate that $\Delta R_{2,agent}$ provides relative microvascular volume distribution and is a useful method to investigate angiogenesis.

Assuming that the susceptibility effect remains within a pixel, the pixel $\Delta R_{2,agent}^*/\Delta R_{2,agent}$ value provides microvessel size information (Dennie et al. 1998; Jensen and Chandra 2000). Tropres et al. (Tropres et al. 2001) extended this idea to determine the vessel size index (VSI) as:

$$VSI = 0.425 \cdot \left(\frac{D}{\gamma B_0 \cdot \Delta \chi_{agent}} \right)^{1/2} \cdot \left(\frac{\Delta R_{2,agent}^*}{\Delta R_{2,agent}} \right)^{3/2} \approx A \left(\frac{\Delta R_{2,agent}^*}{\Delta R_{2,agent}} \right)^{3/2} \quad [2]$$

Where VSI (as a unit of mm) is the weighted-mean radius of vessels, D (as a unit of mm^2/s) is the diffusion coefficient, and A is constant. The VSI map is directly related to $(\Delta R_{2,agent}^*/\Delta R_{2,agent})^{3/2}$, and can provide insights into the composition of vessel sizes *in vivo*. Regions with larger VSI values indicate larger vessel sizes. Figures 2D and 2F show the VSI map and cortical profile, respectively; the largest VSI is observed in the surface of the cortex, as expected. Assuming that USPIO remains within vessels, VSI has been used for examining the characteristics of angiogenesis (Dennie et al. 1998; Tropres et al. 2001; Tropres et al. 2004). The exact weighted-mean-radius determined by VSI correlates well with ex vivo micro-CT angiography and histological vessel measurements (Ungersma et al. 2010), but is six times larger in tumor and muscle than that obtained from optical microscopic imaging (Douma et al. 2010). Thus, VSI should be used as a relative measure of vessel diameter, rather than absolute size. For example, Tropres et al. (Tropres et al. 2004) determined that tumorous tissues had lower CBV, but larger VSI compared to normal tissue, indicating that tumors contain less numerous, but larger sized vessels.

Mapping Quantitative Relative CBV Change

USPIO is ideal to use for CBV-based fMRI studies due to its long blood half-life (Berry et al. 1996; Kennan et al. 1998; Mandeville et al. 1998; van Bruggen et al. 1998). Functional CBV-weighted signal changes accompany BOLD signal changes. Thus, the signal change induced by stimulation before and after USPIO needs to be further examined. The functional BOLD signal change before USPIO (ΔS_{pre}) is described as:

$$\Delta S_{pre} = S_0 \cdot e^{-R_{2,pre}^* TE_{pre}} (-TE_{pre} \cdot \Delta R_{2,BOLD}^*) = S_{pre} (-TE_{pre} \cdot \Delta R_{2,BOLD}^*) \quad [3]$$

Where $\Delta R_{2,BOLD}^*$ is linearly dependent on baseline venous CBV, B_0 and oxygenation level changes. The maximal ΔS_{pre} is achieved at $R_{2,pre}^* \cdot TE_{pre} = 1.0$ and, therefore, the optimal TE is usually set to the tissue T_2^* . Higher baseline venous CBV increases $R_{2,pre}^*$ and $\Delta R_{2,BOLD}^*$, but decreases baseline signal intensity (S_{pre}). Thus, at large vessel regions, a relative functional signal change ($\Delta S_{pre}/S_{pre}$) can be high, but absolute functional BOLD signal change (ΔS_{pre}) decreases at higher fields due to increased $R_{2,pre}^*$ and reduced S_{pre} .

After USPIO is injected into blood, the baseline signal decreases and the functional signal change contains both CBV-induced USPIO susceptibility changes and deoxyhemoglobin-related BOLD signal changes (Kennan et al. 1997; Kennan et al. 1998; Zhao et al. 2006). The post-agent functional signal change (ΔS_{post}) is described as:

$$\Delta S_{post} = S_0 \cdot e^{-R_{2,post}^* TE_{post}} \left(-TE_{post} \cdot \Delta R_{2,BOLD}^* - TE_{post} \cdot C \cdot r_2^* \cdot \Delta CBV \right) \quad [4]$$

Where $R_{2,post}^* = R_{2,pre}^* + \Delta R_{2,agent}^* = R_{2,pre}^* + C \cdot r_2^* \cdot CBV$, C is the MION blood concentration, and r_2^* is the relaxivity per mM of MION (s^{-1}/mM). Notice that [4] is almost independent of B_0 . The relative functional signal change ($\Delta S_{post}/S_{post}$) has a BOLD contribution ($\Delta S_{pre}/S_{pre}$) and an absolute CBV change term. The BOLD contribution to $\Delta S_{post}/S_{post}$ can be corrected using pre-USPIO BOLD data. Since C and r_2^* are identical across pixels, the BOLD-corrected functional signal change is directly related to absolute blood volume change. Then, relative CBV change $\Delta CBV/CBV$ can be determined as

$$\frac{\Delta CBV}{CBV} = \frac{(\Delta S_{pre}/S_{pre})/TE_{pre} - (\Delta S_{post}/S_{post})/TE_{post}}{\Delta R_{2,agent}^*} \quad [5]$$

At higher magnetic fields, the BOLD contribution is not negligible (Lu et al. 2007) and should be corrected in order to quantify relative CBV changes (Kennan et al. 1998; Zhao et al. 2006). The underlying assumption of the BOLD correction is that the BOLD signal change is identical between pre- and post-contrast agent conditions. This assumption is valid only when the intravascular BOLD contribution is minimal, because USPIO suppresses all intravascular signal contributions. If the intravascular BOLD contribution before USPIO injection is significant, the BOLD contribution to CBV-weighted fMRI using Eq. [5] is overestimated, thus the relative CBV (rCBV) change ($\Delta CBV/CBV$) is slightly overestimated.

Examples of how to obtain $\Delta CBV/CBV$ are shown in Figures 3 and 4 (Harel et al. 2002). TE was set to 25 ms and 15 ms for the BOLD and CBV-weighted fMRI studies at 4.7 T, respectively. The baseline T_2^* of cat gray matter was ~51 ms and ~21 ms before and after the 7 mg/kg MION injection, respectively. During a visual stimulation period, the largest BOLD signal changes (yellow) occurred mainly in the upper layers/cortical surface, while smaller signal changes occurred in deeper layers (Fig. 3A). Following MION injection, the MRI signal decreased during neural stimulation, indicating an increase in CBV (Fig. 3B). The CBV-weighted signal was confined to the primary visual areas and closely followed the gray matter contour. In contrast to the BOLD signals, maximum CBV-weighted signals were centered over the gray matter, while a significantly smaller signal was detected at the cortical surface. However, CBV-weighted signal is dependent on the dose of MION and CBV changes, as well as BOLD contributions. Basal CBV levels were obtained by measuring ΔR_2^* induced by MION (Fig. 3C). Although CBV-weighted fMRI signal changes are negligible in large vessel areas (Fig. 3B), quantitative relative CBV changes (rCBV map) are significant (Fig. 3D). More importantly, larger stimulation-induced rCBV changes take place at the center of the cortical gray matter. To better exemplify properties of the stimulus-induced functional signal changes, their profiles were plotted across cortical layers (Fig. 4). To correlate the functional maps with the underlying cortical laminar structures, schematic boundaries between cortical layers, based on the literature (Payne and Peters 2002) and previous histological studies (Harel et al. 2006; Kim and Kim 2011), were superimposed (Fig. 4C). Both rCBV and CBV-weighted signals exhibit elevated signals at

middle cortical layers, with smaller changes at upper surface vessels and white matter regions.

As mentioned in the previous section entitled “Mapping Baseline Microvascular Volume and Vessel Size Index”, spin-echo and VSI fMRI approaches can be used to characterize the size of vessels that are functionally responding. To obtain insight into the hemodynamic regulation, we can assume that baseline $CBV_{total} = CBV_{micro} + CBV_{macro}$, where CBV_{micro} and CBV_{macro} are the micro- and macro-vascular volumes, respectively. Since a portion of small arterioles and venules can contribute to these spin echo (SE) measurements, the boundary between micro- and macro-vessels is not well-defined. Changes in relative total and microvascular volumes were measured, where stimulation-induced $\Delta rCBV_{total} = (\Delta CBV_{micro} + \Delta CBV_{macro}) / (CBV_{micro} + CBV_{macro})$, while $\Delta rCBV_{micro} = \Delta CBV_{micro} / CBV_{micro}$. If a change in relatively large vessels is dominant, then $\Delta rCBV_{micro}$ (measured by SE fMRI) should be very small, as compared to $\Delta rCBV_{total}$ (measured by GE fMRI). If a change in the capillary volume is dominant, $\Delta rCBV_{micro}$ should be larger than $\Delta rCBV_{total}$. Note that SE measurements underestimate true microvascular volume change because the sensitivity of vessels larger than the tuned diameter decreases. We reported a 5.6% $\Delta rCBV_{micro}$ and a 10% $\Delta rCBV_{total}$ in the middle of the cortex (Zhao et al. 2006), which indicates that most responding vessels are larger than the tuned vessel diameter (capillaries). Similarly, since VSI provides an index of a weighted-vessel size, comparison of VSI between the baseline and functional conditions will provide information on the responding vessel sizes. Zhao et al. (Zhao et al. 2006) showed an average ratio of stimulation to baseline VSI values (relative VSI) in the middle of the cortex to be 2.80. In other words, if the weighted-vessel radius was 2 μm in the middle cortex, then the corresponding weighted-vessel radius responding to stimulation would be $\sim 6 \mu m$. This indicates that dilated vessels responding to neural stimulation are larger than capillaries.

Similar studies were performed in rats by Mandeville et al. (Mandeville et al. 2007). In their studies, higher baseline CBV was consistent with higher baseline $\Delta R_{2,*}^{agent} / \Delta R_{2,agent}$ and lower $\Delta rCBV_{total} / \Delta rCBV_{micro}$ resulting in a higher baseline VSI and almost constant functional VSI. This indicates that the large vessel responses are not dominant, which is similar to the conclusion made by Zhao et al. (Zhao et al. 2006). However, functional VSI is much less than baseline VSI, which is consistent with the results at the cortical surface in Zhao et al. (Zhao et al. 2006), but not with the results within the cortex. When $\Delta S_{post} / S_{post}$ is calculated from SE fMRI after an USPIO injection, it relates to an absolute change in microvascular CBV, which is always less than the total CBV change. Since most vessels are larger than the tuned diameter, its diameter change sub-quadratically contributes to SE measurements (see sensitivity profile in (Boxerman et al. 1995)). The microvascular CBV change measured by SE fMRI with USPIO underestimates the true change (Mandeville et al. 2007). Thus, SE fMRI should not be used for accurate determination of the absolute CBV response.

Sensitivity and Specificity of CBV-weighted fMRI vs. BOLD

The functional sensitivity of the CBV-weighted fMRI signal (Eq. [4]) is closely dependent on the dose of USPIO, baseline CBV, and BOLD contamination as well as stimulus duration (Leite et al. 2002). Since the BOLD contrast increases with B_0 , and magnetization of iron oxides is saturated above ~ 1.0 T (Shen et al. 1993), the stimulation-induced signal of CBV-weighted fMRI decreases at higher B_0 due to the counteractive effect of BOLD relative to CBV response. Generally, with lower B_0 , the functional sensitivity gain over conventional BOLD fMRI increases. B_0 -dependent sensitivity properties were investigated by Mandeville et al. (Mandeville et al. 1998).

Reported sensitivity values were measured under a steady state condition. The CBV-weighted fMRI sensitivity is ~6 times greater than the GE BOLD fMRI signal at 2 T (12 mg Fe/kg, TE = 25 ms), 1.5 – 2 times (16 mg/kg, TE = 25 ms) at 4.7 T in rats (Mandeville et al. 1998), and 3 times at 3 T in monkeys (8–10 mg/kg, 20 ms for MION vs. 30 ms for BOLD) (Vanduffel et al. 2001; Leite et al. 2002). At 7 T, 15–20 mg/kg USPIO improves functional contrast-to-noise ratio (CNR) by 70% in rats compared to BOLD (TE = 14 ms for BOLD and USPIO) (Van Camp et al. 2005). At 9.4 T, the improvement of CNR is 1.2 – 1.5 times in the cat visual cortex (MION of 10 mg/kg, TE of 10–15 ms for CBV-weighted fMRI vs. TE of 20 ms for BOLD fMRI) (Zhao et al. 2003). Mandeville et al. (Mandeville et al. 2004) reported that CNR in CBV-weighted GE fMRI with 28 mg Fe/kg and TE of 5 ms improved twofold compared to GE BOLD with TE of 10 ms in the rat brain during cocaine stimulation. Very recently, Qiu et al. used USPIO for 3-T human fMRI studies, showing 2–3 times CNR improvement over GE BOLD in the human visual cortex using a 7 mg/kg dose (Qiu et al. 2012). To maximize the sensitivity gain, TE_{post} should be set to $1/R_{2,post}^*$. The BOLD contribution to CBV-weighted fMRI could also be minimized by using a short TE value.

The sensitivity of CBV-weighted fMRI (Eq. [4]) also depends on the baseline CBV because the baseline signal intensity is related to $\exp(-TE_{post} \cdot C \cdot r_2^* \cdot CBV)$. This baseline CBV-dependent functional sensitivity was examined by Mandeville and Marota (Mandeville and Marota 1999). When the USPIO dose and TE are optimized for one baseline CBV value, namely CBV_{ref} , then ΔS_{post} is modulated by $\exp(-CBV/CBV_{ref})$; thus, regions with a large baseline CBV have decreased functional sensitivity. This baseline CBV-dependent function is referred to as a vascular filter (Mandeville and Marota 1999).

What is the consequence of the vascular filter on CBV-weighted fMRI? Regions with large vessels lose their baseline signals faster than regions with cortical microvessels, thus less absolute signal changes are induced by the same absolute CBV change. This phenomenon is similar to large vessel regions at long TEs or at very high fields; even if the relative percent change ($TE\Delta R_{2,BOLD}^*$) is quite high, an absolute signal change (ΔS_{pre}) is low due to the low baseline signal intensity. This mechanism has been proposed to be a major reason to improve the spatial localization (Mandeville and Marota 1999). Additionally, large BOLD contributions at vessel areas reduces the CBV-weighted signal change. Thus, activation foci in CBV-weighted images obtained as statistical values (or absolute intensities) can shift to deeper cortical areas compared to BOLD fMRI (see Fig. 3A vs. 3B and Fig. 4A). Supporting findings are reported in the rat somatosensory cortex (Mandeville and Marota 1999) and the monkey visual cortex (Smirnakis et al. 2007).

The improved spatial localization of CBV-weighted fMRI may be due to true physiological responses, rather than merely due to the vascular filter function. This hypothesis can be tested by multiple ways: 1) to quantify relative CBV change and compare it with the CBV-weighted response, and 2) to compare measurements with different filter functions. Firstly, to remove baseline signal dependencies from the activation maps, percent signal changes from baseline ($\Delta S/S$) are calculated (see Fig. 4B), then the BOLD contribution is corrected. Peak positions in CBV-weighted ΔS (blue trace in Fig. 4A), CBV-weighted $\Delta S/S$ (blue trace in Fig. 4B), and rCBV changes (green trace in Fig. 4B) within the cortex remain the same. Secondly, SE vs. GE fMRI with USPIO show peak responses in the middle of the cortex and show independence from the vascular filter functions (Zhao et al. 2006). Thirdly, three MION doses of 5, 10 and 15 mg Fe/kg were used at TE = 10 ms at 9.4 T. Peak positions of the CBV-weighted fMRI response remain the same with different MION doses (see Fig. 5). A similar finding was reported by Keilholz et al. (Keilholz et al. 2006), in which spin-echo fMRI was performed on the rat somatosensory cortex with 10, 20 and 30

mg Fe/kg at 11.7 T. The spatial peak of percent signal changes in CBV-weighted fMRI is invariant of the dose of MION and pulse sequence, indicating that the improved spatial location of CBV-weighted fMRI is due to true physiological responses.

Sensitivity of CBV-weighted fMRI vs. Dose of USPIO

The choice of USPIO dose is critical to maximize the functional sensitivity. Two conditions can be considered; i) $TE_{post} = TE_{pre} = 1/R_2^*_{pre}$, and ii) $TE_{post} < TE_{pre}$. For condition #1, the USPIO dose is optimal when the baseline signal decreases to e^{-1} of the pre-contrast intensity (Fig. 5a in (Mandeville and Marota 1999)) at $TE_{post} \cdot C \cdot r_2^* \cdot CBV = TE_{post} \cdot \Delta R_{2,agent}^* = 1.0$. For condition #2, if TE_{post} is set to as short as possible, then the USPIO dose is determined by $R_2^*_{post} = 1/TE_{post}$ (see Fig. 5b in (Mandeville and Marota 1999)). The sensitivity gain of condition #2 compared to condition #1 is $\exp(-TE_{post}/TE_{pre})$ (Fig. 5b in (Mandeville and Marota 1999)). Most CBV-weighted fMRI studies use the latter condition.

During long experimental times, USPIO blood concentration decreases and consequently, functional CBV-weighted $\Delta S/S$ similarly decreases. Figure 6 shows time courses of CBV-weighted fMRI signals more than 2 hours after administration of 10 mg Fe/kg MION (Lee et al. 2002). For this specific and early application of MION in our laboratory, additional dextran solution (to increase the blood half-life of dextran-coated MION) was not infused, unlike the remainder of our cat experiments. Positive BOLD responses and negative CBV weighted responses were consistently obtained from area 18 of the cat visual cortex. As MION concentration in blood decreased by clearance processes, the CBV contrast diminished accordingly. To remove iron concentration-dependent variations, an index of baseline Fe concentration ($\Delta R_{2,agent}^*$) should be measured, and used for determination of the relative CBV change (see Eq. [5]). Thus, baseline $\Delta R_{2,agent}^*$ at each time course was calculated from baseline data. When rCBV changes were calculated with BOLD correction (Fig. 6B), CBV time courses were similar. Especially when the concentration of MION in blood is low, the BOLD correction is essential to determine functional rCBV changes.

Characterizations of CBV-weighted Functional Responses

Evoked CBV changes have an initial fast increase followed by slow increases during long stimulation (Mandeville et al. 1999a; Silva et al. 2007). Note that even though functional CBV-weighted changes are negative, its absolute changes are used for comparing with BOLD responses. The impulse response function (IRF) of CBV-weighted fMRI differs from that of BOLD fMRI. Silva et al. (Silva et al. 2007) measured IRF in the rat somatosensory cortex at 11.7 T, and found that the CBV response starts and peaks ~ 0.5 s earlier than BOLD. Leite et al. (Leite et al. 2002) systematically determined IRFs of CBV-weighted and BOLD fMRI of visual cortex in awake monkey at 3 T. When 4-s stimulation was used, both CBV-weighted and BOLD responses peaked at a similar time, but the CBV-weighted signal returned to the baseline after the stimulus offset more slowly compared to BOLD fMRI. However, when the stimulation duration was 60 s, the first-order time constants of responses were 4.5 s for BOLD and 13.5 s for CBV-weighted fMRI. This indicates that 1) a long stimulation duration increases functional sensitivity of CBV-weighted fMRI relative to BOLD fMRI (Leite et al. 2002), and 2) the CBV-weighted signal responding to long stimulation cannot be explained by a time-invariant linearity function with the IRF obtained from short stimulation (Lu et al. 2005).

Since CBV-weighted fMRI provides higher sensitivity than BOLD fMRI, it can be used for high-resolution fMRI studies in animals. In order to examine time-dependent CBV-weighted fMRI, CBV-weighted fMRI images were obtained at 2 s intervals following each stimulation onset (see Fig. 7). The CBV change initially has significant radial vessel

contributions (see Fig. 7A–B), following dominant responses in the middle of the cortex (see Fig. 7D), indicating that a later response is highly specific (Jin and Kim 2008). CBV-weighted fMRI with $98 \times 98 \times 1000 \mu\text{m}^3$ resolution and 6 mg Fe/kg, Palmer et al. (Palmer et al. 1999) successfully separated localized functional sites induced by forepaw and hindpaw stimulation in α -chloralose anesthetized rats at 4.7 T. In CBV fMRI experiments with $156 \times 156 \times 2000 \mu\text{m}^3$ resolution at 3 T after 12 mg Fe/kg injection (Lu et al. 2004), rat whisker barrel cortex had the highest response to whisker stimulation in layer 4, similar to c-Fos expression maps. These studies indicate that the functional CBV response is quite specific to small functional structures.

Specificity of the CBV response at a cortical columnar scale has been examined by our laboratory (Harel et al. 2002; Zhao et al. 2005; Fukuda et al. 2006). The CBV-weighted fMRI approach with 10 mg Fe/kg and $156 \times 156 \times 1000 \mu\text{m}^3$ resolution was applied to map cat orientation columns at 9.4 T (Zhao et al. 2005; Fukuda et al. 2006), which could not be obtained with conventional BOLD fMRI under the same animal model and experimental conditions (Kim et al. 2000). Figure 8 shows the subtraction maps of 0° and 90° stimulation. Clearly, activations responding to 0° stimulation (red crosses) are not in the same location as those of 90° stimulation. Regions of large CBV changes responding to two orthogonal orientation gratings were highly complementary, indicating that parenchymal CBV responses are quite specific to neural active sites in a steady state condition (Zhao et al. 2005; Fukuda et al. 2006). To further examine functional CBV responses at the columnar and laminar level, we employed the well-established ocular dominance column model (Hubel and Wiesel 1962). Following 7 mg Fe/kg administration, functional CBV-weighted images were acquired with $156 \times 156 \times 2000 \mu\text{m}^3$ resolution and TE of 15 ms at 4.7 T (Harel et al. 2002). Figure 9 shows 3-D statistical maps for binocular and monocular CBV-weighted activation maps from two cats to demonstrate the consistency of our findings. During binocular stimulation, a more homogeneous pattern of increased CBV was observed along the middle cortical layers. On the contrary, during monocular stimulation, local patches of increased CBV-weighted signals were observed. In all cases, the contralateral hemisphere to the stimulated eye exhibited a larger number of activated pixels ($58.1 \pm 3.7\%$; $n = 5$). Patches of localized increases in blood volume were arranged in an orderly fashion across the middle layers throughout the primary visual cortex. The mean distance between the centers of adjacent peaks at the medial parts of the lateral gyrus (area 17) was $0.57 \pm 0.14\text{mm}$ ($n = 4$ cats, total of 124 pairs). Territories dominated by the left eye and the right eye clearly alternated. These results indicate that CBV is regulated at a columnar and laminar level.

Application to Neurovascular and Neuroscience Research

The use of USPIO is ideal to determine dynamic CBV changes induced by ischemia and global stimulation. The earliest applications were to measure CBV changes during ischemia (Hamberg et al. 1996; de Crespigny et al. 2001; Kim et al. 2005). Many vascular reactivity studies followed, whereby breathing gas was manipulated (Payen et al. 1998; Payen et al. 2000; Broux et al. 2002; Julien-Dolbec et al. 2002).

USPIO was applied to obtain neural activation-induced fMRI (Kennan et al. 1998; Mandeville et al. 1998; van Bruggen et al. 1998; Palmer et al. 1999). Even though USPIO was used for preliminary human fMRI studies (Qiu et al. 2012), it is currently not approved as a blood-pool agent for humans. Thus, we focus our literature review on animal studies. A common application of CBV-weighted fMRI is to determine the underlying BOLD mechanism and to enhance fMRI sensitivity in animals. Since BOLD fMRI is related to a combination of CBF, CMRO_2 and CBV responses, measurements of the CBV response can help to determine the CBV contribution to BOLD (Kennan et al. 1998) and to determine the

cerebral metabolic rate of O₂ utilization (CMRO₂) change (Mandeville et al. 1999b; Hyder et al. 2001; Wu et al. 2002; Shen et al. 2008; Shih et al. 2011). To determine the CMRO₂ change with BOLD biophysical models, it is crucial to determine the relationship between $\Delta\text{CBF}/\text{CBF}$ and $\Delta\text{CBV}/\text{CBV}$ (Grubb et al. 1974), which can be expressed as $\Delta\text{CBV}/\text{CBV} = (\Delta\text{CBF}/\text{CBF})^\alpha$, where α is constant. Measured α values are dependent on brain regions and time after the stimulus onset (Wu et al. 2002; Kida et al. 2007; Jin and Kim 2008). At a steady state condition, the α values obtained during neural stimulation are 0.43 (Wu et al. 2002) and 0.19–0.23 (Kida et al. 2007) in the rat somatosensory cortex and ~0.2 in the cat visual cortex (Jin and Kim 2008). In both rat studies (3 T, 12 mg Fe/kg, TE=27.2 ms for (Wu et al. 2002) and 7 T, 8 mg Fe/kg, TE=20 ms for (Kida et al. 2007)), the BOLD contribution to CBV-weighted fMRI was not corrected, thus the CBV change was slightly underestimated. An important point is that the BOLD signal is directly related to the venous CBV change, not the total CBV change. fMRI with USPIO measures total CBV change, which is much larger than the venous CBV change (Lee et al. 2001; Kim et al. 2007; Kim and Kim 2011; Zong et al. 2012). Thus, when the total CBV is used, the contribution of CBV to BOLD signals is over-estimated, and consequently the CMRO₂ change is underestimated. To separate total CBV into arterial and venous CBV, arterial CBV measurement techniques (Kim and Kim 2005; Kim and Kim 2006; Kim et al. 2008) can be combined with USPIO studies.

Since the use of USPIO can enhance functional sensitivity, it is often used for monkey fMRI studies (Dubowitz et al. 2001; Vanduffel et al. 2001; Leite et al. 2002). In non-human primate studies, human MR scanners are often used and experimental time is limited. Thus, an increase in the functional sensitivity is of high priority. Wim Vanduffel (Vanduffel et al. 2001), Guy Orban (e.g., (Durand et al. 2007)), and Doris Tsao (e.g., (Tsao et al. 2008)) and their colleagues routinely use USPIO for monkey fMRI studies. These neuroscience applications are beyond the scope of this review article. For rodent fMRI studies, since high-field animal MRI systems are becoming widely available and BOLD fMRI can be used, CBV-weighted fMRI is less frequently used for neuroscience research compared to non-human primate studies. Mueggler et al. (Mueggler et al. 2001) used USPIO to image functional activity in transgenic mice and Zhao et al. used USPIO to image pain responses in the spinal cord and cortex (Zhao et al. 2008; Zhao et al. 2009).

USPIO has been used for pharmacological MRI, which investigates hemodynamic responses induced by drugs, due to its high sensitivity and its measurement of a single physiological parameter. The Bruce Jenkins and Marcus Rudin groups have led pharmacological MRI studies with USPIO. Pharmacological challenges were bicuculline (GABA_A antagonist) (Reese et al. 2000; Mueggler et al. 2001), cocaine (dopamine transporter inhibitor) (Chen et al. 2001; Chen et al. 2011; Mandeville et al. 2011; Perles-Barbacaru et al. 2011), dopamine D2/D3 receptor agonists and antagonists (Chen et al. 2005; Choi et al. 2010), nicotine (Choi et al. 2006), N-methyl-D-aspartate (NMDA) receptor agonists (Panizzutti et al. 2005), acetylcholine-esterase inhibitor (Rausch et al. 2005), and amphetamine (dopamine releaser) (Jenkins et al. 2004; Ren et al. 2009).

Conclusions

CBV mapping with intravascular, long half-life USPIO offers new imaging approaches for animal research. Although USPIO is not currently approved for human CBV studies, Feraheme (AMAG Pharmaceutical) was approved for iron deficiency anemia treatments in humans. Thus, the approval of USPIO as a blood-pool agent in humans may be possible in the near future. With USPIO, baseline CBV and its changes induced by functional activity and pharmacological intervention can be reliably and easily determined with high sensitivity, even at low magnetic fields. CBV-weighted fMRI provides enhanced sensitivity,

reduced large vessel contribution, and improved spatial specificity compared to BOLD fMRI, and measures a single physiological parameter, which is easily interpretable.

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List of abbreviations

BOLD	blood oxygenation-level dependent
CBF	cerebral blood flow
CBV	cerebral blood volume
CMRO₂	cerebral metabolic rate of O ₂ utilization
fMRI	functional magnetic resonance imaging
MION	monocrystalline iron oxide nanoparticle
rCBV	relative cerebral blood volume
SPIO	superparamagnetic iron oxide
USPIO	ultrasmall superparamagnetic iron oxide
VSI	vessel size index

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Figure 1. Determination of CBV distribution

obtained from T_2^* -weighted images of the cat brain with and without MION (Kim and Kim 2011).

(A) A coronal slice of a T_1 -weighted image was acquired using a four-segmented turbo-FLASH technique with pixel resolution = $78 \mu\text{m} \times 78 \mu\text{m} \times 2 \text{ mm}$, flip angle = $\sim 10^\circ$, TE = 5 ms, TR = 10 ms, intersegment delay = 4 s, and an inversion time (TI) of 1.4 s at 9.4 T. White matter is hyperintense, while the red arrow indicates the myelin-rich stripe of Gennari, a prominent feature in layer IV, which is confirmed by histology (Kim and Kim 2011). A T_2^* -weighted image with TR = 40 ms, TE = 10 ms, and intersegment delay = 100 ms was obtained before (B) and after a bolus injection of 12 mg Fe/kg dextran-coated MION (C). (D) The baseline CBV-related $\Delta R_{2, \text{agent}}^*$ map was calculated by MR signal changes induced by MION (B vs. C). $\Delta \Delta R_{2, \text{agent}}^*$ value (in gray bar) is linearly related to baseline CBV; CBV is the highest at the surface of the cortex, and the lowest in white matter. Penetrating intracortical vessels can be easily visualized, and layer 4 (indicated by the red arrow) has a hyperintense band indicating high CBV.

Figure 2. Baseline total and microvascular CBV, and vessel size index (Zhao et al. 2006).

(A) T₁-weighted anatomical image shows two quadrangular regions within the visual area 18 (outlined in red) for the subsequent cortical depth-dependent analysis. Each region was 700–900 μm wide and ~1.7 mm deep, extending from the surface of the cortex to the white matter. D: dorsal, L: lateral. Maps of $\Delta R_{2,*}$ and $\Delta R_{2,agent}$ were obtained from gradient-echo and spin-echo MRI data with an injection of 10 mg Fe/kg MION at 9.4 T, and indicate distributions of total (B) and microvascular CBV (C). While an area with the largest total CBV is located near the surface of the cortex, the region with the highest microvascular CBV is located within the middle cortical layer. (D) The relative vessel size index generated by $(\Delta R_{2,*} / \Delta R_{2,agent})^{3/2}$ shows a comparatively high ratio of large to small vessels at the cortical surface and within white matter. (E–F) One profile was generated for each animal from the quadrangular ROIs in area 18 in Fig. 2A, then data was averaged across five animals. The surface of the cortex is at zero, with cortical depth represented by increasing distances. An approximate location of cortical layers was determined by relative distances of those layers in area 18 (Payne and Peters 2002) and is differentiated by colored bands; the middle cortical layer (approximately layer IV) is located in the region between 0.7 mm to 1.15 mm from the surface of the cortex. (E–F) Cortical depth profiles reflect the distribution of total CBV ($\Delta R_{2,*}$; squares) and microvascular-weighted CBV signals ($\Delta R_{2,agent}$; circles) (E), and VSI (F). The area with largest total CBV is located at the surface of the cortex, while the region of highest microvascular CBV is located within the middle cortical layer (blue highlighted region).

Figure 3. BOLD contribution to CBV-weighted fMRI signal

(A and B) Absolute functional signal changes (ΔS maps) of BOLD and CBV-weighted signals obtained with 7 mg Fe/kg at 4.7 T, respectively. Purple/blue color (B) indicates a decrease in MRI signal. Maximum BOLD changes responding to visual stimulation occur in large vessel regions, while the largest MION signal changes occur in middle cortical areas. Changes in R_2^* ($\Delta R_{2,*}^{\text{agent}}$) induced by contrast agents (C) are linearly related to the baseline CBV. (D) Calculated relative CBV map with the correction of the BOLD contribution. The pattern of increased CBV in middle layers was preserved. Green contour indicates the cortical surface and black contour indicates white matter. Scale = 1mm.

Figure 4. Laminar distribution of BOLD, CBV-weighted, and rCBV signal changes (Harel et al. 2002).

Signal profiles of Fig. 3 data were plotted across the cortical layers of the lateral gyrus indicated by an arrow on the anatomical image. For comparison, all signals were plotted as the positive change. (A) Absolute signal changes of BOLD, CBV-weighted and CBV-weighted with BOLD corrections. (B) Relative signal changes (%) of BOLD, CBV-weighted and CBV with BOLD correction. (C) An enlarged segment of (B) corresponding to the medial section of both lateral gyri. Schematic boundaries between cortical layers were superimposed. GM, gray matter; WM, white matter. scale = 1mm.

Figure 5. Dose-dependent CBV-weighted fMRI changes

Since the dose of iron can modulate neural activity-induced signal changes, CBV-weighted fMRI signals were measured with 5, 10, and 15 mg Fe/kg using gradient-echo data acquisition at 9.4 T. The anatomic EPI image (A) delineates gray matter from white matter. For better visualization, lateral gyri are shown as pink contours in both hemispheres. In order to show effects of MION doses on baseline $\Delta R_{2,*agent}$ (s⁻¹) and CBV-weighted fMRI (%), cortical profiles were obtained (E and F). When the dose of MION increases from 5 mg/kg to 15 mg/kg, a more steep $\Delta R_{2,*agent}$ profile from the cortical surface is created, but the functional peak position is similar. Blue-highlighted region in F indicates layer 4.

Figure 6. Experimental time-dependent fMRI and rCBV time courses during the decay of MION contents (Lee et al. 2001).

fMRI responses of the cat visual cortex induced by visual stimulation were performed from the green region of interest (inset image) before and after an injection of 10 mg/kg MION at 4.7 T ($TE = 20$ ms, $TR = 0.5$ s). MION was removed more quickly from blood when an additional dextran solution was not infused in cats (note that all other cat studies used additional dextran solution). The negative change in A indicates that the CBV-weighted response is much larger than the positive BOLD contribution. rCBV changes were calculated using BOLD and MION time courses with Eq. [5], showing that the time-dependent variations in CBV-weighted fMRI can be removed. Horizontal bars underneath time courses indicate the stimulus duration.

Figure 7. Time-dependent functional maps of CBV-weighted fMRI signals

CBV-weighted fMRI of the cat visual cortex was obtained with $156 \times 156 \times 2000 \mu\text{m}^3$ during a 10-s visual stimulation after a 10 mg/kg injection at 9.4 T. The functional t -value maps were calculated from 2 to 3 s (A), 3 to 4 s (B), 4 to 5 s (C), and 9 to 10 s (D) after the stimulation onset. A contiguous cluster size of 3 active pixels was imposed. The t -value threshold was $t > 5$ for (A) and was adjusted between 10 to 13.5 to provide a similar number of activated pixels for (B–D). The middle cortical layer is indicated by red arrows. Clearly, the later CBV response is more specific to the middle of the cortex. Blue/purple bar, t -value.

Figure 8. CBV-weighted fMRI responses at columnar resolution obtained with two orthogonal stimulation orientations
(Zhao et al. 2005).

(A) Images with $156 \times 156 \mu\text{m}^2$ in-plane resolution were obtained from a 1-mm thick slice parallel to the surface of the cortex during moving grating stimulation of either 0° or 90° orientation at 9.4 T. LS: lateral sulcus, mg: marginal gyrus, WM: white matter. Raw gray-scale functional maps were initially determined by subtraction of images obtained during 0° (B) or 90° (C) stimulation from pre-stimulus control images without thresholding. The bright pixels in these single-condition maps have the largest (negative) signal changes induced by stimulation. The center of each patch on the 0° map (B) is marked with a red '+' sign; these symbols were then overlaid on the 90° map. A, anterior; M, medial; WM, white matter; mg, marginal gyrus; LS, lateral sulcus; a.u., arbitrary unit; green arrowheads, midline.

Figure 9. CBV-weighted functional maps induced by binocular and monocular visual stimulation

(Harel et al. 2002).

CBV-weighted fMRI of cat visual cortex was obtained with 7 mg Fe/kg MION at 4.7 T. Two examples of statistical maps are shown for binocular (top row) and monocular CBV-weighted activation maps (middle and lower rows, right and left eyes, respectively). During binocular stimulation, a more homogeneous pattern of increased CBV was seen along the middle cortical layers. On the contrary, in monocular stimulation, local patches of increased CBV-weighted signals were observed. R, right hemisphere; L, left hemisphere; D, dorsal; V, ventral; and color bar, t-values. Scale = 1mm.