Contributions of dynamic venous blood volume versus oxygenation level changes to BOLD fMRI

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Blood-oxygenation-level-dependent (BOLD) fMRI has contributions from venous oxygenation and venous cerebral blood volume (CBV) changes. To examine the relative contribution of venous CBV change (ΔCBVv) to BOLD fMRI, BOLD and arterial CBV changes (ΔCBVa) to a 40-s forepaw stimulation in six α-chloralose anesthetized rats were measured using a magnetization transfer-varied fMRI technique, while total CBV change (ΔCBVt) was measured with injection of iron oxide nanoparticles. ΔCBVt was obtained by subtracting ΔCBVv from ΔCBVt. We observed a fast ΔCBVa response with a time constant of 2.9 ± 2.3 s and a slower ΔCBVv response with a time constant of 13.5 ± 5.7 s and an onset delay of 6.1 ± 3.3 s. These results are consistent with earlier studies under different anesthesia and stimulus, supporting that fast CBVa and slow CBVv responses are generalizable. Assuming the observed post-stimulus BOLD undershoot is at least partly explained by the CBVv contribution, the relative contribution of the ΔCBVv- and oxygenation-change-related components to the BOLD response was estimated. The relative CBVv contribution increases with time during stimulation; whereby it is ~0.14 during initial 10 s and reaches a maximum possible value of ~0.45 relative to the oxygenation contribution during the 30–40 s period after stimulus onset. Our data indicates that the contribution of venous oxygenation change to BOLD fMRI is dominant for short stimulations.

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Introduction

Blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) is a widely used non-invasive tool for studying brain functions. Despite its name, BOLD fMRI signal is de-

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responses during neural activation under α-chloralose are the same as our previous findings under isoflurane anesthesia (Kim and Kim, 2011).

To characterize the dynamic BOLD_{ΔV} versus BOLD_{ΔCBV} contributions to BOLD response and evaluate their anesthesia dependence, we measured total CBV (ΔCBV_{v}) and arterial CBV changes (ΔCBV_{a}) in α-chloralose anesthetized rats by contrast agent (Mandeville et al., 1998; Kennan et al., 1998) and a magnetization transfer (MT)-varied fMRI technique (Kim et al., 2008; Kim and Kim, 2010), respectively. Then, ΔCBV_{v} was obtained as the difference between ΔCBV_{t} and ΔCBV_{a}. Assuming the observed post-stimulus BOLD undershoot is at least partly explained by the ΔCBV_{t} contribution (0–100%), we determined the BOLD_{ΔCBV} and BOLD_{ΔV} time courses. To investigate the anesthesia dependence of the relative BOLD_{ΔV} and BOLD_{ΔCBV} contributions, the same analysis was also carried out for our earlier cat visual-stimulation data obtained under isoflurane anesthesia (Kim and Kim, 2011).

Theories

MT-varied fMRI for ΔCBV_{v} measurement

Details of the theoretical background and implementation of the MT-varied fMRI were reported previously (Kim et al., 2008; Kim and Kim, 2010). Briefly, in BOLD fMRI studies, neural stimulation-induced signal changes from arterial blood and tissue can be separated by their different magnetization transfer (MT) properties. The tissue signal is dependent on the MT effect induced by off-resonance RF pulses. In contrast, the arterial blood pool experiences only a minimal MT effect due to the inflow of fresh blood spins unaffected by the MT-inducing pulses. If capillary water freely exchanges with tissue water, this upstream exchange could generate MT effects in venous blood inducing pulses. If capillary water freely exchanges with tissue water, the arterial blood pool experiences only a minimal MT-varied fMRI were reported previously (Kim et al., 2008; Kim and Kim, 2010), respectively.

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Methods and materials

Animal preparation and stimulation

Eleven male Sprague-Dawley rats weighing 300–450 g (Charles River Laboratories, Wilmington, MA, USA) were studied; six for the BOLD, ΔCBV_{a}, and ΔCBV_{v} measurements under α-chloralose anesthesia and five for the determination of arterial blood and tissue R_{2}^{*} values under isoflurane. The animal protocol was approved by the University of Pittsburgh Animal Care and Use Committee. The animals were initially anesthetized with 5% isoflurane in a mixture of O_{2} and air gases (total O_{2} concentration of ~30% throughout the experiments). The animals were intubated and the isoflurane level was subsequently reduced to 2% during the surgical procedure. The femoral artery and vein were catheterized for blood pressure/heart rate monitoring, and for fluid administration and injection of contrast agent, respectively. The rats were then transferred to a custom MRI-compatible cradle equipped with a water-circulating heating pad. The heads of the animals were carefully secured in a stereotaxic restrainer before placement in the magnet.

For BOLD, ΔCBV_{a}, and ΔCBV_{v} measurements, 30 mg/kg bolus of α-chloralose was injected and isoflurane was gradually reduced to 0% within the first hour after bolus injection. Then continuous α-chloralose infusion started after the first hour at 30 mg/kg/h, mixed with 0.05 ml/kg/h of dextrose and 1.2 mg/kg/h of pancuronium bromide throughout the experiments. For R_{2}^{*} measurements, animal anesthetism was maintained with 1.4% isoflurane without α-chloralose because, in contrast to α-chloralose anesthesia, isoflurane dilates blood vessels in the brain (Farber et al., 1997) and thus enhances blood MR signal. Rectal temperature was continuously maintained at 37.5±0.5 °C with a feedback controlled heating pad (Digi-Sense Temperature Controller R/S, Cole-Parmer, IL, USA). The end-tidal CO_{2} was maintained at 3.5–4.5% and arterial blood gas was periodically measured.

For forepaw stimulation, two needle electrodes were inserted under the skin between digits 2 and 3, and between digits 3 and 4 of the left forepaw (Silva et al., 1999). Stimulation was applied to the animal through a constant-current stimulator (A366D, World Precision Instruments Inc., Sarasota, FL, USA) that was gated by a pulse generator (Master 8, AMPI, Israel) with the following optimized parameters (Silva et al., 1999): current = 1.5–2 mA depending on the rat by ensuring no blood pressure change during the stimulation, pulse duration = 333 μs, and repetition rate = 3 Hz. The stimulation duration was 40 s and the inter-stimulation intervals were ~2 min.

MRI methods

All MRI experiments were performed on a 9.4 T/31 cm magnet with an actively shielded 12-cm gradient coil, which is interfaced to a Unity INOVA console (Varian, Palo Alto, CA, USA). A 2-cm diameter surface coil was used for all functional studies, while two actively detunable RF coils were used for baseline arterial R_{2}^{*} measurement: a neck coil for ASL (arterial spin labeling) and a head coil for imaging. The homogeneity of the magnetic field was optimized by localized shimming. Preliminary multi-slice scout BOLD fMRI studies were performed to localize the primary somatosensory cortex (S1), then a single 2-mm thick coronal slice showing robust BOLD response in S1 was selected for further studies. All images were acquired with the GE-EPI
sequence. Two separate studies were performed: 1) $R^2_g$ of arterial blood was measured at baseline (no activation) and 2) functional studies were performed to measure BOLD, arterial CBV, and total CBV changes.

**Baseline measurements of cortical arterial blood and tissue $R^2_g$**

$R^2_g$ of tissue and cortical arterial blood was measured for $\Delta CBV_a$ quantification (see Eq. (1)). Since the arterial blood transit time from labeling plane to capillary is 600–700 ms in rats (Kim and Kim, 2006), we chose arterial spin labeling duration of 700 ms without a post-labeling delay. Thus, labeled spins mostly fill the arterial vasculature during the 700 ms of labeling period and do not exchange with tissue due to the ~500-ms water exchange time between capillary and tissue (Orrison et al., 1995). Labeled and control images were alternatively acquired using the single-shot GE EPI with matrix size = $64 \times 32$, field of view (FOV) = 3.0 × 1.5 cm$^2$, repetition time (TR) = 2.5 s, and echo time (TE) = 6, 10, 15, and 20 ms. TE-dependent signal intensity was obtained from two 3 × 3-pixel (1.4 × 1.4 mm$^2$) regions of interest (ROI) centered over the S1 of both hemispheres. Then, $R^2_g$ values of tissue and cortical arterial blood were determined from the slopes of linear fits of log(unlabeled control signal) and log(difference between labeled and control signals) vs. TE, respectively.

**Measurements of $\Delta CBV_a$ and $\Delta CBV_t$/$CBV_0$**

BOLD and $\Delta CBV_a$ measurements with MT-varied fMRI were followed by $\Delta CBV_a$/$CBV_0$ studies without MT effects after injection of a susceptibility contrast agent, where $CBV_0$ is the baseline $CBV_a$. All images were acquired with TR = 1 s, TE = 20 ms for MT-varied BOLD and TE = 10.5 ms for $\Delta CBV_a$/$CBV_0$ studies, flip angle = 50° in S1, in-plane matrix = $64 \times 64$, and FOV = 2.3 × 2.0 cm$^2$. Each fMRI run consisted of 40 s pre-stimulation, 40-s forepaw stimulation, and 80 s (for $\Delta CBV_a$ and $\Delta CBV_t$) or 120-s (for CBV$_0$) post-stimulation periods, and repeated approximately 20 times for signal averaging.

MT-varied fMRI (Kim et al., 2008; Kim and Kim, 2010) was acquired using single-shot GE-EPI with the following MT-inducing pulse parameters: offset frequency = 5000 Hz, pulse duration = 880 ms, and MT ratios (MTR = $(1 - S_{MT}/S_0)$, where $S_{MT}$ and $S_0$ are the intensities with and without MT pulses, respectively) = 0, −0.5, and −0.75 in the S1 region.

For the determination of stimulus-induced CBV change ($\Delta CBV_t$/$CBV_0$), the FDA-approved susceptibility contrast agent Feraheme (AMAG Pharmaceuticals, Inc., MA) was injected intravenously with a concentration of ~10 mg Fe/kg body weight. The steady-state condition was achieved within 5 min after the injection. In order to reduce TE, a two-shot EPI technique was used. For the calculation of Feraheme-induced $R^2_g$ changes ($\Delta R^2_g$), gradient-echo EPI images were acquired with 11 different TE values between 11 and 50 ms before and after Feraheme injection.

**Functional MRI data processing**

**General analysis**

Data analyses were performed by custom MATLAB (Mathworks, Natick, MA, USA) routines. For each animal, all runs with identical experimental conditions were averaged. The mean time courses were then corrected for possible baseline linear drifts by fitting a linear function to the last 15-s of the pre-stimulation and the last 20-s of the post-stimulation periods, and subtracting the resulting fit from the original time courses (Bandettini et al., 1993).

BOLD fMRI signals were obtained from the fMRI data with MTR = 0 (no MT effect), and $\Delta CBV_a$ was determined from the fMRI data with MTR values of 0, −0.5, and −0.75. For normalization of the MT-varied fMRI time courses for $\Delta CBV_a$ calculation, $S_0$ was determined by extrapolating to the first time point ($t = 0$) by fitting the 2nd–7th time points of the MR signal intensity time courses with MTR = 0 with the theoretical steady-state-approaching curve, because the first image was acquired for EPI reference scan with zero phase encoding. Then, normalized functional signal changes ($\Delta S_{MT}/S_0$) were linearly fitted against normalized mean baseline signals ($S_{MT}/S_0$), and $\Delta CBV_a$ (units of ml blood/g tissue) was calculated from the intercept using Eq. (1).

The $\Delta CBV_a$/$CBV_0$ ratio was calculated from the Feraheme-induced baseline $R^2_g$ change and the fMRI data without MT effect. Baseline $R^2_g$ values were calculated by fitting a single-exponential function of TE-dependent image intensity versus TE, then the baseline $R^2_g$ change induced by Feraheme injection ($\Delta R^2_{contrast}$) was determined from the difference of $R^2_g$ values before and after injection. Stimulation-induced $R^2_g$ changes before and after Feraheme injection (denoted as $\Delta R^2_{stim}$ and $\Delta R^2_{stim, contrast}$, respectively) were calculated as $-\ln (\Delta S/TE)$, where $\Delta S$ is the stimulation-induced MRI signal change at MTR = 0. The fractional total CBV change ($\Delta CBV_t$/$CBV_0$) was then calculated as:

$$\frac{\Delta CBV_t}{CBV_0} = \frac{-\Delta R^2_{stim, contrast} - \Delta R^2_{stim}}{\Delta R^2_{contrast}}$$

where $\Delta R^2_{stim}$ was subtracted from $\Delta R^2_{stim, contrast}$ to correct for the BOLD contribution to the stimulation-induced $R^2_g$ change.

**Generation of BOLD, $\Delta CBV_a$ and $\Delta CBV_t$/$CBV_0$ maps**

Functional maps were generated with AFNI (Cox, 1996). All images were smoothed with a 2D Gaussian filter ($\sigma = 0.15$ mm). Functional signal changes at the three MT levels, $\Delta CBV_a$ and $\Delta CBV_t$/$CBV_0$ amplitudes were calculated as their mean responses of the 5–40th time points after stimulus onset. All color maps were displayed for pixels with $P < 0.001$ in the fMRI responses at all three MTR levels and in the total CBV-weighted response.

**Quantitative ROI analysis of BOLD, $\Delta CBV_a$ and $\Delta CBV_t$/$CBV_0$**

Since a rat brain atlas (Paxinos and Watson, 1986) shows a forelimb S1 of ~1.5 × 1.5 mm$^2$ in coronal plates 0.2 and 0.3 mm anterior to bregma, a 4 × 5 pixel (1.4 × 1.6 mm$^2$) ROI centered over anatomically-defined area on the hemisphere contra-lateral to the stimulated forepaw was defined for all CBV$_0$ and CBV quantifications. Time courses were obtained from the ROI in each animal from raw data, and $\Delta CBV_a$ and $\Delta CBV_t$/$CBV_0$ time courses were calculated as described above. To reduce noise levels, time courses were smoothed with a 3-point ordered statistical filter (Cox, 1996) by replacing the nth data point by 0.15 × min(d$_{n-1}, d_n, d_{n+1}$) + 0.7 × median(d$_{n-1}, d_n, d_{n+1}$). Where $d_n$ is the value of nth data point in the original time courses and the functions min, median, and max return the minimum, median, and maximum values of their arguments, respectively.

To compare $\Delta CBV_a$ and $\Delta CBV_t$, baseline $CBV_0$ value (CBV$_0$) was assumed within a physiological range; an upper bound was 5 ml/100 g, while a lower bound was estimated by matching the time courses of the initial $\Delta CBV_a$ (= fractional CBV change × $CBV_0$) and $\Delta CBV_t$ responses, assuming that the initial $\Delta CBV_a$ response mainly originates from the dilatation of arteries (Kim et al., 2007; Vazquez et al., 2010; Drew et al., 2011). Since the $\Delta CBV_a$ response reached a peak at 5–6 s after stimulus onset, $CBV_0$ was calculated from the ratio of the mean $\Delta CBV_a$ and mean fractional CBV$_t$ changes at the 5–6 second data points after stimulus onset. Then, $\Delta CBV_t$ values were calculated as the difference of the $\Delta CBV_a$ and $\Delta CBV_t$ time courses (Kim and Kim, 2010).

In order to characterize the dynamics of functional CBV changes, the time course of each blood volume component was fitted by a 40-s boxcar with unit height convoluted with the following single-exponential impulse response function (IRF):

$$f(t) = \frac{a}{t} \exp\left(-\frac{t-t_0}{\tau}\right) h(t-t_0).$$

(4)
where \( h(t - t_0) = 1 \) for \( t \geq t_0 \) and is 0 otherwise. The parameter \( a \) is equal to the steady-state response to a very long stimulation, and \( \tau \) is the time constant. The response delay \( t_0 \) is either assumed to be zero or treated as a free parameter in the fit (see Results for details).

**Determination of \( \Delta \text{CBV}_t \)-induced BOLD response (\( \text{BOLD}_{\Delta \text{CBV}_t} \))**

To estimate \( \text{BOLD}_{\Delta \text{CBV}_t} \), the post-stimulus BOLD undershoot was assumed to be originated at least partially from the sustained \( \Delta \text{CBV}_v \). Thus, \( \text{BOLD}_{\Delta \text{CBV}_t} = c \times \Delta \text{CBV}_v \times \text{BOLD} \) undershoot, where \( c \) is a factor relating \( \Delta \text{CBV}_v \) to \( \text{BOLD}_{\Delta \text{CBV}_t} \), and \( \text{BOLD}_v \) is the fraction of \( \text{BOLD}_{\Delta \text{CBV}_v} \) in the BOLD signal during the post-stimulus BOLD undershoot, ranging between 0 and 1. When \( \Delta \text{CBV}_v = 1 \), the post-stimulus BOLD undershoot is caused entirely by \( \Delta \text{CBV}_v \). The value of \( c \) can be calculated as the product of \( \text{f}_{\text{CBV}v} \) and the ratio of \( \text{BOLD} \) to \( \Delta \text{CBV}_v \) responses during the post-stimulus undershoot. Then \( \text{BOLD}_{\Delta \text{CBV}_t} \) was calculated over the whole time course as \( (c \times \Delta \text{CBV}_v \times \text{f}_{\text{CBV}v} \times \text{BOLD}) \), and the difference between the experimental BOLD data and \( \text{BOLD}_{\Delta \text{CBV}_t} \) is the BOLD signal induced by oxygenation level changes (\( \text{BOLD}_{\Delta \text{CBV}_t} \)).

The same analysis was also carried out for our previous published BOLD and \( \Delta \text{CBV}_v \) time courses in cats under isoflurane anesthesia (Kim and Kim, 2011). In that study, the stimulation consisted of 40-s binocular, full-field, black-and-white square-wave moving gratings, which was presented between 50-s pre-stimulation and 100-s post-stimulation periods of static gratings. For both studies, the factor \( c \) was calculated using the mean BOLD and \( \Delta \text{CBV}_v \) responses near the dip of \( \Delta \text{CBV}_v \). The value of \( c \) can be calculated as the product of \( \text{f}_{\text{CBV}v} \) and the ratio of \( \text{BOLD} \) to \( \Delta \text{CBV}_v \) responses during the post-stimulus undershoot. Then \( \text{BOLD}_{\Delta \text{CBV}_t} \) was calculated over the whole time course as \( (c \times \Delta \text{CBV}_v \times \text{f}_{\text{CBV}v} \times \text{BOLD}) \), and the difference between the experimental BOLD data and \( \text{BOLD}_{\Delta \text{CBV}_t} \) is the BOLD signal induced by oxygenation level changes (\( \text{BOLD}_{\Delta \text{CBV}_t} \)).

**Results**

All animals were maintained within a normal physiological range (\( \text{PaCO}_2 = 31.6 \pm 4.5 \) (mean ± SEM) mm Hg; \( \text{PaO}_2 = 131.1 \pm 9.5 \) mm Hg, pH = 7.48 ± 0.01). No significant arterial blood pressure change was observed during stimulation. The measured \( \text{R}^2_0 \) of tissue and cortical arterial blood was \( 35.8 \pm 4.3 \) s\(^{-1} \) (mean ± SEM, \( n = 5 \)) and \( 50.7 \pm 5.3 \) s\(^{-1} \), respectively, in the somatosensory cortical area. This gives \( e^{−(\text{R}^2_0 − \text{R}^2_{\text{tissue}})} = 0.74 \pm 0.10 \) at TE = 20 ms, which was incorporated for all \( \Delta \text{CBV}_v \) calculations.

**BOLD vs. arterial CBV vs. venous CBV change induced by stimulation**

Fig. 1B shows fractional fMRI signal change maps of one animal (Fig. 1A), and the group-averaged fMRI time courses (\( n = 6 \)) of the S1 ROI (Fig. 1B) at the three MTR levels. As expected, baseline image intensities decreased with MTR (Fig. 1A), but percent signal change increased with MTR (Fig. 1B), which agrees well with the previous results (Kim et al., 2008; Kim and Kim, 2011; Kim and Kim, 2010). During the post-stimulus period (see inset time courses), prominent undershoots of the signal intensities below the pre-stimulus baseline were observed with similar amplitude at the three MTR levels in all animals.

Fig. 2A shows the \( \Delta \text{CBV}_a \) and \( \Delta \text{CBV}_v/\text{CBV}_0 \) maps in a representative animal, where activation is clearly observed in right S1. Fig. 2B displays the group-averaged \( \Delta \text{CBV}_a \) and \( \Delta \text{CBV}_v/\text{CBV}_0 \) time courses. \( \text{CBV}_a \) increased rapidly immediately following stimulus onset and was maintained at a plateau during the remainder of the 40-s stimulation period, while \( \text{CBV}_t \) had an initial rapid increase followed by a slow increase during the remainder of the stimulation period. After stimulus offset, \( \text{CBV}_a \) quickly returned to pre-stimulus baseline and was followed by a slight undershoot, while \( \text{CBV}_v \) slowly decreased to baseline. The dynamics of \( \Delta \text{CBV}_a/\text{CBV}_0 \) time course agrees well with previous findings in rats under similar \( \alpha \)-chloralose anesthetic conditions (Mandeville et al., 1998; Silva et al., 2007).

In order to obtain absolute \( \Delta \text{CBV}_v \), two different conditions were assumed: i) \( \text{mean} \Delta \text{CBV}_v = \text{mean} \Delta \text{CBV}_t \) at the peak (5–6 s after stimulus onset), and ii) \( \text{CBV}_v = \text{mean} \text{CBV}_v = 5 \text{ml/100 g} \). When we assumed that \( \Delta \text{CBV}_v = \text{mean} \Delta \text{CBV}_t \) at the peak, we obtained a \( \text{CBV}_v \) value of 3.9 ml/100 g, which is within the range (1.6–4.1 ml/100 g) of \( \text{CBV}_v \) for the rat’s gray matter previously reported in the literature (Tropres et al., 2001; Kim et al., 2007; Schwarzbauer et al., 1997; Lin et al., 1997;
Adam et al., 2003). ΔCBV<sub>t</sub> time courses were determined as (ΔCBV<sub>v</sub>/CBV<sub>v0</sub> × CBV<sub>v0</sub> = 0) convoluted with a boxcar function. (D) Corresponding ΔCBV<sub>a</sub> responses were obtained by subtracting ΔCBV<sub>a</sub> from ΔCBV<sub>v</sub>. Black lines are fits using a single exponential impulse response (Eq. (4) with τ<sub>a</sub> as a free parameter) convoluted with a boxcar. The black horizontal bars in (B)–(D) denote the stimulation period. Best fits are given in Table 1. Error bars: standard deviations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

![Figure 2](image2.png)

**Fig. 2.** Maps of CBV<sub>a</sub> and relative total CBV response (ΔCBV<sub>a</sub> and ΔCBV<sub>v</sub>/CBV<sub>v0</sub>) amplitudes [(A) and group-averaged ΔCBV<sub>a</sub> and ΔCBV<sub>v</sub>/CBV<sub>v0</sub> (B), ΔCBV<sub>a</sub> and ΔCBV<sub>v</sub> (C), and ΔCBV<sub>t</sub> (D)] response time courses. (A) The green box denotes the region of interest (ROI) in the somatosensory cortex. (C) ΔCBV<sub>v</sub> was estimated by assuming two possible values of baseline CBV (CBV<sub>v0</sub>) (3.9 and 5 ml/100 g tissue). Black lines are fits using a single exponential impulse response (Eq. (4) with τ<sub>v</sub> = 0) convoluted with a boxcar function. (D) Corresponding ΔCBV<sub>a</sub> responses were obtained by subtracting ΔCBV<sub>a</sub> from ΔCBV<sub>v</sub>. Black lines are fits using a single exponential impulse response (Eq. (4) with τ<sub>a</sub> as a free parameter) convoluted with a boxcar. The black horizontal bars in (B)–(D) denote the stimulation period. Best fits are given in Table 1. Error bars: standard deviations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

![Figure 3](image3.png)

**Fig. 3.** The percentage of ΔCBV<sub>a</sub> in ΔCBV<sub>v</sub> during different periods of the stimulation. Two total baseline CBV<sub>v0</sub> values of 3.9 and 5 ml/100 g are assumed. The error bars are standard errors of the mean.

**Table 1**

<table>
<thead>
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<th>Baseline CBV&lt;sub&gt;v0&lt;/sub&gt;</th>
<th>ΔCBV&lt;sub&gt;v&lt;/sub&gt; (no delay)</th>
<th>ΔCBV&lt;sub&gt;v&lt;/sub&gt; (with delay)</th>
<th>ΔCBV&lt;sub&gt;a&lt;/sub&gt;</th>
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<td>14.2 4.1</td>
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</table>

Characteristics of functional CBV<sub>a</sub>, CBV<sub>v</sub>, and CBV<sub>t</sub> responses. Delays τ<sub>a</sub>, time constants (τ<sub>v</sub>) and amplitudes (α) in Eq. (4) were obtained by fitting the group-averaged time courses (Figs. 2C and D) using Eq. (4) convoluted with a unit boxcar stimulus function. Two baseline CBV<sub>v0</sub> conditions were used. The units of the baseline CBV<sub>v0</sub> and α are ml-blood/100 g tissue.
consistent with the steady-state amplitudes of the group-averaged ΔCBVt time course (p = 0.28). The fitted steady-state mean amplitude of the ΔCBVv response was 0.90 ± 0.21 ml/100 g, also consistent with the steady-state amplitude of the group-averaged ΔCBVv time course with CBV0 = 3.9 ml/100 g (p = 0.31). The fitted steady-state amplitude of the ΔCBVv response was 0.65 ± 0.17 ml/100 g when t0 = 0 and was 0.63 ± 0.11 ml/100 g when t0 ≠ 0, which were consistent with the corresponding fitted values of the group-averaged ΔCBVv time course with CBV0 = 3.9 ml/100 g (p > 0.05).

Contributions of venous oxygenation level versus venous CBV change to BOLD

The BOLD response shows a prominent post-stimulus undershoot which returns to baseline at a time point similar to the ΔCBVv response, suggesting that the slow return of the BOLD undershoot is solely caused by BOLD undershoot, i.e. fCBVv > 0. Figs. 4A and B display BOLDΔv and BOLDΔCBVv time courses for the fCBVv range of 0–1 for this rat study and our earlier cat data (Kim and Kim, 2011), respectively. In Figs. 4A and B, CBV0 values were set to match initial ΔCBVv and ΔCBVt responses; CBV0 = 3.9 ml/100 g in Fig. 4A and CBV0 = 5.08 ml/100 g in Fig. 4B after correction of the tissue–arterial blood ΔCBVv difference (Kim and Kim, 2011). The upper and lower boundary of the red region is BOLDΔv at fCBVv = 1 and 0, respectively, while the upper and lower boundary of the green region is BOLDΔCBVv at fCBVv = 0 and 1, respectively. Figs. 4C and D show ratios of mean BOLDΔCBVv to mean BOLDΔv time courses, respectively, for periods of 0–10 s, 10–20 s, 20–30 s, 30–40 s, and 40–120 s after stimulation onset for fCBVv = 0.33, 0.67, and 1. In Fig. 4C, the ratios for CBV0 = 3.9 ml/100 g and 5 ml/100 g correspond to gray bars and superimposed white bars, respectively, while the ratios were calculated for CBV0 = 5.08 ml/100 g only in Fig. 4D. In both studies, BOLDΔCBVv was less than 15% of BOLDΔv during the first 10-s period and increased with time during the later periods of stimulation. When fCBVv = 1, its absolute value reaches ~45% of BOLDΔv during the last 10-s period of stimulation, which corresponds to a 45% reduction of the BOLD response compared to the case of no ΔCBVv change. Furthermore, when fCBVv = 1, the ratio of mean BOLDΔCBVv to mean BOLDΔv over the 10–40 s period is -0.34 ± 0.07 (CBV0 = 3.9 ml/100 g) to -0.40 ± 0.06 (CBV0 = 5 ml/100 g) for rat data and -0.34 ± 0.09 for cat data.

Discussions

R2* of arterial blood and tissue

In our study, we found that the cortical arterial blood R2* (50.7 ± 5.3 s⁻¹) is higher than the tissue R2* (35.8 ± 4.3 s⁻¹). The tissue R2* agrees well with a recently reported R2* of 35.3 s⁻¹ in the gray matter of human brain (Budde et al., 2011) at the same field strength of 9.4 T. Tissue T2 in the rat somatosensory cortex at 9.4 T is 37.7 ± 0.7 ms (Meng et al., 2011) to 38.6 ± 2.1 ms (Lee et al., 1999), while cortical arterial blood T2 is 30.6 ± 4.0 ms in vivo (Meng et al., 2011), consistent with a higher cortical arterial blood R2* compared to tissue. However, femoral arterial blood T2 measured in vitro is 40.8 ± 3.4 ms (Lee et al., 1999). The shorter cortical arterial blood T2 compared to the intravascular arterial T2 might be explained by the motion of arterial blood during measurements and slightly reduced oxygenation in cortical arteries (e.g., 82–88%) compared to the systemic level (e.g., 97%) (Vazquez et al., 2010).

In contrast to a larger R2* of the cortical arterial blood compared to the tissue at 9.4 T, the opposite is true at low fields. At 1.5 T, R2* is in the range of 4–7 s⁻¹ for fully oxygenated in vitro arterial blood (Spees et al., 2001; Li et al., 1998). For blood samples with Y and hematocrit levels similar to our study (Y = 0.82 and Hct = 0.4), the R2* is approximately equal to 7 s⁻¹ (Spees et al., 2001), while some other studies found R2* ranging from 9 to 10.5 s⁻¹ (Li et al., 1998; Silvennoinen et al., 2003; Chien et al., 1994). On the other hand, R2* in gray matter of human brain is about 15 s⁻¹ at 1.5 T (Gati et al., 2002).

Fig. 4. Decomposition of BOLD responses into venous CBV (BOLDΔCBVv) and venous blood oxygenation level (BOLDΔv) change related components in the current rat (A and C) and earlier cat studies (B and D). (A) and (B): The top boundary of red region and the bottom boundary of the green region are BOLDΔv and BOLDΔCBVv, time courses, respectively, when BOLD undershoot is solely caused by BOLD undershoot, i.e. fCBVv = 1. The arrows denote the directions the two time courses would move with decreasing fCBVv. The lower boundary of the red region and the upper boundary of the green region corresponds to fCBVv = 0. Black horizontal bars: stimulation period. (C) and (D): The ratio of BOLDΔCBVv to BOLDΔv during different periods of the stimulation for assumed fCBVv values of 0.33, 0.67, and 1. In (C), the gray and superimposed white bars correspond to CBV0 = 3.9 and 5 ml/100 g, respectively. In (D), CBV0 is set to 5.08 ml/100 g. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Comparison to earlier results under the same anesthetic condition

In this study, we found an initial fast CBV response with a time constant of 2.9 ± 2.3 and a subsequent slower CBV response with a time constant of 13.5 ± 5.7 s in α-chloralose anesthetized rats. The CBV response was delayed 6.1 ± 3.3 s from the stimulation onset. Our results are consistent with earlier studies under the same anesthesia conditions (Silva et al., 2007; Mandeville et al., 1999; Lu et al., 2005), where both fast and slow components were observed in the total CBV or CBV-weighted responses. In (Mandeville et al., 1999), the fast and slow CBV-weighted response components have time constants of 1.5 ± 0.8 s and 14 ± 13 s, respectively, and the onset of the slow component is delayed by 8 s relative to the stimulus onset. In (Silva et al., 2007), the total CBV impulse response was modeled as a sum of two gamma variate functions, where the fast function had a time to peak (TTP) of 1.29 s and a full width at half maximum (FWHM) of 1.18 s, and the slow function had a TTP of 7.3 s and a FWHM of 18.2 s. To convert those values to slow response delays and time constants defined in Eq. (4), we note that the TTP corresponds roughly to the response delay in our impulse response function and the FWHM is roughly related to the single exponential time constant by \( \tau = \text{FWHM}/(2 \ln 2) \), which is obtained by setting the FWHM of the gamma variate function equal to the FWHM of a symmetric single exponential function \( f(t) = \exp(-|t|/\tau) \). Therefore, the time constants of the fast and slow components in Silva et al. (2007) are 0.85 s and 13.1 s, respectively, and the delay of the slow component is 7.3 s.

The relative contributions of the fast and slow components in our study are also in good agreement with the earlier studies. In Mandeville et al. (1999) and Silva et al. (2007), the slow component of the CBV response contributes approximately 40% and 67%, respectively, of the total CBV response by the end of 30-s stimulation, close to our result where \( \Delta \text{CBV}_v \) was 55 ± 12% of \( \Delta \text{CBV}_t \) at 20–30 s after stimulus onset when \( \text{CBV}_0 = 3.9 \text{ ml/100 g} \) (see Fig. 3). In Lu et al. (2005), fast and slow components were clearly visible in their total CBV-weighted response to 32-s whisker stimulation in the barrel cortex. The slow component contributed roughly 50% of the total CBV change by the end of the 32-s stimulation.

One interesting note is that the prediction of the total CBV response to long stimulation based on its response to short stimulation will underestimate the actual response due to ignoring slow \( \Delta \text{CBV}_v \), which was indeed observed in Lu et al. (2005).

Anesthesia dependence of hemodynamic responses

Table 2 summarizes the time constants of the slow and fast CBV responses studied in different anesthesia conditions and species. Although different anesthetics induce different baseline vascular and physiological conditions, fast and slow CBV responses were consistently observed. A human fMRI study with direct \( \Delta \text{CBV}_v \), measurement also found a slower \( \Delta \text{CBV}_v \), response than CBF change (CBF change is highly correlated with \( \Delta \text{CBV}_v \); Kim et al., 2007) (Chen and Pike, 2009).

The peak amplitudes of the \( \Delta \text{CBV}_v \) and \( \Delta \text{CBV}_a \) in α-chloralose anesthetized rats are higher than those in isoflurane anesthetized cats: 0.35 ml/100 g versus 0.24 ml/100 g for \( \Delta \text{CBV}_v \) and 0.46–0.68 ml/100 g versus 0.18 ml/100 g (at CBV0 = 5.08 ml/100 g) for \( \Delta \text{CBV}_a \) (Kim and Kim, 2011).

Table 2: Comparison of the single exponential time constants of the fast and slow CBV impulse response components in different studies. The fast and slow components correspond to the arterial and venous CBV responses, respectively, in our study. SMA = somatosensory cortex.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Cortical area</th>
<th>Anesthesia</th>
<th>( \tau ) (fast or ( \Delta \text{CBV}_v )) (s)</th>
<th>( \tau ) (slow or ( \Delta \text{CBV}_v )) (s)</th>
<th>Delay of slow component (s)</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandeville et al. (1999)</td>
<td>Rat</td>
<td>SMA</td>
<td>α-chloralose</td>
<td>1.5 ± 0.8</td>
<td>14 ± 13</td>
<td>8</td>
<td>( \Delta \text{CBV}_v )</td>
</tr>
<tr>
<td>Leite et al. (2002)</td>
<td>Monkey</td>
<td>Visual</td>
<td>Awake</td>
<td>4.5</td>
<td>13.5</td>
<td>1.5</td>
<td>( \Delta \text{CBV}_v )</td>
</tr>
<tr>
<td>Silva et al. (2007)</td>
<td>Rat</td>
<td>SMA</td>
<td>α-chloralose</td>
<td>0.85</td>
<td>13.1</td>
<td>7.3</td>
<td>( \Delta \text{CBV}_v )</td>
</tr>
<tr>
<td>Kim and Kim (2011)</td>
<td>Cat</td>
<td>Visual</td>
<td>Isoflurane</td>
<td>2.5</td>
<td>18.4–40.9</td>
<td>Fixed to 0</td>
<td>( \Delta \text{CBV}_v ), ( \Delta \text{CBV}_a )</td>
</tr>
<tr>
<td>Drew et al. (2011)</td>
<td>Mouse</td>
<td>Primary vibrissa</td>
<td>Awake</td>
<td>10 ± 10</td>
<td>40 ± 35</td>
<td>Fixed to 0</td>
<td>( \Delta \text{CBV}_v ), ( \Delta \text{CBV}_a )</td>
</tr>
<tr>
<td>This study</td>
<td>Rat</td>
<td>SMA</td>
<td>α-chloralose</td>
<td>2.4</td>
<td>14.2–15.0</td>
<td>4.1–6.5</td>
<td>( \Delta \text{CBV}_v ), ( \Delta \text{CBV}_a )</td>
</tr>
</tbody>
</table>

The increase of \( \text{CBV}_v \) with time during stimulation might be responsible for 1) the reduced BOLD response after initial overshoot as observed in the current and some other fMRI studies (Obata et al., 2004; Kim and Kim, 2011; Hoge et al., 1999; Frahm et al., 1996), and 2) the nonlinearity of BOLD fMRI responses. 1) Based on Balloon model simulations (Buxton et al., 1998), Obata et al. (2004) demonstrated that, due to increased \( \Delta \text{CBV}_v \), time, the BOLD response decreases after initial overshoot even when the assumed CBF response continuously increases with time during stimulation. Thus, the effect of dynamic \( \Delta \text{CBV}_v \), changes needs to be considered in interpreting the dynamic properties of BOLD responses to long stimulations. 2) The predicted BOLD response to long stimulation based on...
the BOLD IRF with minimal $\Delta CBV$ contribution to short stimulation would be higher than the experimental BOLD with the $\Delta CBV$, contribution. This explanation was consistent with the findings of Miller et al. (2001), although other sources of nonlinearity should also be considered such as neural adaption and nonlinearity in CBF responses.

Conclusions

In summary, we measured dynamic BOLD and compartment-specific CBV responses to electrical forepaw stimulation in $\alpha$-chlordes flurane-anesthetized rats. The measured $\Delta CBV$ and $\Delta CBV$ time courses in rats under $\alpha$-chloralose anesthesia show a fast $\Delta CBV$ response and a much slower and delayed $\Delta CBV$ response. Our findings support the anesthesia independence of the fast $\Delta CBV$ and slow CBV dynamic properties. The obtained $\Delta CBV$ and BOLD time courses allowed us to estimate a range of the $\Delta CBV$ and oxygenation change related components in the BOLD response. The $[BOLD]_{\Delta CBV}$ is negligible during the initial 10 s of stimulation but may become significant toward the end of the stimulation period.

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References


