

Effects of hypoxia, hyperoxia, and hypercapnia on baseline and stimulus-evoked BOLD, CBF, and CMRO₂ in spontaneously breathing animals

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Functional magnetic resonance imaging (fMRI) was used to investigate the effects of inspired hypoxic, hyperoxic, and hypercapnic gases on baseline and stimulus-evoked changes in blood oxygenation level-dependent (BOLD) signals, cerebral blood flow (CBF), and the cerebral metabolic rate of oxygen (CMRO₂) in spontaneously breathing rats under isoflurane anesthesia. Each animal was subjected to a baseline period of six inspired gas conditions (9% O₂, 12% O₂, 21% O₂, 100% O₂, 5% CO₂, and 10% CO₂) followed by a superimposed period of forepaw stimulation. Significant stimulus-evoked fMRI responses were found in the primary somatosensory cortices. Relative fMRI responses to forepaw stimulation varied across gas conditions and were dependent on baseline physiology, whereas absolute fMRI responses were similar across moderate gas conditions (12% O₂, 21% O₂, 100% O₂, and 5% CO₂) and were relatively independent of baseline physiology. Consistent with data obtained using well-established techniques, baseline and stimulus-evoked CMRO₂ were invariant across moderate physiological perturbations thereby supporting a CMRO₂-fMRI technique for non-invasive CMRO₂ measurement. However, under 9% O₂ and 10% CO₂, stimulus-evoked CBF and BOLD were substantially reduced and the CMRO₂ formalism appeared invalid, likely due to attenuated neurovascular coupling and/or a failure of the model under extreme physiological perturbations. These findings demonstrate that absolute fMRI measurements help distinguish neural from non-neural contributions to the fMRI signals and may lend a more accurate measure of brain activity during states of altered basal physiology. Moreover, since numerous pharmacologic agents, pathophysiological states, and psychiatric conditions alter baseline physiology independent of neural activity, these results have implications for neuroimaging studies using relative fMRI changes to map brain activity.

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Introduction

Functional magnetic resonance imaging (fMRI) commonly measures relative (percent) changes in blood-oxygenation-level dependent (BOLD) contrast, cerebral blood flow (CBF) and cerebral blood volume (CBV). These relative fMRI responses are highly sensitive to baseline physiological parameters such as CBF, CBV, tissue oxygenation, and oxidative metabolism (Corfield et al., 2001; Ramsay et al., 1993). Factors that affect these physiological parameters are numerous and include anesthetics, respiration rate, blood pressure, endogenous hormones, emotional states, diseases, drugs of abuse, and many commonly ingested substances such as caffeine (Dager et al., 1999), nicotine (Jacobsen et al., 2002), and alcohol (Levin et al., 1998). These observations suggest caution when comparing fMRI results across subjects whose baseline physiological parameters may differ. They also suggest that absolute fMRI signal change (i.e., change relative to a single fixed baseline state serving as a control), rather than relative fMRI signal change (i.e., change relative to its own respective baseline state), is a more accurate index of brain activity in situations where baseline physiology is markedly perturbed, such as in pharmacologic or disease-induced states. In order to unambiguously identify key baseline physiological factors that modulate fMRI responses, baseline and stimulus-evoked CBF and BOLD signals must be systematically evaluated under various perturbations of baseline cerebral blood flow and tissue oxygenation, with such perturbations being accomplished via inspiration of hypoxic, hyperoxic, or hypercapnic gases. It is hypothesized that, by determining absolute and relative stimulus-evoked changes in CBF and BOLD signals under various physiological perturbations in the same subjects and in the same experimental setting, the validity of using relative stimulus-evoked fMRI signal change as an indicator of neuronal activity under different basal physiological conditions can be tested. In addition, the cerebral metabolic rate of oxygen (CMRO₂) (Davis et al., 1998; Hoge et al., 1999; Liu et al., 2004; Mandeville et al., 1998), calculated from these measure-

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ments using Davis' CMRO₂ formalism, could be utilized to investigate metabolic changes under different baseline physiologies with and without functional stimulation.

Davis et al. (1998) introduced an eloquent formalism based on the BOLD biophysical model (Ogawa et al., 1993) to determine CMRO₂. The advantage of Davis' formalism over existing formalisms is that there are no a priori assumptions regarding resting capillary or venous oxygen saturation, blood volume fraction, blood flow, and metabolic rate of oxygen. All these parameters and other physiological quantities are lumped into the constant *M* which can be measured on a pixel-by-pixel basis. A modified CMRO₂ model has been proposed to take into account the arterial–venous blood volume contributions and includes a non-steady state determination of CMRO₂ (Wu et al., 2002). While Hoge et al. (1999), Mandeville et al. (1999), and others have reproduced and extended Davis' findings. However, no studies have been conducted to support the CMRO₂-MRI model under physiologically perturbed baseline states. Since it is well established that moderate and transient perturbations in arterial oxygen and carbon-dioxide partial pressures (PaO₂ and PaCO₂) per se do not change CMRO₂ (Kety and Schmidt, 1948; Novack et al., 1953), it is hypothesized that CMRO₂ values derived using Davis' formalism would be invariant under such perturbations. The same approach can be used to further test the integrity CMRO₂-MRI formalism under relatively more severe physiological perturbations. In addition, the effect of baseline conditions on stimulus-evoked neural activity can be assessed by performing somatosensory stimulation in the presence of different gas modulations.

Experiments demonstrating the self-consistency of the Davis formalism are arguably best performed in animal models under well-controlled conditions and for these reasons the established forepaw stimulation rat model is ideal. Essentially all fMRI studies of forepaw stimulation in rats use α -chloralose as the anesthetic (Duong et al., 2000; Mandeville et al., 1998; Silva et al., 1999) which has been shown to minimally perturb neural activity and hemodynamic coupling (Ueki et al., 1992). However, achieving a stable anesthesia over long durations with α -chloralose is relatively difficult and generally requires mechanical ventilation and invasive blood-gas sampling; thus, it is less suited for prolonged experiments that require repeated fMRI measurements. Our lab recently demonstrated that forepaw stimulation in rats under isoflurane anesthesia and spontaneously breathing conditions is well suited for repeated fMRI measurements because the animals can maintain stable physiology throughout the experiment (Liu et al., 2004). Of equal importance is the use of a quantitative (absolute) CBF technique based on the two-coil continuous arterial spin-labeling method in which BOLD and CBF can be simultaneously measured (Duong et al., 2000; Silva et al., 1999). This technique produces relatively high CBF contrast and eliminates inter-trial variations in CBF and BOLD responses associated with sequential measurements of these parameters, and enables calculation of CMRO₂ under different baseline physiological conditions with and without forepaw stimulation using Davis' formalism. The main goals of this study were: (i) to characterize the effects of inspired hypoxic, hyperoxic, and hypercapnic gases on baseline and forepaw-stimulation induced changes in CBF, BOLD, and CMRO₂ under spontaneously breathing conditions, and (ii) to use these findings to test the self-consistency of Davis' CMRO₂-MRI technique under relatively moderate and severe physiological perturbations.

Materials and methods

Animal preparation

Nine male Sprague–Dawley rats (300–340 g) were initially anesthetized with the vaporizer set to 2.0% isoflurane. Needle electrodes were inserted subcutaneously into the forepaws. In 5 out of 9 rats, the femoral artery was catheterized for continuous recording of heart rate (HR) and mean arterial blood pressure (MABP). Blood gases were sampled once for each gas condition. Respiration rate (RR) was derived from the slow modulations on top of the cardiac waveforms. Animals were secured in an MR-compatible rat stereotaxic headset with custom-designed ear-, nose-, tooth-, and shoulder-bars. The vaporizer was then set to 1.15–1.25% isoflurane throughout the remainder of the study under spontaneously breathing conditions. Rectal temperature was monitored and kept at $37.0 \pm 0.5^\circ\text{C}$.

Forepaw stimulation during modulated gas conditions

Each rat was subjected to six separate and randomly administered inspired gas conditions. These gases were: 9%, 12%, 21% (room air) and 100% O₂ premixed with balance N₂, and 5% and 10% CO₂ premixed with 21% O₂ and balance N₂. 21% O₂ was used as a fixed reference state, 9% O₂ and 10% CO₂ gas challenges were categorized as “severe” and the remaining gas challenges were categorized as “moderate” based subsequent to their effects on cerebral hemodynamics and metabolism. Note also that the terms hypoxia, hyperoxia, and hypercapnia as used herein refer to inspired gas conditions rather than arterial blood gas conditions (see Discussion). Gas was equilibrated for ~3 min before starting each fMRI trial, which consisted of 2-min baseline (gas condition only) and 2-min stimulation (gas condition + forepaw stimulation) periods. Forepaws were stimulated simultaneously in series using a 6-mA current with 0.3-ms pulse duration at 3 Hz which has been previously titrated to yield optimal fMRI responses without inducing significant, sustained changes in MABP, HR, and blood gases (Liu et al., 2004). Two repeated trials of each gas condition were typically made on each animal and an approximately 15-min break was given between trials. The entire experiment took ~4–5 h.

MR experiments

MRI was performed on a 4.7-T/40-cm horizontal magnet (Oxford, UK) equipped with a Biospec Bruker console and a 20-G/cm gradient (ID = 12 cm, 120- μs rise time) (Billerica, MA). A surface coil (2.3-cm ID) was used for brain imaging and a neck coil (Duong et al., 2000; Silva et al., 1999) for perfusion labeling. Coil-to-coil electromagnetic interaction was actively decoupled. Anatomical images were acquired using the fast spin-echo pulse sequence with TR = 2 s, 16 echo trains, effective TE = 104 ms, matrix = 128×128 , FOV = $2.56 \times 2.56 \text{ cm}^2$, eight 1.5-mm slices, and 16 averages.

Simultaneous CBF and BOLD measurements were made using the two-coil continuous arterial spin-labeling technique (Duong et al., 2000; Silva et al., 1999) with single-shot, gradient-echo, echo-planar-imaging (EPI) acquisition. Paired images were acquired alternately—one with, and the other without, arterial spin labeling. MR parameters were: data matrix = 64×64 , FOV = $2.56 \times 2.56 \text{ cm}^2$, the same eight 1.5-mm slices as in anatomical scans, TE = 20 ms, TR = 2 s, and 60 pairs of images.

Data analysis

Image analysis employed codes written in Matlab (MathWorks Inc, Natick, MA) and the STIMULATE software (University of Minnesota). BOLD time-series images were obtained from non-labeled images of the CBF measurements. CBF images (S_{CBF}) with intensities in mL/g/min were calculated at each time point as described elsewhere (Duong et al., 2000; Sicard et al., 2003). Cross-correlation analysis was performed on the BOLD and CBF data sets to obtain percent-change activation maps.

To avoid bias to a particular current or type of activation map, region-of-interest (ROI) analysis was performed to obtain fMRI time courses. Cross-correlation (CC) CBF and BOLD maps were computed and averaged across all gas conditions (CC coefficients were similar except for stimulation under 10% CO_2 which was lower). Bilateral ROIs enclosing the forepaw primary somatosensory cortices (6–9 pixels each hemisphere on each slice, with 2–4 slices typically showing activation) were drawn on the average CC maps with reference to anatomy. Time courses from each rat were derived from the same ROIs without using activation-map masks.

CMRO_2 , CBF, and BOLD signals are related by Davis et al. (1998),

$$\frac{\Delta \text{BOLD}}{\text{BOLD}_0} = M \left(1 - \left(\frac{\text{CMRO}_2}{\text{CMRO}_{2|0}} \right)^\beta \left(\frac{\text{CBF}}{\text{CBF}_0} \right)^{\alpha-\beta} \right), \quad (1)$$

where M is the proportionality constant and parameters with subscript zero indicate baseline values. Grubb's factor (α) of 0.38 (Grubb et al., 1974; Mandeville et al., 1999) and β of 1.25 (Boxerman et al., 1995; Davis et al., 1998) were used, which were taken to be constants reflecting the effects of blood volume and deoxyhemoglobin concentration on the BOLD signal, respectively. M values were calculated from the hypercapnia data by setting $\text{CMRO}_2/\text{CMRO}_{2|0}$ to unity since brief and mild hypercapnia does not alter CMRO_2 (Kety and Schmidt, 1948; Novack et al., 1953). Using the derived M values, $\text{CMRO}_2/\text{CMRO}_{2|0}$ was calculated. Additional details including error propagation have been described elsewhere (Liu et al., 2004).

Average CMRO_2 for 10% CO_2 baseline was determined with M value derived from 5% CO_2 baseline and vice versa. M values derived from 5% and 10% CO_2 challenges were not statistically different and so were averaged together. Averaged M value was then used to determine average baseline CMRO_2 of the other gas challenges (9%, 12%, 21%, and 100% O_2) and all average stimulus-evoked CMRO_2 changes within the forepaw primary somatosensory cortices. Absolute CBF changes were calculated in units of mL/g/min. "Absolute" baseline and stimulus-evoked

BOLD and CMRO_2 changes were derived by normalizing all values with respect to that obtained under 21% O_2 baseline period which served as the fixed baseline; in other words, absolute BOLD and CMRO_2 signal changes, as defined herein, are changes relative to a single fixed state. "Relative" stimulus-evoked changes in BOLD, CBF, and CMRO_2 for each gas condition were computed by dividing the difference between baseline and stimulus-evoked values of a gas challenge by the baseline value of said gas challenge. Lastly, note that for simplicity, changes in T_2^* -weighted signal changes due to forepaw stimulation or alterations of basal physiology are referred to as "BOLD changes."

Statistical analysis

Pairwise comparisons of physiological parameters between gas conditions, and between baseline and stimulation periods were made in the same animals. All reported values in text and graphs are mean \pm SD. All statistical tests were performed using paired t test (two tails) with P value < 0.05 taken to be statistically significant, unless otherwise specified.

Results

Physiological measurements

Physiological parameters are summarized in Table 1. HR, MABP, and RR during 21% O_2 were consistent with those reported previously under similar experimental conditions (Liu et al., 2004) and were stable during the breaks between gas challenges. All of the following changes are relative to 21% O_2 and are statistically significant ($P < 0.05$) unless otherwise noted. Inhalation of hypoxic gas decreased HR, MABP, PaCO_2 , PaO_2 , and arterial oxygen saturation (SaO_2), and increased RR and arterial pH. Inhalation of hyperoxic gas decreased HR, RR and arterial pH, increased PaCO_2 and SaO_2 , and produced no significant change in MABP. Hypercapnia decreased arterial pH and SaO_2 , increased RR, PaO_2 and PaCO_2 , and produced no significant changes in MABP and HR. The inspiration of hypoxic, hyperoxic, or hypercapnic gases each produced alterations in both PaCO_2 and PaO_2 which is typical of the spontaneously breathing condition. While the stimulation current used herein had been previously shown to cause small and transient changes in MABP (< 5 mm Hg) and HR (Liu et al., 2004), no statistically significant transient stimulus-evoked changes in these parameters was found in this study. Both studies found no statistically significant sustained changes in MABP and HR in response to stimulation.

Table 1

Heart rate (HR), mean arterial blood pressure (MABP), respiration rate (RR), and blood gases under different gas conditions (mean \pm SD, $n = 5$ of 9 rats)

Gas conditions	HR (beat/min)	MABP (mm Hg)	RR (breath/min)	pH	PaCO_2 (mm Hg)	PaO_2 (mm Hg)	SaO_2 (%)
9% O_2	365 \pm 10*	80 \pm 08 ^a	75 \pm 3 ^a	7.55 \pm 0.01 ^a	26 \pm 1 ^a	33 \pm 2 ^a	75 \pm 3 ^a
12% O_2	372 \pm 12*	113 \pm 09 ^a	70 \pm 2 ^b	7.48 \pm 0.01 ^a	32 \pm 1 ^a	40 \pm 1 ^a	81 \pm 3 ^a
21% O_2	383 \pm 11	135 \pm 09	67 \pm 2	7.42 \pm 0.01	37 \pm 1	88 \pm 2	97 \pm 0.4
100% O_2	370 \pm 10*	140 \pm 11	60 \pm 3 ^a	7.40 \pm 0.01*	40 \pm 1 ^b	324 \pm 7 ^a	99 \pm 0.0 ^a
5% CO_2	382 \pm 05	129 \pm 08	69 \pm 3 ^b	7.30 \pm 0.02 ^a	49 \pm 2 ^a	102 \pm 3 ^a	97 \pm 0.4
10% CO_2	380 \pm 10	132 \pm 04	69 \pm 3*	7.15 \pm 0.01 ^a	70 \pm 2 ^a	112 \pm 2 ^a	96 \pm 0.4*

HR, MABP, and RR were not statistically different between baseline and forepaw stimulation periods ($P > 0.05$) and were grouped together.

* $P < 0.05$ relative to 21% O_2 .

^a $P \leq 0.0005$ relative to 21% O_2 .

^b $P \leq 0.005$ relative to 21% O_2 .

CBF and BOLD under different gas exposures

Fig. 1 shows representative anatomic images, quantitative CBF images, CBF percent-change maps, and BOLD percent-change maps from one animal. Bilateral activations in the forepaw primary somatosensory cortices were observed in both CBF and BOLD activation maps. Activations in the motor cortices, secondary somatosensory cortices, and subcortical structures were inconsistently observed and were of smaller magnitude. Signal changes in the sagittal sinus also were sometimes observed.

Group-averaged basal and stimulus-evoked absolute CBF values during each gas condition are summarized in Fig. 2. Baseline CBF during 21% O₂ was 1.10 ± 0.04 mL/g/min, consistent with a previous study (Liu et al., 2004). Absolute baseline CBF grew progressively larger from 9%, 12%, 21%, to 100% O₂, and rose further during hypercapnia (5% to 10% CO₂). However, the absolute stimulus-evoked CBF increases were similar under 12%, 21%, 100% O₂, and 5% CO₂ and were independent of basal CBF. Due to differences in absolute baseline CBF, relative stimulus-evoked CBF changes varied across different gas conditions although the stimuli were identical. Under stronger physiological perturbations (9% O₂ and 10% CO₂), both absolute and relative stimulus-evoked CBF increases were markedly smaller.

Absolute baseline BOLD signal increased from 9%, 12%, 21%, 100% O₂, 5% to 10% CO₂ (Fig. 3), following a similar trend as changes in absolute baseline CBF. Absolute stimulation-induced BOLD increases were similar under 12%, 21%, and 100% O₂, and 5% CO₂. Due to differences in absolute baseline BOLD, relative stimulus-evoked BOLD increases varied across different gas conditions although the stimuli were identical. Under stronger gas perturbations (9% O₂ and 10% CO₂), absolute and relative stimulus-evoked BOLD increases were markedly smaller.

CMRO₂ under different gas exposures

The *M* values in the forepaw primary somatosensory cortices obtained from 5% and 10% CO₂ challenges were 0.12 ± 0.02 and 0.14 ± 0.02 , respectively. These *M* values were higher than those reported previously (Liu et al., 2004) because of a longer echo time

used herein. For baseline period, absolute (CMRO₂/CMRO_{2|0}) basal CMRO₂ values with respect to air were close to unity across all gas conditions (12% O₂: 1.04 ± 0.17 ; 100% O₂: 1.08 ± 0.06 ; 5% CO₂: 1.05 ± 0.08 ; 10% CO₂: 0.90 ± 0.2), with the exception of under 9% O₂ which showed a 32% reduction (Fig. 4).

Absolute stimulus-evoked CMRO₂ changes under 9%, 21%, 100% O₂, and 5% CO₂ were not statistically different from each other, with the exception of being significantly smaller under 10% CO₂. In contrast, relative stimulus-evoked CMRO₂ changes varied significantly across different gas conditions. For example, the relative stimulus-evoked CMRO₂ change during 9% O₂ was significantly larger than that during air, although the absolute stimulus-evoked CMRO₂ changes were essentially identical for the two conditions.

Discussion

Potential drawbacks of the isoflurane-anesthetized forepaw-stimulation model

The use of isoflurane anesthesia has some drawbacks. First, isoflurane suppresses neural activity which could explain the higher stimulation current needed relative to that used under α -chloralose (1.5–2.0 mA; Duong et al., 2000; Silva et al., 1999) which could potentially cause pain-induced changes in MABP and HR, as well as activation outside the somatosensory pathway. However, the applied current was previously optimized to elicit robust fMRI responses without producing sustained changes in MABP and HR (Liu et al., 2004) which is interpreted as an absence of painful stimuli (Spenger et al., 2000). This anesthesia protocol used herein was also found to eliminate conscious response to noxious stimuli (tail pinching; Liu et al., 2004). Thus, although smaller magnitude activations were sometimes present outside the somatosensory pathway, they were unlikely the result of pain. Of note, in an fMRI study performed at 4.7 T, Spenger et al. (2000) also report highly variable activations outside the somatosensory pathway (with no changes in MABP and HR) during forepaw stimulation in rats under α -chloralose anesthesia previously

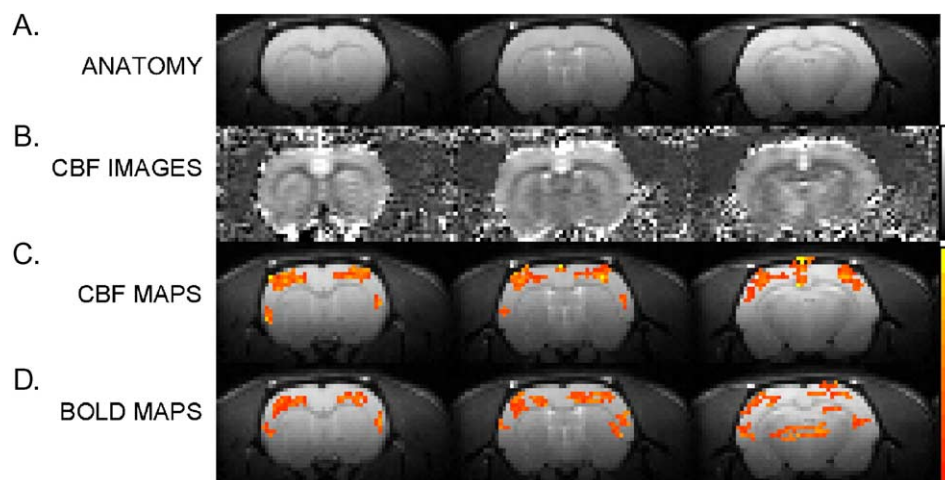


Fig. 1. (A) Representative anatomical images, (B) absolute cerebral blood flow (CBF) images, (C) CBF activation maps, and (D) BOLD activation maps from an anesthetized rat with both forepaws simultaneously stimulated under 21% O₂. Maps were overlaid on anatomic images. Map percent-thresholds were set to clearly display activations in the primary and secondary somatosensory cortices; activations of the motor cortex and subcortical areas were sometimes present but not displayed. Grayscale bar: CBF ~0–3 mL/g/min. Color bar: CBF ~75–200%, BOLD ~2–7.5%.

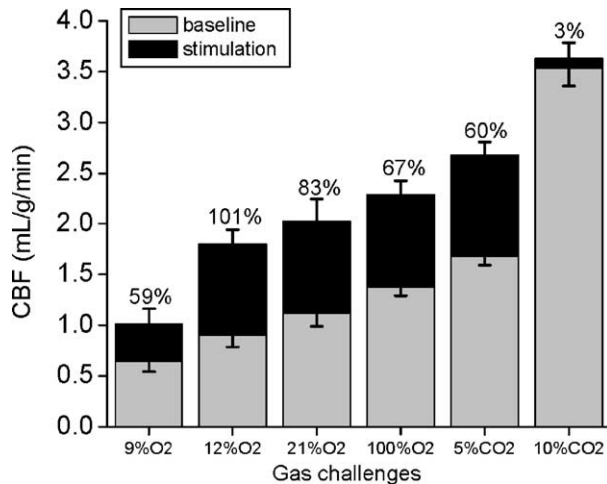


Fig. 2. Absolute CBF under different gas modulations before and during forepaw stimulation under different gas conditions (mean \pm SD, $n = 9$). Data were obtained from ROIs of the forepaw primary somatosensory cortices. Relative to 21% O₂ (air), absolute stimulus-evoked CBF increases were not statistically different during 12% and 100% O₂, and 5% CO₂ ($P > 0.05$) but were reduced under 9% O₂ and 10% CO₂ ($P < 10^{-4}$). Stimulus-evoked percent-change values are displayed numerically.

optimized to eliminate pain response to electrical stimulation. Second, isoflurane also depresses cerebrovascular reactivity to CO₂ relative to awake conditions (Sicard et al., 2003) which may explain the stronger hypercapnic challenges required to derive M . In animal studies, 5% and 10% CO₂ challenges are commonly used with other anesthetics (Luo et al., 2003; Wu et al., 2002), and the former is frequently used in human studies (Davis et al., 1998; Hoge et al., 1999). Third, isoflurane is a cerebrovasodilator which increases basal CBF (Hendrich et al., 2001; Matta et al., 1999), thus potentially reducing the head room for forepaw stimulation- or hypercapnia-evoked CBF and/or BOLD increases. Lastly, inhala-

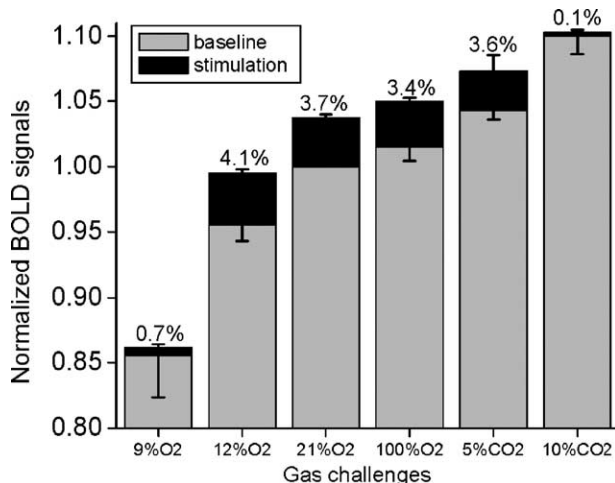


Fig. 3. Normalized (absolute) BOLD signals under different gas modulations before and during forepaw stimulation (mean \pm SD, $n = 9$). Data were obtained from ROIs of the forepaw primary somatosensory cortices. All BOLD signals were normalized with respect to 21% O₂ without stimulation. Relative to 21% O₂ (air), absolute stimulus-evoked BOLD increases were not statistically different during 12% and 100% O₂, and 5% CO₂ ($P > 0.05$) but were reduced under 9% O₂ and 10% CO₂ ($P < 10^{-6}$). Stimulus-evoked percent-change values are displayed numerically.

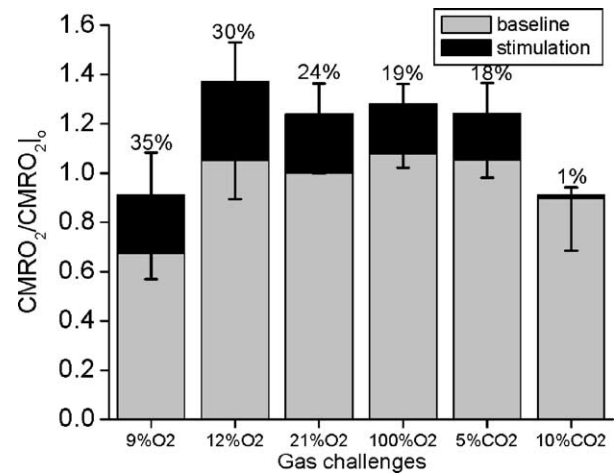


Fig. 4. Normalized (absolute) CMRO₂ changes under different gas modulations before and during forepaw stimulation (mean \pm SD, $n = 9$). All absolute CMRO₂ changes were calculated with respect to 21% O₂ baseline period. The absolute stimulus-evoked CMRO₂ changes were similar across gas conditions except under 10% CO₂, whereas the stimulus-evoked CMRO₂ percent-changes, displayed numerically, were highly dependent on baseline CMRO₂.

tion anesthesia may become unstable over time due to change in the equilibrium between inspired and alveolar gas tensions; however, this disequilibrium is negligible with isoflurane due to its low blood solubility (White et al., 1974).

The spontaneous breathing condition has drawbacks not found with mechanical ventilation. Spontaneous breathing could result in higher physiological noise in the fMRI signals, but this can be corrected. Respiratory adaptations to inspired gases quickly alter both PaO₂ and PaCO₂; although not a drawback for this particular study, such autoregulatory responses would make it nearly impossible to separate the effects of altered arterial O₂ and CO₂ on cerebral hemodynamics and metabolism. Lastly, spontaneous breathing under light anesthesia is not completely equivalent to the freely-breathing awake condition as low-dose isoflurane is also known to preserve but delay or attenuate autoregulatory responses to changes in MABP (Lee et al., 1994; Ludders, 1992; Olsen et al., 1994; Strebel et al., 1995), O₂ (Hirshman et al., 1977; Sollevi and Lindahl, 1995), and CO₂ (Sicard et al., 2003). Despite these potential drawbacks, the forepaw stimulation rat model under isoflurane and spontaneous breathing condition is easy to set up, produces robust fMRI responses, and allows the animals to maintain stable physiology for a prolonged period.

Limitations of the CMRO₂ model and future improvements

The Davis model uses CBF and BOLD measurements to derive CMRO₂. This derivation relies on the constants α and β which are pixel-specific, the former empirically obtained from whole monkey brain. However, Mandeville et al. (1999) reported α of 0.4 in rat forepaw somatosensory cortex and Davis et al. (1998) showed that CMRO₂ is weakly dependent on α as well as β . The calculated CMRO₂ changes herein were relatively independent for β ranging from 1 to 1.5 (data not shown). Thus, the values of these constants and the use of ROI analysis are unlikely to alter the overall conclusions of this study.

In addition, exchanging CBV with CBF in the CMRO₂ model assumes temporal synchrony of CBV and CBF, which may not be

valid. However, the relatively small CBV–CBF uncoupling in the time domain is unlikely to be an issue for studies with relatively low temporal resolution. Further improvements in Davis' CMRO₂ MRI technique can be made by validating the assumptions in the CMRO₂ formalism, demonstrating the reproducibility of the CMRO₂ maps, cross-validating with microPET-CMRO₂ measurements, experimentally determining α and β constants on a pixel-by-pixel basis in the rat brain, and pixel-by-pixel mapping of CBF, BOLD, M , and CMRO₂ at higher temporal and spatial resolution. Lastly, though not a problem with the model per se, using multiple echoes to quantify T₂ or T₂* relaxation time constants would allow a more objective assessment of the stability of the baseline BOLD signal in prolonged studies.

BOLD, CBF, and CMRO₂ under moderate gas challenges

During hypercapnia, absolute baseline CBF and BOLD increases were in agreement with previous studies (Liu et al., 2004; Sicard et al., 2003). Transient hyperoxia induced hypoventilation which resulted in mild increases in PaCO₂, CBF, and BOLD signals. This hyperoxia-induced elevation of PaCO₂ dominated the vasoconstrictive effects associated with chronic hyperoxia (Omae et al., 1998) and likely explains the difference between the results of this study and those in which a transient hyperoxic challenge was administered to mechanically ventilated rats in which PaCO₂ was artificially maintained at normocapnic levels (Piantadosi, 1999). Transient hypoxia induced hyperventilation which reduced PaCO₂ and thus decreased CBF, consistent with data obtained on spontaneously breathing, isoflurane-anesthetized (Weiss et al., 1983) or awake (Poulin et al., 2002) rats. The hypoxia-induced reduction of PaCO₂ dominated the commonly reported compensatory CBF increases (Shockley and LaManna, 1988) during inspiration of hypoxic gas in mechanically ventilated rats in which PaCO₂ was artificially controlled (Javaheri, 1986). Isoflurane-induced attenuation of the respiratory response to hypoxia (Hirshman et al., 1977; Sollevi and Lindahl, 1995) may have also contributed to baseline CBF findings under hypoxia. Another explanation for these fMRI signal changes is that baseline neural activity changed across the moderate gas conditions. Though our data cannot conclusively demonstrate otherwise, this is unlikely given that prior well-validated studies showed no or negligible changes in neural activity (Artru and Michenfelder, 1980; Kety and Schmidt, 1948) and metabolites (Emoto et al., 1988) under similar gas conditions.

One major finding of this study is that the absolute stimulus-induced CBF and BOLD changes were invariant across moderate gas perturbations whereas relative stimulus-evoked CBF and BOLD changes varied remarkably across these same gas perturbations. For example, though absolute stimulus-evoked CBF was virtually the same under 9% and 21% O₂, relative stimulus-evoked CBF changes were 35% and 24%, respectively. Thus, inter- or intra-subject differences in relative stimulus-evoked BOLD or CBF changes do not necessarily indicate differences in stimulus-evoked neural activity, but may rather be due to differences in baseline physiology. These findings suggest that caution must be exercised when interpreting relative fMRI signal changes between studies, subjects, or even within a subject. Knowledge of absolute baseline CBF and BOLD is critical for proper interpretation of relative stimulus-evoked fMRI signals.

However, the BOLD signal has limited utility for intra- or inter-study comparisons of basal physiology or stimulus-evoked

change. Since the fixed reference baseline BOLD (T₂*) signal is non-quantifiable and influenced by many biological and non-biological physical parameters (e.g., field strength), it could not be used to assess differences in basal cerebral physiology between studies. In essence, absolute CBF would be of greater utility for the above comparisons.

As mentioned, comparisons of fMRI signal changes across laboratories are generally difficult due to differences in animal preparation, field strength, MR parameters, and the nature of the stimulus, among other factors. Nonetheless, our results are in general agreement with those of prior forepaw stimulation studies. Mandeville et al. (1998) reported forepaw stimulation-induced BOLD increases of 2% at 2 T and 5% at 4.7 T. Duong et al. (2000) described stimulus-evoked CBF change of 98%, Liu et al. (2004) reported a blood flow change of 58%, and Silva et al. (1999, 2000) reported changes of 87–124%. Of note, Ureshi et al. (2004) observed smaller (20–40%) relative CBF changes than reported herein with a hindpaw stimulation paradigm.

Hypoxia, hyperoxia, and hypercapnia significantly modulated the absolute baseline CBF and BOLD signals. However, absolute baseline CMRO₂ remained invariant across moderate gas conditions (12% and 100% O₂, and 5% CO₂) relative to air. Furthermore, absolute stimulus-evoked CMRO₂ magnitude increases under these modulations were remarkably similar. Since it is well established that these moderate gas challenges do not change baseline CMRO₂, these data offer strong support for Davis' CMRO₂ model by demonstrating its internal consistency.

BOLD, CBF, and CMRO₂ under severe gas challenges

The absolute and relative stimulus-evoked CBF and BOLD changes under 10% CO₂ were essentially zero, consistent with Posse et al. (2001) who reported essentially no stimulus-evoked BOLD fMRI responses during relatively severe hypercapnia (end-tidal CO₂ = 70 mm Hg) in humans. The near-absence of absolute and relative stimulus-evoked fMRI responses during 10% CO₂ found herein was likely a consequence of marked perturbation of baseline vascular capacitance, namely, blood vessels might have reached near maximal dilation during baseline, preventing further increase during stimulation. The basal CBF during 10% CO₂ was 3.5 mL/g/min, similar to that during 15% CO₂ (data not shown), suggesting that basal CBF during 10–15% CO₂ is near maximum.

In principle, the reduced basal CBF during 9% O₂ hypoxia relative to air could be due to reduced metabolic demand and/or hypoxia-induced reduction of PaCO₂. As previously mentioned, most studies demonstrate that brief hypoxia does not alter energy metabolism and neuronal activity while others report only subtle changes in tissue carbohydrates and metabolites. However, the reduced baseline CMRO₂ during 9% O₂ observed herein supports a metabolic depression-mediated reduction in basal CBF. Interestingly, calculation of the baseline BOLD signal during 9% O₂ assuming no CMRO₂ change yielded a –9.2% BOLD change (relative to 21% O₂ baseline) whereas a –14% BOLD change was observed, supporting the alternate hypothesis of a PaCO₂-mediated reduction in basal CBF during hypoxia. Experiments using mechanical ventilation to maintain normal PaCO₂ during inspiration of hypoxic gas would quantitatively distinguish between metabolic- and PaCO₂-driven reductions in baseline CBF.

During 9% O₂ hypoxia, the absolute stimulus-evoked CBF and BOLD changes were small relative to that during air. This could be due to hypoxia-induced reduction in neuronal response to stimulation and/or hypoxia-induced attenuation of neurovascular coupling. The former is unlikely because the absolute stimulus-evoked CMRO₂ magnitude increase was comparable to that under air, whereas the latter possibility is supported by this and other (Hoffman et al., 1991; Strebel et al., 1995) studies.

Since hypercapnia does not significantly alter neural metabolism, stimulus-evoked CMRO₂ changes should be largely preserved. For this reason, the significant reduction in stimulus-evoked CMRO₂ response under the 10% CO₂ is interesting and unexpected. Though it cannot be ruled out that the 10% CO₂ challenge produced a true reduction of stimulus-evoked CMRO₂, it is conceivable that this finding is the result of a breakdown of the CMRO₂ model. Specifically, inhalation of 10% CO₂ produced near-maximum basal vasodilation (i.e., “ceiling effect”) that precluded substantial stimulus-evoked increases in CBF and BOLD which, in turn, may have led to an underestimated stimulus-evoked CMRO₂ change. This issue must be further investigated using a validated technique for measuring CMRO₂.

Additive versus proportional hypothesis

Two hypotheses have been proposed to account for the confounding effects of baseline CBF and BOLD signals on the stimulus-evoked fMRI responses. The additive hypothesis states that the stimulus-evoked fMRI signal change is constant and independent of the basal CBF or tissue oxygenation (Brown et al., 2003; Friston et al., 1990; Hoge et al., 1999; Li et al., 2000; Ramsay et al., 1993), resulting in a constant absolute fMRI signal change. The proportional hypothesis states that the stimulus-evoked fMRI change is proportional to the basal CBF or tissue oxygenation, resulting in a constant relative CBF or BOLD change (Cohen et al., 2002; Kemna and Posse, 2001; Shimosegawa et al., 1995). Recently, models describing a more complex dependence of BOLD on baseline CBF (Mulderink et al., 2002) and a “feed-forward” mechanism for neurovascular coupling (Uludag et al., 2003) have been proposed to account for the confounding effects of global CBF and BOLD signals on the stimulus-evoked fMRI responses. However, these previous studies measured only relative BOLD (Cohen et al., 2002; Friston et al., 1990; Kemna et al., 2001; Ramsay et al., 1993; Shimosegawa et al., 1995) and/or relative CBF (Hoge et al., 1999; Li et al., 2000) changes, and thus their conclusions were indirect. In this study, both absolute and relative stimulus-evoked CBF and BOLD signal changes were measured under multiple physiological perturbations in the same group of animals, conclusively demonstrating the validity of the additive hypothesis. Under stronger perturbations in baseline CBF (10% CO₂) and tissue oxygenation (9% O₂), neither the additive nor the proportional hypotheses appeared valid, likely because of large perturbations in vascular coupling and/or the nonlinear relationship between changes in BOLD and CBF predicted by the biophysical BOLD model during such conditions.

Implications to fMRI studies that measure relative CBF and BOLD changes

In human fMRI studies, it has been widely observed that some subjects show large BOLD percent-changes (good res-

ponders) while others show substantially weaker BOLD percent-changes (poor responders) to identical stimuli under identical experimental conditions. While many factors could account for this observation, one potential explanation, as suggested by Davis et al. (1998), is that there are inter-subject differences in baseline physiological parameters. Our results indicate that the relative stimulus-evoked fMRI responses are indeed critically dependent on baseline CBF and tissue oxygenation, and further suggest that the notion of good- versus poor-responders could be in part due to the limitation of measuring relative changes in fMRI signals.

These results also have important implications in fMRI of disease states that perturb basal CBF and/or cerebral neurovascular coupling, such as in stroke (Shen et al., 2004), Alzheimer’s disease (Niwa et al., 2000), diabetes (Rosengarten et al., 2004), aging (D’Esposito et al., 2003), as well as extra- and intracranial artery disease (Hamzei et al., 2003). For example, during stroke, oligemic and penumbral brain regions could experience mild to severe hypoxia, regional hypoperfusion to hyperemia/hyperperfusion, loss of neurovascular coupling, and/or various degrees of perturbed metabolism (Shen et al., 2004). These pathological conditions could markedly affect the baseline physiology and, thus, the relative stimulus-evoked fMRI signals. Another implication is in pharmacological fMRI. Following drug administration, for example, the baseline physiological state of the brain could be different regionally or globally due to drug-induced changes in respiration rate, blood pressure and/or volume, and vascular tone (Luo et al., 2003). These alterations, which are independent of the drug-induced changes in neural activity, could markedly affect the relative stimulus-evoked fMRI signals. Consequently, relative fMRI signal changes under pathological conditions and pharmacological challenges need to be interpreted with caution and failure to account for baseline physiological factors could lead to misinterpretation of the fMRI data derived from relative changes. Absolute measurements, as defined herein, are expected to be helpful in dissecting various physiological contributions to the fMRI signals, lending to an accurate measure of neural activity under physiologically perturbed conditions.

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