Forepaw-Stimulation CBF and BOLD Response Under Hypoxia, Hyperoxia and Hypercapnia

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Introduction The BOLD fMRI signal is critically dependent on the underlying physiologic conditions. Basal CBF (1,2) and basal arterial pO_2 are expected to modulate the magnitude of the stimulus-evoked BOLD responses. Two hypotheses have been postulated to explain these findings. The *proportional* hypothesis describes the functionally induced CBF change as being proportional to the basal CBF, resulting in a constant percent CBF change. The *additive* hypothesis describes the absolute CBF change as being constant and independent of the basal CBF. These hypotheses remain controversial and the quantitative extent to which the underlying physiology modulates the BOLD response is unknown. In this study, experiments were designed to test these hypotheses by systemically evaluating the functionally evoked BOLD and CBF fMRI response under hypoxic, hyperoxic and hypercapnic conditions. Forepaw stimulation in rats was performed during sustained exposure to different inhaled gases (9, 12, 21, 100%O_2, and 5, 10%CO_2) in order to modulate the baseline CBF and oxygenation saturation under carefully controlled conditions. This was made possible with a new forepaw stimulation model in isoflurane-anesthetized, spontaneously breathing rats because the animals could *autoregulate* their physiology and maintain it at a stable level over a prolong period of time (3). Both BOLD and CBF were measured (simultaneously) using the continuous arterial spin-labeling technique with multislice echo-planar-imaging acquisition.

Methods Five male SD rats (300-375g), anesthetized with 1.0% isoflurane throughout the study, breathed spontaneously without mechanical ventilation. Forepaw stimulation was performed under 5% and 10% CO_2 (21% O_2 with balanced N_2) and under 9, 12, 21 and 100% O_2 (balanced N_2). Both forepaws were stimulated simultaneously in series using optimized parameters (6mA with 0.3 ms pulse duration at 3 Hz, with electrodes placed under the skin) previously established under identical conditions in which no substantial stimulus-induced changes in MABP were observed (3). 15 mins break was given between different gas exposures.

Combined CBF and BOLD were measured using the continuous arterial spin-labeling technique with single-shot, gradient-echo, echo-planar-imaging (EPI) (4.7T). An actively decoupled surface coil was used for brain imaging and a neck coil for perfusion labeling. MR parameters were: data matrix=64x64, FOV=2.56x2.56cm^2, eight 1.5-mm slices, TE=15ms, and TR=2s. For each set of measurements, 30 pairs of images (2mins) were acquired during baseline, 30 pairs during stimulation, and 30 pairs during baseline. Anatomical images (128x128, RARE) were also acquired.

ROI analysis of the forepaw primary somatosensory cortices was performed. To avoid bias to any particular gas condition, all data in the same animal were averaged to generate an average activation map on which ROI's were drawn with reference to anatomical images as a guide. The percent-change time course for each gas condition was obtained without using the activation-map mask. Quantitative baseline CBF and stimulus-evoked percent changes in CBF and BOLD under each gas condition were evaluated.

Results & Discussions Representative CBF activation maps (obtained from averaging all gas conditions in one animal) showed robust bilateral activations in the forepaw primary somatosensory cortices, as expected (**Fig. 1)**. Quantitative CBF values during baseline and stimulation periods under different inhaled gas conditions are summarized in **Fig. 2**. Under basal conditions, CBF at 21%O_2 (air) was 0.93±0.07 ml/g/min (mean±SEM). Under 12 and 9%O_2, CBF decreased slightly relative to 21%O_2, consistent with the presence of hyperventilation which decreased blood-gas CO_2 and thus CBF (5). Under 100%O_2 (which induced mild hypoventilation), CBF was slightly higher relative to CBF under 21%O_2 although this did not reach statistical significance. At 5 and 10%CO_2, basal CBF markedly increased, as expected.

Following forepaw stimulation, the magnitude of quantitative CBF increases was essentially the same ($P>0.05$) for all gas conditions, except under 10%CO_2. These results support the *additive* hypothesis. Under 10%CO_2, a "ceiling effect" in the CBF response was observed (*i.e.*, small quantitative CBF increase given the already high basal CBF). **Fig. 3** shows the results expressed in percent changes (ΔCBF). Under 21% O_2, stimulus-evoked ΔCBF was ~60%. Stimulus-evoked ΔCBF was higher under 12% O_2 and even higher under 9%O_2but smaller under 100%O_2, as expected based on their respective basal CBF. Stimulus-evoked ΔCBF was reduced under 5%CO_2 and was essentially abolished under 10%CO_2.

Stimulus-evoked BOLD percent changes (ΔBOLD) are shown in **Fig. 4**. Stimulus-evoked ΔBOLD under 21%O_2 was 1.4±0.4%, similar to 100%O_2. Stimulus-evoked ΔBOLD response was slightly reduced under 5%CO_2, markedly reduced under 12 and 9%O_2, and was essentially abolished under 10%CO_2. The stimulus-evoked BOLD responses largely behaved as expected based on their respective basal CBF. Together, these results clearly disprove the *proportional* hypothesis because the stimulus-evoked CBF and BOLD percent changes were not constant (Fig. 3 and 4).

Fig. 1

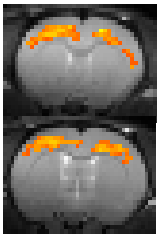


Fig. 2

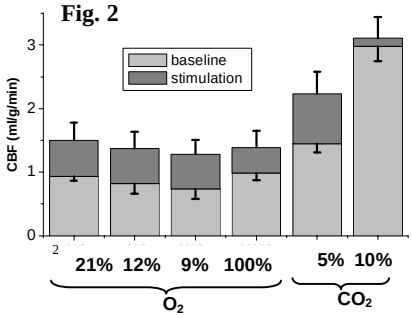


Fig.3

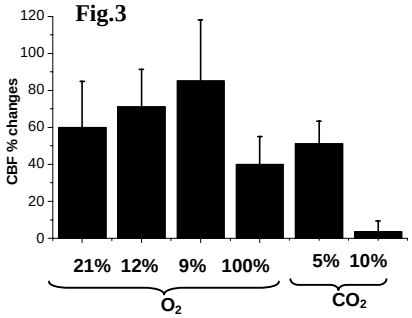
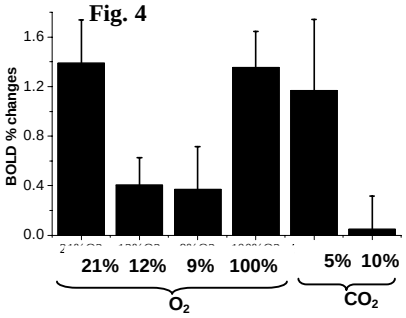


Fig. 4



Conclusions This study presents a systematic investigation of the quantitative CBF, relative CBF and BOLD changes in responses to forepaw stimulation under different basal O_2 and CO_2 conditions. Our data support the *additive* hypothesis which states that absolute CBF changes are constant and independent of the basal CBF for small deviations from normal physiological conditions. With large perturbations in CBF, such as under 10%CO_2, neither the additive nor the proportional model was valid.

References: 1) Kemna et al. NeuroImage 2001, 14:642. 2) Cohen et al. JCBFM 2002, 22:1042. 3) Liu et al. submitted, 2003. 4) Sicard et al., JCBFM 2003, 23:427. 5) Duong & Kim, MRM 2001, 45:61.