Dynamic Oxygen-Consumption Imaging of Forepaw Stimulation in Isoflurane-Anesthetized Rats

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Introduction Under normal and resting physiological conditions in the brain, essentially all (> 99%) the energy required for ATP production is supplied by oxidative metabolism, and the cerebral metabolic rate of oxygen ($CMRO_2$) is tightly coupled to cerebral blood flow (CBF) and cerebral metabolic rate of glucose ($CMR_{glucose}$). During task-induced increase in neuronal activity, CBF and $CMR_{glucose}$ increases had been consistently shown to be similar (~50%) (1,2). However, the magnitude of the stimulus-evoked $CMRO_2$ changes remains controversial. Stimulus-evoked $CMRO_2$ increases had been reported to be negligible (1, 3), substantial but smaller than the increases in CBF and $CMR_{glucose}$ (4-6), to significantly larger than CBF increase (200-400%) (7). In this study, we examined the feasibility of imaging oxygen consumption in association with forepaw stimulation in *spontaneously breathing* rats under *isoflurane anesthesia*. This new forepaw stimulation model has many advantages because the animals could maintain their own physiology at stable level for repeated measurements. *Simultaneous* measurements of BOLD and CBF were made using the continuous arterial spin-labeling technique with multislice echo-planar imaging acquisition. Stimulus-evoked $CMRO_2$ changes, calculated using Davis's biophysical BOLD model (4), were evaluated *dynamically* and *on a pixel-by-pixel basis* under graded forepaw stimulation currents.

Methods Six male SD rats (300-375g) were anesthetized with 2% isoflurane during placement of a femoral arterial catheter and needle electrodes under the forepaw skin. Isoflurane at 1% was used during imaging. Rats breathed spontaneously without mechanical ventilation. Respiration rate (RR), heart rate (HR), and mean arterial blood pressure (MABP) were recorded continuously. Blood gas was sampled once during imaging. Hypercapnia used 10% CO_2. Forepaw stimulation used 4, 6 and 8 mA with 0.3 ms pulse duration at 3 Hz. In 4 rats, left and right paws were stimulated separately. In 2 rats, both forepaws were stimulated simultaneously in series.

Combined CBF and BOLD measurements were made on a 4.7T Bruker scanner using the continuous arterial spin-labeling technique with single-shot, gradient-echo, echo-planar-imaging (EPI) acquisition. An actively decoupled surface coil (2.3-cm ID) 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 CBF measurements, 30 pairs of images (2mins) were acquired during baseline and 30 pairs during hypercapnic challenge or forepaw stimulation. Anatomical images (128x128, RARE) were also acquired.

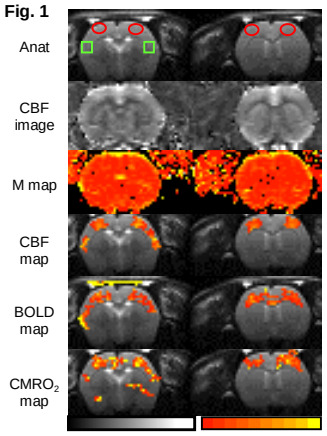
Cross-correlation analysis was used to obtain percent-change activation maps. $CMRO_2$ calculation used the biophysical BOLD model of Davis *et al.* (4). $CMRO_2$, CBF and BOLD signals are related by $\Delta BOLD/BOLD_o = M\{ 1-(CMRO_2/CMRO_{2|o})^{\beta} (CBF/CBF_o)^{\alpha-\beta} \}$ where parameters with subscript zero indicate baseline values. M maps were calculated from the hypercapnia data by setting $CMRO_2/CMRO_{2|0}$ to one since mild hypercapnia does not alter $CMRO_2$ (8). We used α of 0.38 (9) and β of 1.5 (4). To avoid bias to a particular current's activation map, group-average percent changes were evaluated using ROI analysis (without mask of activation map). ROIs enclosing the somatosensory cortices were drawn with reference to anatomical images and *average* maps of all currents only as a guide.

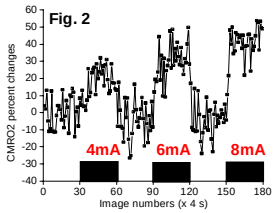
Results & Discussions At 4 mA, there were no statistically significant transient or sustained changes in MABP, HR and RR during stimulation relative to baseline. At 6mA, there were small transient changes in HR (P = 0.06) and MABP (<5mmHg, P=0.01) immediately following stimulation onset; however, there were not significant changes during the sustained stimulation period (P>0.05). At 8mA, there was a substantial transient increase in HR (20bpm, P<0.008) and MABP (20mmHg, P<0.008), which remained substantially elevated (15bpm and 10mmHg, P<0.008) during stimulation. Blood gases of all rats were within normal physiological ranges.

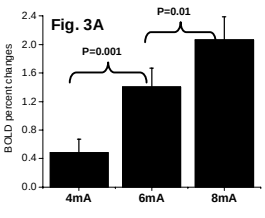
Representative (2 slices) anatomical images, CBF images, M maps, BOLD, CBF and $CMRO_2$ activation maps of one rat (6mA, 2 paws simultaneously stimulated) are shown in **Fig. 1**. M maps were heterogeneous with larger M values found around the lateral ventricles and the brain-skull interface where vascular density is high. In contrast to forepaw stimulation under α-chloralose, activities within the secondary and sub-cortical structures were usually detected in isoflurane-anesthetized animals. CBF, BOLD and $CMRO_2$ maps show robust bilateral activations. $CMRO_2$ time courses (concatenate of 3 currents from one rat) showed robust responses (**Fig. 2**). The group-average percent changes are summarized in **Fig. 3**. At 4, 6 and 8 mA, ΔBOLD were 0.5±0.2, 1.4±0.3, and 2.0±0.3% (mean±SD, n = 6), respectively; ΔCBF were 23±6, 58±9, and 87±14%, respectively; $\Delta CMRO_2$ were 14±4, 24±6, and 43±11%, respectively. The current that showed a robust response without substantial change in MABP (6mA) was ~3 times higher than those reported using α-chloralose (10), likely due to isoflurane being a potent anesthetic.

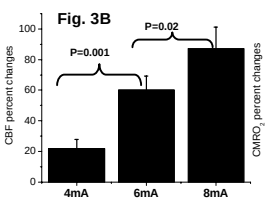
Correlation plots of BOLD *versus* CBF percent changes, and $CMRO_2$ *versus* CBF percent-changes at 4, 6 and 8 mA for individual animals are roughly linear (**Fig. 4**). Although data points of different stimulation currents from different animals on the scatterplots overlapped slightly, they were reasonably segregated with 4 mA data points clustered at smaller percent changes while 8 mA data points clustered at larger percent changes. Fractional changes in CBF and $CMRO_2$ coupled linearly with a ratio of 2.2:1, consistent with those reported previously (4,5). The magnitude of the $CMRO_2$ changes are consistent with previous studies that showed partial coupling of CBF and oxygen consumption changes (4-6) but inconsistent with those that showed little or no oxygen consumption changes (1,3) during increased neural activity.

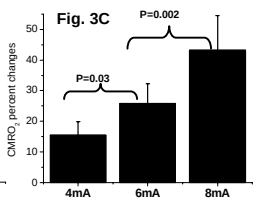
Conclusions $CMRO_2$ measurement was improved by simultaneously imaging CBF and BOLD and using the high-contrast continuous arterial spin-labeling technique. Isoflurane-anesthetized rat model for forepaw stimulation under spontaneous breathing yielded stable physiology over prolong period. Stimulus-evoked multi-slice $CMRO_2$ maps were dynamically acquired on a pixel-by-pixel basis. The optimal current was ~6 mA under 1% isoflurane which did not induce substantial change in MABP, HR and RR. The magnitude of the $CMRO_2$ changes is consistent with partial coupling of CBF and $CMRO_2$ during increased neuronal activity. $CMRO_2$ imaging is expected to have important applications in fMRI of disease states and pharmacological fMRI where baseline physiology is expected to be perturbed.

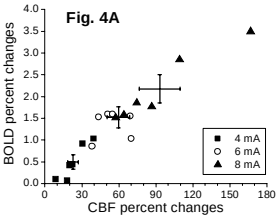


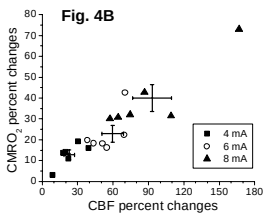












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