Dynamic compartment-specific cerebral blood volume and BOLD responses to electrical forepaw stimulation in α-chloralose anesthetized rats

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Introduction: Studying the compartment-specific dynamic cerebral blood volume responses is important for quantitative fMRI studies and understanding the dynamic properties of blood-oxygenation-level-dependent (BOLD) fMRI responses. MRI [1] and direct optical [2] measurements in isoflurane anesthetized rats show that the total CBV response is dominated by arterial dilation,while venous vessels do not change much during 15-s electrical forepaw stimulation. When relatively long stimulation duration (40-s) is applied in cat visual cortex [3], initial rapid arterial vasodilation is followed by slower prolonged venous dilation. Most of functional CBV MRI studies in animal models have been accomplished by α-chloralose anesthesia [4-7]. It is known that different anesthesia induces different baseline conditions of vessel physiology, which results in different functional signal changes [8]. Thus, the characteristics of CBV_a vs. CBV_v responses during neural activation under α-chloralose could be different with our previous findings under isoflurane anesthesia [3]. In this study, we measured BOLD, arterial, venous, and total cerebral blood volume responses (ΔCBV_a,ΔCBV_v, and ΔCBV_t) to electrical forepaw stimulation in α-chloralose anesthetized rats using a magnetization transfer (MT)-varied fMRI technique [9] and injection of paramagnetic contrast agent.

Methods and Materials: Six male Sprague-Dawley rats weighing 300-450 g were studied; All MRI experiments were performed on a 9.4 T magnet interfaced to a Unity INOVA console (Varian, Palo Alto, CA, USA). A 2-cm diameter surface coil was used. A single 2-mm thick coronal slice covering the primary somatosensory area (S1) was acquired with gradient-echo (GE) echo-planar imaging (EPI) with the following parameters: matrix=64×64, FOV = 2.3 × 2.0 cm^2, TE/TR = 20/1000 ms. For BOLD and ΔCBV_a measurements, each TR consists of 880 ms of off-resonance MT pulse with 5,000Hz offset, 20 ms delay, and 100 ms slice excitation and data acquisition. The power levels of the MT pulses were adjusted to achieve magnetization transfer ratios (MTR) of 0, ~0.5, and ~0.75 in the S1. For the measurement of stimulus-induced CBV_t change ($\Delta CBV_t/CBV_0$), FDA-approved susceptibility contrast agent Feraheme (AMAG Pharmaceuticals, Inc., MA) was injected intravenously with a concentration of 10 mg Fe/kg body weight. Images were acquired with the same imaging parameters except TE = 10.5 ms and without MT effect. Each fMRI trial consisted of consecutive 20 s pre-stimulation, 40 s forepaw stimulation, and 80 (for arterial CBV) or 120 s (for total CBV) post-stimulation periods, repeated approximately 20 times for signal averaging. For ΔCBV_a calculation, signal changes (ΔS_{MT}) at different MTR were normalized by the fully recovered signal (S_0) and $\Delta S_{MT}/S_0$ were linearly fitted against normalized mean baseline signals (S_{MT}/S_0) and the intercept at $S_{MT}/S_0 = 0$ were obtained. ΔCBV_a is then obtained by multiplying the intercept by the tissue-to-blood partition coefficient of 0.9 ml/g [10] and correcting for T_2^* differences between tissue and arterial blood [11]. $\Delta CBV_t/CBV_0$ was calculated as ($\Delta R_{2,sf}^*$ - $\Delta R_{2,s}^*$) /$\Delta R_{2,f}^*$, where $\Delta R_{2,s}^*$ and $\Delta R_{2,sf}^*$ are stimulus-induced R_2^* changes before and after Ferahem injection, respectively, and $\Delta R_{2,f}^*$ is the Feraheme-induced R_2^* changes at baseline. Venous CBV responses (ΔCBV_v) were calculated as the difference of the ΔCBV_t and ΔCBV_a time courses by assuming different CBV_0 values (see results). BOLD at the three MTR, ΔCBV_a, and $\Delta CBV_t/CBV_0$ amplitudes were calculated as their mean responses between the 5th and 40th time points after stimulus onset and their color maps were displayed for pixels with P<0.001 in the BOLD responses at all three MTRs and in the total CBV-weighted responses.

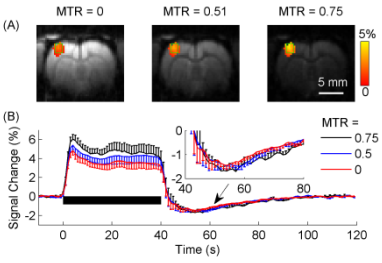

Figure 1: BOLD response amplitude (A) and group-averaged time courses (B) in the magnetization transfer (MT)-BOLD fMRI experiment for arterial cerebral blood volume changes (ΔCBV_a). (A) The green boxes denote the region of interest (ROI) in the primary somatosensory cortex. The black horizontal bar denotes the time period when the stimulus was on.

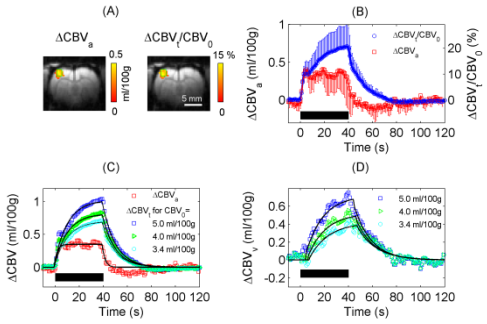

Figure 2: Maps of CBV_a and $\Delta CBV_t/CBV_0$ response amplitudes (A) and group averaged ΔCBV_a and $\Delta CBV_t/CBV_0$ (B) ΔCBV_a and ΔCBV_t (C) and ΔCBV_v (D) response time courses. Corresponding ΔCBV_v responses were obtained by subtracting the ΔCBV_a from the ΔCBV_t. The ΔCBV_t were calculated by assuming three possible values of baseline CBV (CBV_0) (3.4, 4, and 5 ml/100g tissue). The black lines are fits using a single exponential impulse response convoluted with a boxcar function.

Results and Discussion: Figure 1(A) shows maps of BOLD amplitude at the three MTR levels overlaid on corresponding baseline EPI images of one animal. Fig. 1(B) shows the group-averaged time courses (n = 6) of the S1 ROI. Baseline image intensities decreased with MTR, but percentage functional changes increased with MTR. During the post-stimulus period (see inset time courses), decreased BOLD signals below pre-stimulus baseline were clearly observed and were similar at three MTR levels in all animals. From these three time courses, ΔCBV_a time course was determined.

Figure 2 shows ΔCBV_a and $\Delta CBV_t/CBV_0$ maps and group-averaged ΔCBV_a, ΔCBV_v and ΔCBV_t ROI time courses. The dynamics of the ΔCBV_t time course agree well with the previous findings in rats under α-chloralose anesthetic condition [5, 7]. By assuming that the initial response of ΔCBV_t is dominantly due to the ΔCBV_a response, we obtained a CBV_0 value of 3.4±0.6 ml/100g. To investigate the effect of uncertainty in CBV_0, in Fig. 2D we also calculated ΔCBV_v assuming CBV_0 values of 4 and 5 ml/100g. The ΔCBV_v response is much slower than the ΔCBV_a response for all assumed CBV_0 values, consistent with our previous findings in isoflurane-anesthetized cats which showed initial rapid arterial vessel dilation, and later slower prolonged venous dilation [3]. However, the amplitudes of ΔCBV_a and ΔCBV_v in α-chloralose anesthetized rats are much higher than in isoflurane anesthetized cats: 0.33 ml/100g versus 0.18 ml/100g for ΔCBV_a and 0.36 − 0.69 ml/100g versus 0.13 − 0.25 ml/100g for ΔCBV_v.

Least-square fits to the group-averaged ΔCBV_a, ΔCBV_t, and ΔCBV_v responses were performed by convolution of boxcar with an exponential decay impulse response $f(t) = \exp\left(-\frac{t-t_0}{\tau}\right)h(t-t_0)$ (Figs. 2C and 2D). The response delay (t_0) was set to zero for fits to ΔCBV_a and ΔCBV_t (Fig. 2C). Since the ΔCBV_v response seemed to deviate from zero after several seconds from the stimulus onset, it was fitted by treating t_0 as a free parameter (black lines in Fig. 2D). The time constants for the ΔCBV_a, ΔCBV_t responses are 2.4 and 10.7 s, respectively. The time constants for the ΔCBV_v response are 14.2-15.8 s and $t_0 = 4.1 - 7.7$ s. The time constants and delays are in general agreement with earlier results under different anesthesia and stimulation conditions [3, 12, 13].

Conclusions: Out study found a fast ΔCBV_a response with a time constant of 2.4 s and a much slower ΔCBV_v response with a time constant of ~15 s and an onset delay of 4 – 8 s in rats under α-chloralose anesthesia. Our findings support the anesthesia independence of the fast CBV_a and slow CBV_v dynamic properties.

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