Insights into the Origin of Spontaneous Coherent BOLD fluctuations in a Resting Rat Brain under Varied Isoflurane Anesthesia Depth

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Introduction The coherent fluctuations of blood oxygen level dependent (BOLD) signals have been widely observed in many brain networks of different species at the resting state, and such coherence was hypothesized to result from the underlying anatomical and functional connectivity of brain networks. However, the real mechanism of this observation remained elusive, and even its neural origin is still in doubt[1]. In the previous study[2], the strong coupling has been observed between simultaneously-recorded electroencephalogram (EEG) and cerebral blood flow (CBF) signals from the isoflurane-anesthetized rat brain at the resting state; and moreover, the CBF signals recorded from the bilateral primary somatosensory cortex (S1FL) also show strong inter-hemispheric correlations. Given the well-known relationship of CBF and BOLD signals, those results strongly suggested the existence of coherent BOLD fluctuations in isoflurane-anesthetized rat brains and its neural origin. To further verify this hypothesis, the resting-state BOLD signals were acquired from isoflurane-anesthetized rat brain under three different anesthesia levels as applied in the previous study[2], and the spatiotemporal correlation analysis was performed to examine coherent BOLD fluctuations and its dependency on anesthesia levels. The results are then compared with those of the previous study to elucidate the correlations among the spontaneous EEG, BOLD and CBF fluctuations, and anesthesia depth in a resting brain.

Methods Six Sprague-Dawley rats were first anesthetized with ~2% (v/v) isoflurane in a mixture of O_2 and N_2O gases (2:3). Femoral artery and vein were catheterized for physiologic monitoring and/or blood sampling. All the experiments were performed on a 9.4T horizontal magnet (Magnex Scientific, UK) interfaced with a Varian INOVA console (Varian Inc., Palo Alto, CA). The multi-slice T_1-weighted anatomical images were acquired to identify the rat primary somatosensory cortex and appropriately choose locations of the fMRI image slices. Then, the gradient-echo planar image (GE-EPI) was used to acquire 5 consecutive coronal fMRI slices (FOV = 3×3 cm^2; TR/TE = 612/16.5 ms; 64×64 image matrix size; 1 mm thickness) covering the primary somatosensory cortex. All the BOLD signals were acquired when the rats were in uniform darkness which we regarded as the resting-state. For three rats, the acquisition was repeated for the light (~1.8% isoflurane), mild (~2.0% isoflurane) and deep (~2.2% isoflurane) anesthesia conditions, and only light and mild anesthesia conditions were conducted for the other three rats. For each anesthesia condition, the GE-EPI acquisition was repeated 2~4 runs, and each run included 500 image volumes. The GE-EPI signals were also acquired for 2~4 runs after the rats were sacrificed by KCl bolus injection. The time courses of all GE-EPI pixels were normalized by their means, and then band-pass filtered (0.005~0.1 Hz) in frequency domain to remove the DC component, the linear signal drift, and the high frequency noises. Then, a 2×2-pixel cluster was chosen as the reference region (the black square in Fig. 1) from the right S1FL region, which was similar to the locations of the EEG electrodes and LDF probes used in our previous study[2], whose time course was then extracted and cross-correlated with the time courses of all GE-EPI pixels to generate a correlation map for each run. Finally, the correlations maps under the same anesthesia condition were averaged across runs to give the final correlation map for that anesthesia condition.

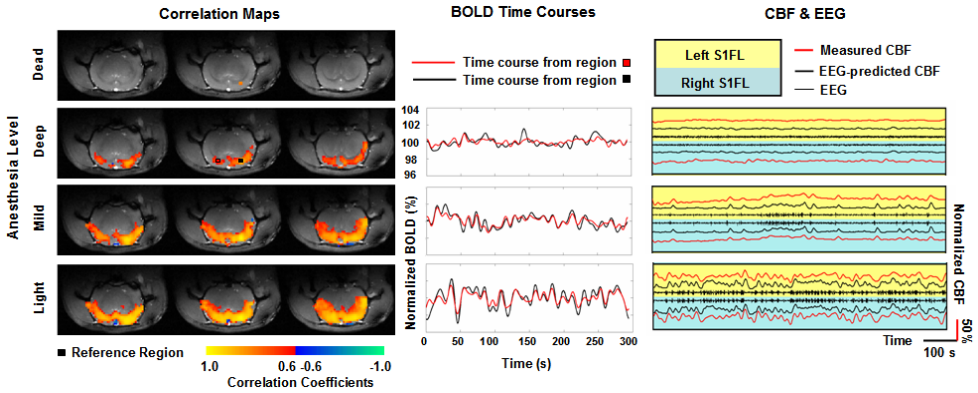

Fig. 1 Correlation maps (left column), BOLD time courses (middle column), and coupled CBF&EEG signals (right column[2])

Result The correlation maps for different anesthesia levels (left column in Fig. 1) clearly show a coherent resting-state network covering the S1HL, S1FL, S1DZ, S1BF, and S2 regions of the somatosensory cortex. Moreover, the coherence strength of this network decreased as the anesthesia level changing from mild to deep, and completely vanished when the rats were sacrificed. The BOLD time courses extracted from two ROIs in bilateral S1FL regions (middle column in Fig. 1) show that both the amplitude and the inter-hemispheric correlation of BOLD fluctuations decreased as the anesthesia depth increased, which is very similar to the change of CBF measured in the previous study[2] (illustrated in right column of Fig.1). To quantitatively compare coherent BOLD fluctuations observed in the present study with EEG-coupled CBF signals in the previous study, both the standard deviation (quantifying the amplitude of fluctuations) and inter-hemispherical correlation coefficients (quantifying the strength of coherence) of BOLD and CBF signals were calculated for each anesthesia condition and shown in Fig. 2. Both statistics of BOLD and CBF signals show very similar dependency on anesthesia levels.

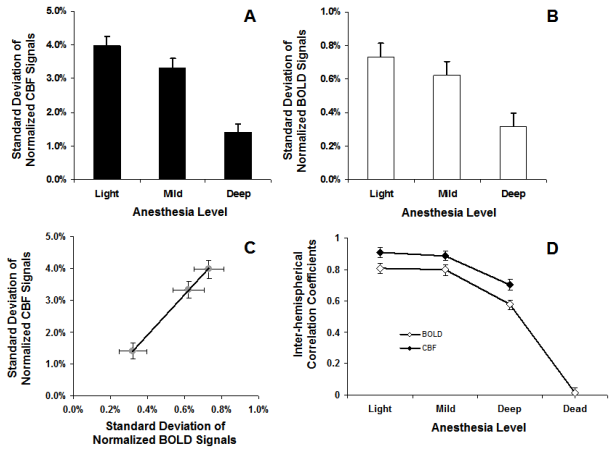

Fig. 2 Statistics quantifying the amplitude (A,B,C) and the inter-hemispherical correlation (D) of BOLD and CBF fluctuations. Error bars: standard errors.

Discussion and Conclusion Our results clearly demonstrate the existence of coherent BOLD fluctuations in the somatosensory system of isoflurane-anesthetized rats at rest, which is consistent with the former finding in isoflurane-anesthetized monkeys[3]. Moreover, a strong dependency of coherent BOLD fluctuations on anesthesia level was also observed within a critical range of ~1.8% to 2.2% isoflurane (Figs. 2A, 2B and 2C), which is different from the monkey study[3]. By comparing the temporal characteristics of BOLD signals and their anesthesia dependency with those of CBF signals measured in our previous study showing a strong coupling between spontaneous CBF and EEG signals, as well as based on the tight coupling relationship between BOLD and CBF signals as summarized by Fig. 2, we can draw an important conclusion: the spontaneous BOLD fluctuations observed in the present study result from the underlying CBF fluctuations which are driven by the spontaneous neuronal electrical activities in a resting brain. The results reveal a strong, temporal neuro-CBV-BOLD coupling relationship in the resting brain which provides essential mechanism of the resting brain connectivity.

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