Electrophysiological Investigation of the Basis of the Resting-State fMRI Signal

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Introduction Resting-state fMRI has been applied to study functional connectivity in large-scale neuronal networks of both awake human and anesthetized nonhuman primates (1,2). Changes in functional connectivity under a variety of pathological conditions have been reported (e.g. 3,4). These studies collectively suggest that, rather than simple physiological artifacts induced by cardiac pulsations or respiration as was originally suspected, these precisely patterned spontaneous fluctuations have a neural basis (5). Nevertheless, the linkage between neuronal activity and resting-state fMRI signal remains largely unknown, underscoring the critical need for animal models to investigate this phenomenon.

In previous studies using CBV-weighted fMRI, we observed coherent spontaneous fluctuations in bilateral primary somatosensory cortices (S1FLs) of α-chloralose anesthetized rats (5,6), which were subject to anesthetic dose modulation. Several labs also reported functional connectivity in different rat brain systems (7-10). The purpose of the present study was to investigate the neuronal correlates of fluctuations in the resting-state fMRI signal. We employed an anesthesia dose-modulation strategy, and hypothesized that the underlying electrophysiological signal driving interhemispheric synchronized spontaneous fluctuations within bilateral S1FL would have the same dose-dependent pattern as the resting-state fMRI signal, and would uniquely distinguish it from that between the S1FL and a control site.

Methods fMRI experiments were conducted on a Bruker 9.4T scanner. Superparamagnetic contrast agent Ferumoxtran-10 (Advanced Magnetics, Inc, MA) was administered (15 mg/kg, I.V.) to achieve CBV contrast to enhancing fMRI signal sensitivity and functional specificity. Animal preparation was similar to our previous report (11), and was approved by the Animal Care and Use Committee of the National Institute on Drug Abuse. Briefly, rats (n = 6) were artificially ventilated under α-chloralose anesthesia. The anesthesia level was systematically modulated by changing α-chloralose doses: animals received a loading dose (80 mg/kg, I.V.), continuous infusion (30 mg/kg) was initiated 30 min after the loading dose. The second (70 mg/kg) and third (100 mg/kg) doses of α-chloralose were administered manually (I.V.), at a time interval of 50 min between injections. Image acquisition started 15 min after the beginning of the 30 mg/kg infusion, and the 70 and 100 mg/kg bolus injections, respectively. Scan parameters were: FOV = 3.5 × 3.5 cm^2, matrix size = 64×64, TE = 15 ms, TR = 1426 ms, 7 slices with thickness of 1.5 mm. A total of 270 volumes were collected in 385 sec. Data were low-pass filtered (f < 0.1 Hz), and were analyzed at a group-level using AFNI with the seed-point analysis strategy. Electrophysiological experiments were conducted on a separate group of rats (n=8) following a design parallel to the MRI experiments. Two ball-shaped Ag/AgCl recording electrodes were stereotaxically placed on the intact dura at the center of bilateral S1FL; a third electrode was placed on the visual cortex to serve as a control site. EEG signal was amplified (1000 X), band-pass (0.3-1000 Hz) and notch filtered at 60 Hz, and digitally sampled at 2000 Hz. In one animal, EEG signal was band-pass filtered at 0.3-10 kHz, and sampled at 32 kHz. Results were very similar. EEG data were analyzed in MATLAB and EEGLAB. Time–frequency analysis within individual frequency bands (δ: 1-4Hz; θ: 5-8Hz; α: 9-14Hz; β: 15-30Hz; γ: 30-50Hz; γ_h: 50-100Hz) was conducted using Thomson's multitaper analysis method.

Results Figure 1 depicts the effect of α-chloralose dose on resting-state functional connectivity. Figure 1A is a 3D representation of color-coded group t-statistical maps thresholded at p<0.025. The seed voxels were chosen from the left hemisphere. Functional connectivity within the left hemisphere persisted during all three doses of α-chloralose; while the connectivity within the right hemisphere decreased as the anesthesia dose increased. Interhemispheric synchrony of the spontaneous fluctuations was significantly modulated by α-chloralose as shown in Fig. 1B.

Figure 2 shows statistical comparisons of power correlations between electrode pairs and anesthesia levels. For the delta band, at 30 mg/kg α-chloralose, the power correlation between the left and right S1FL electrodes (LF, RF) was significantly higher than at the two higher doses (Wilcoxon signed-ranks test, p < 0.04). No significant dose difference was found between either the LF or the RF electrode and the right visual cortex (RV). Among electrode pairs, the differences were most pronounced at 30 mg/kg, where the power correlation between LF-RF electrodes were greater than those between LF-RV and between RF-RV pairs (Wilcoxon signed-ranks test, p<0.05). Other bands showed little or no dose-dependency or region-specificity. Figure 3 illustrates anesthetic dose modulations of the δ-band EEG power and resting-state fMRI signal in bilateral S1FL. Similar patterns of anesthetic dose modulation on these two signal types were observed.

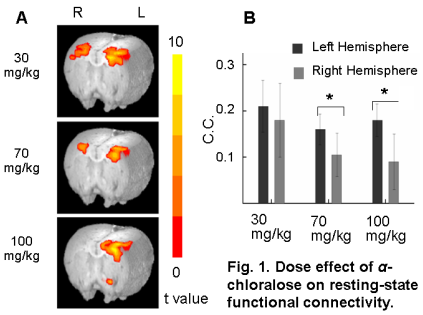

Fig. 1. Dose effect of α-chloralose on resting-state functional connectivity.

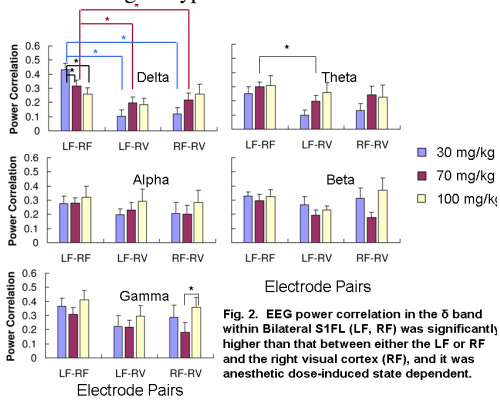

Fig. 2. EEG power correlation in the δ band within Bilateral S1FL (LF, RF) was significantly higher than that between either the LF or RF and the right visual cortex (RV), and it was anesthetic dose-induced state dependent.

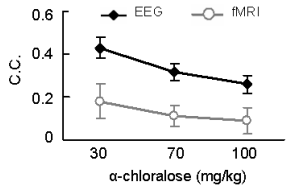

Fig. 3. Similar patterns of anesthetic dose modulation on δ-band EEG power and resting-state fMRI signals in bilateral S1FL.

Discussion By employing CBV-weighted fMRI to enhance both sensitivity and functional specificity, we detected resting-state functional connectivity in bilateral S1FL, which was subject to anesthetic dose modulations. We further demonstrated that only the delta band EEG component from bilateral S1FL was modulated in an anesthetic dose-dependent fashion, which uniquely differentiated it from the EEG components in other bands as well as the EEG signal from a control site. Such region-specificity and anesthetic dose-dependency strongly suggest that the synchronized delta oscillations underlie/correspond to the resting-state fMRI signal.

References 1. Biswal BB et al, *MRM* 1994;34:537-41. 2.Vincent JL et al., *Nature 2007;*447:83-6. 3. Li S-J et al., *Radiology* 225:253-9. 4. Lowe MJ et al., *Radiology* 2002; 224:184-92. 5. Leopold DA et al., *Cereb Cortex* 2003;13:422-33. 6. Lu H et al., *ISMRM* 2006; p.532. 7. Lu H et al., *ISMRM* 2007; p.3212. 8.Williams KA et al., *ISMRM* 2006; p. 2119. 9. Zhao F. et al. *ISMRM* 2007; p. 1980. 10. Kim YR et al. *ISMRM* 2007; p. 70. 11. Lu H et al. *MRM* 2004;52:1060-8.