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Coherent slow cortical potentials reveal a superior localization of resting-state functional connectivity using voltage-sensitive dye imaging

Bing Li [1], Rui Liu [1], Qin Huang, Jinling Lu, Qingming Luo, Pengcheng Li *

Britton Chance Center of Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan 430074, PR China
Key Laboratory of Biomedical Photonics of Ministry of Education, Department of Biomedical Engineering, Huazhong University of Science and Technology, 1037 Luoyu Rd., Wuhan 430074, PR China

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ABSTRACT

The resting-state functional connectivity (RSFC) of spontaneous hemodynamic fluctuations is widely used to investigate large-scale functional brain networks based on neurovascular mechanisms. However, high-resolution RSFC networks based on neural activity have not been disclosed to explore the neural basis of these spontaneous hemodynamic signals. The present study examines the neural RSFC networks in mice at high spatial resolution using optical imaging with voltage-sensitive dyes (VSDs). Our results show that neural RSFC networks for the slow cortical potentials (0.1–4 Hz) showed similar correlation patterns to the RSFC networks for the spontaneous hemodynamic signals, indicating a tight coupling between the slow cortical potential and the spontaneous hemodynamic signals during rest, but the bilateral symmetry of the RSFC networks for the slow cortical potentials was significantly lower than that for the spontaneous hemodynamic signals. Moreover, similar asymmetric neural activation patterns could also be found between the bilateral cortexes after stimulating the paws of mice. By increasing anesthetic levels to induce the reduction of consciousness, the RSFC networks for the slow cortical potentials persisted, but those for the spontaneous hemodynamic signals became discrete. These results suggest that the coherent slow cortical potentials underlie the spontaneous hemodynamic fluctuations and reveal a superior localization of RSFC networks. VSD imaging may potentially be used to examine the RSFC of neural activity, particularly under conditions of impaired neurovascular coupling.

Introduction

Coherent spontaneous low-frequency (<0.1 Hz) fluctuations in blood–oxygen-level-dependent signals that can be detected using functional magnetic resonance imaging (fMRI) have been shown to reflect resting-state functional connectivity (RSFC) networks (Biswal et al., 1995; Fox and Raichle, 2007) and closely resemble the signals observed during the performance of functional tasks (Fox et al., 2007; Vincent et al., 2007; Zhang et al., 2013). Experimental data suggest that there is a close relationship between functional connectivity and anatomical connections (Vincent et al., 2007; Wang et al., 2013). Interestingly, RSFC integrity appears to be essential for the development and maintenance of normal brain functions (Greicius, 2008; He et al., 2007; Pizoli et al., 2011), and changes in these networks are often observed in neuropsychiatric disorders, such as schizophrenia (Liang et al., 2006; Lynall et al., 2010; Shen et al., 2010; Zhou et al., 2008), Alzheimer's disease (Sorg et al., 2007; Wang et al., 2006, 2007) and depression (Greicius et al., 2007; Liu et al., 2012; Sheline et al., 2010; Zeng et al., 2012).

There has been a growing interest in investigating the neural substrates of these spontaneous hemodynamic fluctuations using a variety of electrophysiological signals, including local field potential (Nir et al., 2008; Pan et al., 2013; Scholvinck et al., 2010), electrocorticography (He et al., 2008; Nir et al., 2008), electroencephalography (Lu et al., 2007) and magnetoencephalography (de Pasquale et al., 2010; Hipp et al., 2012). Raw filtered neural signals in different frequency bands (He et al., 2008; Pan et al., 2013) and band-limited power of neural signals (de Pasquale et al., 2010; He et al., 2008; Hipp et al., 2012; Lu et al., 2007; Nir et al., 2008; Pan et al., 2013; Scholvinck et al., 2010) have been used to be correlated with the spontaneous hemodynamic fluctuations. However, electrophysiological signals were reported to be possibly affected by the positions where the electrodes were implanted in experiments (Lu et al., 2007; Pan et al., 2013). Moreover, electrophysiological signals from an electrode reflected a weighted spatial average of electrical brain activity (Hipp et al., 2012; Lu et al., 2007; Pan et al., 2013; Scholvinck et al., 2010), and their spatial resolution was limited. Therefore, there is a clear need to measure the RSFC of spontaneous neural activity at high spatial resolution without the use of electrodes to explore the neural basis of the spontaneous hemodynamic fluctuations. Optical imaging using voltage-sensitive dyes (VSDs) has been used to record electrophysiological local field potentials at subcolumnar spatial resolution within large cortical areas (Ferezou et al., 2006; Ma et al., 2012; Mohajerani et al., 2011; Petersen et al., 2003; Scanziani and Hausser,

* Corresponding author at: Britton Chance Center of Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan 430074, PR China. Fax: +86 27 87792034.
E-mail address: pengchengli@mail.hust.edu.cn (P. Li).
[1] These authors contributed equally to this work.

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2009), and it should be possible to use this technology to measure the RSFC networks of spontaneous neural activity and compare them with the networks of the spontaneous hemodynamic signals.

The effects of anesthesia on RSFC networks remain uncertain. Some results suggest that RSFC networks based on hemodynamic signals would be reduced under conditions of deep anesthesia or sleep (Boveroux et al., 2010; Lu et al., 2007), while others show that RSFC networks are maintained under the similar conditions (Horovitz et al., 2008; Larson-Prior et al., 2009). Therefore, the effects of anesthesia on RSFC networks need to be further investigated.

To address these issues, RSFC networks of spontaneous neural activity were visualized using VSD imaging in this work and compared with RSFC networks of spontaneous hemodynamic signals that were visualized using optical intrinsic signal (OIS) imaging. Although OIS imaging has a similar hemodynamic contrast as fMRI, it has the advantage of higher spatial resolution (Bero et al., 2012; Li et al., 2012; White et al., 2011). Lastly, RSFC networks based on both neural activity and hemodynamic signals were investigated under different levels of anesthesia to explore the effects of anesthesia on cortical functional connectivity.

Material and methods

Animal model

A total of 18 adult, male C57BL/6 mice were used in this study. All animal protocols were approved by the Committee for the Care and Use of Laboratory Animals at Huazhong University of Science and Technology. Mice were anesthetized with isoflurane (2% for induction and 1% for surgery) and body temperature was maintained at 37 ± 0.5 °C using a heating pad.

Voltage-sensitive dye imaging

Each mouse was placed in a stereotaxic apparatus, and cranial windows of ~7 × 8 mm (bregma 2.5 to −4.5 mm and lateral 0–4 mm) were opened over the bilateral hemispheres. The dura was carefully removed and the exposed cortex was bathed in VSD RH1691 (optical imaging) dissolved in Hepes-buffered artificial cerebrospinal fluid (ACSF) for 90–120 min. Following dye incubation, the cortex was thoroughly washed, covered with Hepes-buffered ACSF and sealed with a glass coverslip. VSD signals were excited with a red light-emitting diode (627 nm center with a 630/20 nm filter; Luxeon). Images were captured using an Electron Multiplying CCD (EMCCD) camera (14 bit; iXon3; Andor Technology PLC) and a bandpass filter (692 ± 20 nm; Semrock) was placed in the camera lens light path to minimize the contribution of concurrent hemodynamic signals in the VSD images. For the RSFC analyses ($n = 6$), VSD imaging was performed over a period of 180 s to ensure the emergence of stable functional networks (Chu et al., 2012); the sampling rate was 100 Hz. To assess functional activity following hindlimb stimulation ($n = 6$), two needle electrodes were inserted parallel to the hindlimb. Single electrical pulses of 1 mA for 1 ms were used, and the interstimulus interval between electrical stimulations was 30 s. Imaging was performed under 0.5% isoflurane unless otherwise noted.

Optical intrinsic signal imaging

Spontaneous hemodynamic fluctuations were recorded using OIS imaging as described previously (Li et al., 2012). Briefly, the skull of each mouse ($n = 6$) was bilaterally exposed (~7 × 8 mm; bregma 2.5 to −4.5 mm and lateral 0–4 mm) and thinned to ~50% of its original thickness using a high-speed dental drill (Fine Science Tools, USA) under constant saline cooling and then covered with a thin layer of mineral oil to prevent drying. The cortical surface was then illuminated using a halogen lamp (Olympus LG-PS2, Japan). A 12-bit CCD camera (Pixelfly VGA, PCO Computer Optics, Germany) attached to a stereomicroscope (Olympus SZ6045TR Zoom, Japan) was used to acquire the images. Image acquisition was performed by switching the center wavelengths using a Liquid Crystal Tunable Filter (LCTF; VariSpec, Cambridge Research & Instrumentation, USA). The center wavelength of the LCTF used in our system could be varied from 400 to 720 nm in 1-nm increments with a bandwidth of 7 nm. OIS imaging was performed at a sampling rate of 1 Hz for 10 min to acquire two image sequences at 625 and 570 nm in a sequential manner.

Transitions between anesthesia levels

To investigate the effects of anesthesia strength on RSFC, three levels of anesthesia (0.5%, 1% and 1.5% isoflurane) were used during OIS and VSD imaging. The anesthesia levels were changed in a randomized manner for each mouse, and an interval of 15 min was allowed for each transition from one isoflurane level to another (Wang et al., 2010).

Analysis of neural signals

A brain mask was created by excluding non-cortex areas to include only the pixels that represented the brain (Bero et al., 2012). The VSD image sequences acquired for RSFC analysis were band-passed filtered into five frequency bands: 0.1–4 Hz (delta), 4–8 Hz (theta), 8–15 Hz (alpha), 15–30 Hz (beta) and 30–40 Hz (low gamma). Further preprocessing steps included spatial smoothing (median filter, 5 × 5) and a time-series linear detrending. The VSD signals in each frequency band, not the band-limited power/amplitude, were used to compute the RSFC maps. RSFC maps were obtained using a seed-based correlation method by calculating the Pearson's correlation coefficient between the time course of the seed region and every other pixel. The correlation coefficients were transformed using Fisher's z transformation for variance stabilization. All seed regions were selected using a mouse brain atlas (Franklin and Paxinos, 2007). Averaged RSFC maps were generated across all mice in the same group and displayed by setting the correlation coefficient threshold to 0.1.

For the sensory stimulation experiments, 8 trials were averaged separately for both the left and right hindlimbs of each mouse. The VSD responses to stimulation were calculated as the normalized difference relative to the average baseline recorded prior to stimulation ($\Delta F/F_0$).

Analysis of hemodynamic signals

The OIS image sequences were converted to changes in hemoglobin concentrations (oxy-hemoglobin and deoxy-hemoglobin) for every pixel and time point, according to the Lambert–Beer law (Li et al., 2012; Sun et al., 2011). RSFC maps based on oxy-hemoglobin were reported to show greater similarity in their connectivity patterns than those based on deoxy-hemoglobin (Bero et al., 2012; Lu et al., 2010). After spatial smoothing (median filter, 5 × 5), band-pass filtering (0.009–0.08 Hz) and time-series linear detrending, RSFC analyses were performed using the hemodynamic contrast for oxy-hemoglobin as previously described (Li et al., 2012). Consistent with the previous results reported with OIS imaging (Bero et al., 2012; Li et al., 2012; White et al., 2011), both positive correlations and negative correlations were observed in this work. Moreover, the negative correlations were also observed in VSD imaging. But in the present study, we focused on the positive correlations and RSFC maps were displayed by setting the correlation coefficient threshold to 0.1, and the negative correlations were not present.

Comparing the RSFC networks of neural and hemodynamic signals

The similarity between the RSFC maps derived for neural activity and those derived for hemodynamic signals was characterized as