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Mapping functional connectivity based on synchronized CMRO₂ fluctuations during the resting state

Changwei W. Wu^{a,b}, Hong Gu^a, Hanbing Lu^a, Elliot A. Stein^a, Jyh-Horng Chen^{b,*}, Yihong Yang^{a,*}

^a Neuroimaging Research Branch, National Institute on Drug Abuse, NIH, Baltimore, MD, USA

^b Interdisciplinary MRI/MRS Lab, Department of Electrical Engineering, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Article history:

Received 16 October 2008

Revised 26 November 2008

Accepted 23 December 2008

Available online xxxx

Keywords:

Functional connectivity

BOLD

Perfusion

CMRO₂

ABSTRACT

Synchronized low-frequency fluctuations in the resting state functional MRI (fMRI) signal have been suggested to be associated with functional connectivity in brain networks. However, the underlying mechanism of this connectivity is still poorly understood, with the synchronized fluctuations could either originate from hemodynamic oscillations or represent true neuronal signaling. To better interpret the resting signal, in the current work, we examined spontaneous fluctuations at the level of cerebral metabolic rate of oxygenation (CMRO₂), an index reflecting regional oxygen consumption and metabolism, and thus less sensitive to vascular dynamics. The CMRO₂ signal was obtained based on a biophysical model with data acquired from simultaneous blood oxygenation level dependent (BOLD) and perfusion signals. CMRO₂-based functional connectivity maps were generated in three brain networks: visual, default-mode, and hippocampus. Experiments were performed on twelve healthy participants during 'resting state' and as a comparison, with a visual task. CMRO₂ signals in each of the abovementioned brain networks showed significant correlations. Functional connectivity maps from the CMRO₂ signal are, in general, similar to those from BOLD and perfusion. In addition, we demonstrated that the three parameters (M , α and β) in the biophysical model for calculating CMRO₂ have negligible effects on the determination of the CMRO₂-based connectivity strength. This study provides evidence that the spontaneous fluctuations in fMRI at rest likely originate from dynamic changes of cerebral metabolism reflecting neuronal activity.

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Introduction

How functional networks unfold during neural activity has been of long standing interest in contemporary neuroscience. Most of our knowledge about brain networks comes from imaging studies with task manipulations, neglecting the endogenous brain fluctuations during the resting state (Hubel and Wiesel, 1962; Shadlen and Newsome, 1998). However, the human brain consumes approximately 20% of the total body energy to support intrinsic brain activity, while the increase in metabolic demand induced by tasks above the "resting level" are relatively small (<5%) (Attwell and Laughlin, 2001; Raichle and Mintun, 2006; Shulman et al., 2004). Such observations suggest that intrinsic brain activity may reflect an essential, fundamental brain function, including communication and coordination among functionally-related brain regions. In support of this conjecture, recent

studies observed synchronized low-frequency (<0.1 Hz) fluctuations between brain regions during the resting state using blood oxygen level dependent (BOLD)-weighted functional magnetic resonance imaging (fMRI) (Biswal et al., 1995; Greicius et al., 2003; Lowe et al., 1998). This "functional connectivity" phenomenon is robustly detectable among subjects and even across species (Damoiseaux et al., 2006; Lu et al., 2007; Vincent et al., 2007). With its high spatial resolution and the capability of mapping functional connectivity without engaging cognitive tasks, resting-state fMRI has made significant advances to both basic cognitive neuroscience and clinical disorders (Fair et al., 2008; Greicius et al., 2007; Wang et al., 2006; Zhou et al., 2008). Nevertheless, the underlying mechanisms of these spontaneous fluctuations have yet to be fully clarified, limiting its applicability in understanding basic neuronal processing mechanisms.

BOLD has been the choice for the majority of functional connectivity studies, probably due to its relatively high sensitivity. However, since the BOLD signal is an interplay of multiple hemodynamic and metabolic parameters such as cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral metabolic rate of oxygen (CMRO₂) (Kim et al., 1999; Kim and Tsekos, 1997; Mandeville et al., 2001), it would be desirable to assess the origin of functional connectivity using signals more closely related to neuronal activity. Biswal et al. observed CBF-based functional connectivity of the motor

* Corresponding authors. Y. Yang is to be contacted at the Neuroimaging Research Branch, National Institute on Drug Abuse, NIH, 251 Bayview Blvd., Suite 200, Room 7A709, Baltimore, MD 21224, USA. Fax: +1 443 740 2734. J.-H. Chen, Room 706, Ming-Da Building, Department of Electrical Engineering, National Taiwan University, Sec. 4, No. 1, Roosevelt Rd., Taipei 106, Taiwan. Fax: +886 2 2364 8238.

E-mail addresses: jhchen@ntu.edu.tw (J.-H. Chen), yihongyang@intr.nida.nih.gov (Y. Yang).

network using both BOLD- and arterial spin labeling- (ASL-) fMRI techniques in human subjects (Biswal et al., 1997). Chuang et al. improved the CBF-based technique for functional connectivity by minimizing the BOLD contamination in the ASL signal (Chuang et al., 2008). Fukunaga et al. reported that resting-state activity may partially represent a metabolic process by analyzing the simultaneously measured BOLD/perfusion weighted signals during visual stimulation, hypercapnia and resting states (Fukunaga et al., 2008). However, to date there is no direct observation of the relationship of the metabolic signals (e.g. CMRO₂) between functionally-related brain regions during the resting state.

In the present study, we investigate temporal correlation of CMRO₂ signals in functionally related brain areas and the spatial distribution of functional connectivity generated from the CMRO₂-based correlation. Simultaneous BOLD/perfusion acquisitions were conducted during both visual stimulation and resting state, and voxel-wise CMRO₂ signals and CMRO₂-based connectivity maps were generated using a biophysical model (Hoge et al., 1999). Spatial patterns of BOLD-, perfusion- and CMRO₂-based connectivity maps were compared in three well known networks: visual, default-mode and hippocampal.

Methods

Participants

Twelve healthy subjects participated in this study (4 males, age: 27 ± 7 years). They were screened with a questionnaire to ensure no history of neurological illness, psychiatric disorders or past drug usage. Present drug use and pregnancy were assessed with urine testing. Informed consent was obtained from all participants prior to the experiments in accordance with the protocol approved by the Institutional Review Board of the National Institute on Drug Abuse.

MRI experiments

The experiments were conducted on a 3.0 T Siemens Allegra scanner (Siemens, Erlangen, Germany) using a head volume coil. A pulsed arterial spin labeling (PASL) sequence based on a flow-sensitive alternating inversion recovery (FAIR) echo planar imaging (EPI) method was adopted for functional scans. Arterial blood was labeled with alternating slice-selective inversion (label) and non-slice-selective inversion (control, NSIR) scans. Imaging parameters of the PASL sequence were: inversion time (TI)=1400 ms, repetition time (TR)=2000 ms, echo time (TE)=28 ms, flip angle=90° and bandwidth=4112 Hz/pixel. Ten oblique imaging slices (220 × 220 mm² field of view, 64 × 64 in-plane matrix size, and 6 mm slice thickness) were aligned along the anterior commissure (AC)–posterior commissure (PC), allowing for coverage of the entire visual cortex and a large part of the default-mode and hippocampal networks. The slab thickness for the NSIR scans was about 1.4 times the imaging slab (84 mm thickness in total).

Head motion was minimized using individually custom-made foam padding. At the beginning of the resting scan, subjects were instructed to rest with their eyes closed, not to think of anything in particular, and not to fall asleep during the acquisition period. Twelve minutes of continuous fMRI data were acquired for each subject, corresponding to 360 measurements. After the resting scan, visual stimuli were back-projected on a screen inside the scanner using an LCD projector for the following visual-stimulation experiment. Subjects passively viewed a full field, black and white circular checkerboard pattern flashing at 8 Hz during the “stimulation” period and viewed a static cross pattern at the center of the screen during the “resting” period. The block design paradigm started with a 38 s resting period (including dummy scans of 6 s), followed by five cycles of 32 s stimulation and 32 s resting periods. Each run lasted for 358 s, corresponding to 176 measurements.

After the functional scans, a series of non-slice-selective inversion recovery (NSIR) EPI images were acquired at 19 different TIs (30 80 130 180 230 330 430 530 630 730 830 1030 1230 1530 1830 2230 2730 3230 3830 ms) in the same locations as for the functional scans for structural segmentation in the following processing. Regarding the spatial normalization and localization, a set of high-resolution T₁-weighted anatomical images (3D-MPRAGE with 256 × 192 × 160 matrix size; 1 × 1 × 1 mm³ in-plane resolution; TI=1000 ms; TR/TE=2500/4.38 ms; flip angle=8°) was acquired on each subject.

Pre-processing

Functional PASL data were processed using the Analysis of Functional Neuroimaging (AFNI) software package (Cox, 1996). Motion correction was performed by volume registering each 3D volume to a base volume. Linear detrending was applied to eliminate signal drift induced by system instability. Subsequently, the PASL data were processed with a filtering strategy for separating BOLD and perfusion signals (Chuang et al., 2008). The PASL dataset underwent a low-pass filtering (<0.125 Hz, corresponding to $\frac{1}{4TR}$) for BOLD signal and a high-pass filtering (>0.125 Hz) for perfusion signal, respectively, using Chebyshev type II filters in MATLAB (MathWorks, Inc., CA). Both low-passed and high-passed data were then converted into Talairach space and linearly resampled to an isotropic resolution (3 × 3 × 3 mm³). Spatial smoothing was also applied using a Gaussian isotropic kernel (full width at half maximum of 6 mm) to minimize individual variances and enhance signal-to-noise ratio (SNR). After these processing procedures, the label and control (odd and even) images of the low-pass filtered dataset were summed together to form BOLD signal for the following analyses. The high-pass filtered dataset was demodulated by multiplying $\cos(\pi n)$ (n is the scan number) (Chuang et al., 2008), and then every two images were summed together to match the time points of the BOLD signal. Using both the BOLD and perfusion signals, the time series of CMRO₂ was obtained through the following equation (Chiarelli et al., 2007b; Hoge et al., 1999):

$$S_{CMRO_2} \cong \left(1 - \frac{S_{BOLD}}{M}\right)^{1/\beta} (S_{CBF})^{1-\alpha/\beta} \quad (1)$$

where $\alpha=0.38$, $\beta=1.5$ and M is the percentage modulation factor, varying from 5% to 25% (Chiarelli et al., 2007b). In the current study, the M factor was set to be 22% in accordance with Hoge's report (Hoge et al., 1999).

A T₁ map was fitted for each subject using the NSIR-EPI images acquired at multiple TIs, which was then used for structural segmentation into gray matter, white matter, and cerebrospinal fluid (CSF) components using a custom written linear decomposition algorithm in MATLAB. Signals from white matter and CSF were utilized as nuisance covariates to remove potential contaminations of global fluctuations in the following regression process.

Furthermore, to correct for the potential influence of physiological noise, estimations of cardiac and respiratory information were performed using a temporal independent component analysis (ICA) (Beall and Lowe, 2007). Specifically, twelve components were identified by temporal ICA from the resting-state datasets. Among the spatial patterns of the twelve components, the one with highest spatial correlation with a predefined cardiac source map was considered as the major source of cardiac response. The time course from each cardiac-correlated map was averaged to generate a cardiac estimator. The same procedure was done to generate a respiratory estimator. These cardiac and respiratory estimators were then used to regress out potential physiological influence in the calculation of correlation coefficients.

(a) Visual Task **(b) Resting State**

Fig. 1. Normalized time courses of single subject retrieved from the ROIs of each network during (a) visual task and (b) resting state. (R: right side, L: left side; VIS: visual cortex, PCC: posterior cingulate cortex, ACC: anterior cingulate cortex, HPC: hippocampus).

Statistical analysis

Spherical seeds (6 mm in diameter) were prescribed for each of the three networks based on standard Talairach coordinates: 1) posterior cingulate cortex (PCC) [10, −54, 14]; 2) primary visual cortex (VIS) [10, −88, 5]; and 3) hippocampus (HPC) [30, −24, −9]. All spherical seeds were applied to the right side of the brain. Individual analyses of the BOLD, perfusion and CMRO₂ datasets were carried out using the general linear model on the three networks for both visual-task and resting-state on each subject. The average time series from each spherical seed was taken as the major predictor in the regression model. Other nuisance covariates were also considered (the six motion parameters, the estimators of respiratory/cardiac noise and the time-series retrieved from the segmented white matter and CSF mask). Group-level analyses were performed in AFNI using mixed-effects ANOVA on the correlation coefficient value. Corrections for multiple comparisons were executed at the cluster level using Gaussian random field theory (voxel-wise threshold of $p < 0.005$ was applied to the connectivity maps, with a minimum cluster volume threshold of 567 mm³, yielding an overall false positive $p < 0.05$ as determined by Monte Carlo simulation). The group-level analyses generated thresholded t -score maps of connectivity. In addition, to compare the connectivity strengths of brain regions, two regions of interest (ROIs) were selected for each network from the resting-state BOLD signal, including right and left visual cortex (VIS-R and VIS-L) chosen from the result of visual-stimulation BOLD signal; anterior cingulate and posterior cingulate (ACC and PCC) and right and left hippocampus (HPC-R and HPC-L) chosen from the result of resting-state BOLD signal.

Results

BOLD, perfusion and CMRO₂ time courses in the visual cortex, default mode, and hippocampus ROIs, respectively, from a single representative subject are shown in Fig. 1, and the connectivity

strength between the ROIs of all subjects are listed in Table 1. Each time course in Fig. 1 was normalized to its mean. The cross-correlation coefficient (CC) between two selected ROIs is shown in the up-right corner of each panel. In Fig. 1a, the first row of visual network, BOLD signals show obvious activation pattern in accord with the designed stimulation paradigm, and the time courses from the bilateral visual cortices are highly correlated. Perfusion time courses in the visual cortex ROIs, high-pass-filtered to remove BOLD contamination, closely follow the visual task paradigm as well. Although the CNR in perfusion (~ 2.3) was relatively low comparing to that of BOLD (~ 3.1), signals from the bilateral visual cortices are well correlated. CMRO₂ time courses in the visual cortex ROIs, with lower CNR (~ 1.6), still show reasonably consistent patterns with the visual stimulation and slightly lower correlation between the bilateral visual areas. Time courses from the default mode and hippocampus networks do not show temporal patterns following visual stimulation. However, the intra-network correlation is different in the two networks during the visual task. The connectivity strength between PCC and ACC in the default-mode network is low, while the bilateral hippocampus ROIs have strong correlations in the BOLD, perfusion and CMRO₂ signals.

Fig. 1b shows the normalized BOLD, perfusion and CMRO₂ time courses from the same subject during the resting state. The spontaneous fluctuations in the signals from the visual cortices are well correlated, although slightly lower than the correlation during the visual stimulation. Signal fluctuations in the bilateral hippocampal ROIs at rest are similar to those during visual stimulation. However, the correlations between PCC and ACC at rest are much higher than those with external stimulation. It was also observed that among the three fMRI signals, BOLD has the highest correlation between selected ROIs in these networks, followed by perfusion and then CMRO₂.

Functional connectivity maps of the visual, default mode, and hippocampus networks from the entire group are shown in Figs. 2a for visual task and b for resting state overlaid on the averaged anatomical images. Functional connections of relevant brain regions

Table 1
Connectivity strength (CC) of all subjects between selected ROIs of each network during (a) visual task and (b) resting state

Subject #	Visual cortex			Default mode			Hippocampus		
	BOLD	Perfusion	CMRO ₂	BOLD	Perfusion	CMRO ₂	BOLD	Perfusion	CMRO ₂
(a) Visual task									
1	0.99	0.83	0.73	0.22	0.12	0.03	0.76	0.70	0.64
2	0.99	0.85	0.67	0.26	0.28	0.19	0.83	0.49	0.43
3	0.97	0.83	0.74	0.42	0.33	0.25	0.76	0.45	0.45
4	0.98	0.72	0.55	0.49	0.16	0.04	0.62	0.35	0.29
5	0.98	0.71	0.54	0.54	0.13	0.00	0.88	0.69	0.63
6	0.97	0.92	0.86	0.42	0.19	0.11	0.81	0.70	0.70
7	0.99	0.83	0.18	0.33	0.39	0.16	0.79	0.43	0.45
8 ^a	0.99	0.94	0.87	0.24	0.26	0.12	0.87	0.68	0.67
9	0.99	0.90	0.83	0.49	0.44	0.10	0.87	0.73	0.68
10	0.97	0.90	0.88	0.50	0.48	0.42	0.83	0.48	0.35
11	0.99	0.69	0.59	0.42	0.35	0.22	0.76	0.19	0.19
12	0.98	0.63	0.52	0.61	0.34	0.00	0.51	0.64	0.62
Mean±STD	0.98±0.01	0.81±0.10	0.66±0.20	0.41±0.12	0.29±0.12	0.14±0.12	0.77±0.11	0.54±0.17	0.51±0.17
(b) Resting state									
1	0.89	0.74	0.47	0.60	0.12	0.06	0.59	0.61	0.57
2	0.94	0.73	0.32	0.64	0.23	0.15	0.84	0.46	0.40
3	0.94	0.59	0.44	0.29	0.09	0.07	0.68	0.36	0.33
4	0.84	0.54	0.33	0.58	0.07	0.07	0.61	0.39	0.30
5	0.97	0.76	0.57	0.73	0.50	0.43	0.90	0.54	0.51
6	0.92	0.75	0.65	0.79	0.71	0.67	0.85	0.61	0.76
7	0.96	0.78	0.71	0.19	0.18	0.07	0.81	0.54	0.51
8 ^a	0.93	0.70	0.61	0.55	0.38	0.26	0.82	0.70	0.68
9	0.96	0.86	0.79	0.66	0.40	0.18	0.86	0.56	0.51
10	0.95	0.90	0.87	0.74	0.66	0.63	0.87	0.56	0.43
11	0.92	0.43	0.21	0.59	0.28	0.21	0.53	0.14	0.21
12	0.76	0.53	0.41	0.52	0.30	0.10	0.27	0.55	0.53
Mean±STD	0.92±0.06	0.69±0.14	0.53±0.20	0.57±0.18	0.33±0.21	0.24±0.22	0.72±0.19	0.50±0.15	0.48±0.16

^a Representative subject shown in Fig. 1.

Fig. 2. Functional connectivity maps ($p < 0.05$, corrected) of BOLD, perfusion and CMRO₂ over 12 subjects during (a) visual task and (b) resting state. The group-level statistics were spatially overlaid over the anatomical images.

within these networks were observed in all three maps (BOLD, perfusion and CMRO₂), indicating that the synchronized spontaneous oscillations exist at both hemodynamic and metabolic levels. Comparing to perfusion- and CMRO₂-based connectivity maps, BOLD-based connectivity maps have larger spatial extents probably due to higher sensitivity of BOLD. In the visual cortex, the connection from the seed (V1) area to the ipsilateral lateral geniculate nucleus (LGN) is

strong during visual stimulation, but the connection is not detectable during the resting state. Instead, BOLD-based connectivity of the visual area has broad and symmetric spatial extent at rest, inclusive of bilateral insula/superior temporal gyrus (STG). The perfusion- and CMRO₂-based connectivity maps in the visual network demonstrated laterally asymmetric spatial extent, while the BOLD-based maps are generally symmetric. Since the seed was chosen from right V1, the

Fig. 3. The dependence of presumed three parameters on the connectivity strength over the three networks. (a) M (b) α and (c) β .

connection of the seeds can be traced to deep V1 areas on the ipsilateral side, while scattered connections to the contralateral insula/STG regions are also shown. In the default mode network, the connectivity strength between PCC and ACC is strong in the BOLD, perfusion and CMRO₂ maps at rest, but such connection is not observable during visual stimulation. Furthermore, insula and/or STG are also functionally connected to PCC, especially at rest. Once again, the BOLD-based connectivity map is more laterally symmetric than the perfusion- and CMRO₂-based maps. Compared to the other two networks, the connectivity in the hippocampal network has similar spatial patterns during both resting and task states, suggesting that functional connectivity in brain regions that are not generally involved in a task (visual stimulation in this case) may not be affected by that task.

Since CMRO₂ was calculated based on a biophysical model described in Eq. (1) (Chiarelli et al., 2007b; Hoge et al., 1999), we assessed the potential effects of the parameters in the model on CMRO₂ functional connectivity. Specifically, the influence of the three factors, M , α and β , were evaluated based upon the average CC values of corresponding CMRO₂-based connectivity maps. Fig. 3 shows the dependence of the functional connectivity strength on M (Fig. 3a), α (Fig. 3b) and β (Fig. 3c) within typical ranges of each parameter for the three networks. In general, the connectivity strength is insensitive to these parameters when restricted to their typical ranges (<3%).

Discussions

In this study, we investigated the synchrony of spontaneous fluctuations in BOLD, perfusion and CMRO₂ signals at rest, extending the functional connectivity analysis to a metabolic level. CMRO₂ was calculated from the simultaneously acquired BOLD and perfusion signals based on a biophysical model (Chiarelli et al., 2007b; Hoge et al., 1999), and the perfusion signal was processed with high-pass filtering to minimize BOLD contamination (Chuang et al., 2008). Our results show that synchronized spontaneous fluctuations during the resting state exist at both hemodynamic (BOLD and perfusion) and metabolic (CMRO₂) levels in the visual, default mode, and hippocampal networks, suggesting that the correlation of spontaneous fluctuations in fMRI signals in these brain networks originates from dynamic metabolic changes, likely reflecting ongoing neuronal activity.

Functional connectivity during the resting state had distinct characteristics from that in the visual-stimulation state, particularly in the visual and default-mode networks. In the visual system, for instance, LGN is strongly correlated with the seed region in the primary visual cortex (V1) during visual stimulation, but the connection is not observable at rest. It should be noted, however, that the origin of the correlation during external stimulation and resting may not be the same, particularly in the brain regions responding to the stimulation. To address this issue, we concatenated the “on” states of the visual stimulation scans (the first two time points of the on states were discarded to prevent the transient effect of hemodynamic response) and then underwent the same regression procedure, inclusive of the same seed points and the same nuisance covariates, but with reduced time points. Results showed that even though the connectivity seems weaker in the concatenated data probably due to the reduced time points (from 88 to 30), the V1/LGN connection is still observable during the concatenated visual states but undetectable in the resting state. Another example is that, in the default-mode network, functional connection from PCC to ACC is not detectable during visual stimulation, but is robustly shown at rest. The unmatched functional connectivity under rest and task conditions implies that the requirements for appropriate communication and coordination between brain areas in a network, as reflected by the connectivity strength, may be dependent on specific neuronal processing conditions. In contrast, connectivity maps in the hippocampus network were very similar between task and rest conditions, indicating that the visual stimuli had little effect on the underlying connectivity in this network. Together, these results suggest that each brain network may have distinct functional connectivity characteristics, at rest and during the processing for specific tasks, depending on its role in brain network processes.

The connectivity strength (indicated by CC of the time courses and extent of connectivity maps) of the brain networks obtained from BOLD, perfusion and CMRO₂ signals, was strongest from BOLD, followed by perfusion and then CMRO₂. This is probably due to the different sensitivity of the imaging techniques. In this study, BOLD has the highest CNR (~3.1), followed by perfusion (~2.3) and then CMRO₂ (~1.6), estimated from visual cortex signals during visual stimulation. Even when the spatial features of the connectivity maps derived from the different techniques remained similar after their statistical thresholds were adjusted, the spatial extent of the BOLD-based

connectivity maps were consistently larger than that of perfusion and CMRO₂ when the same statistical threshold was used. Further studies on the noise propagation in the determination of CMRO₂, as well as the effects of CNR on the connectivity strength would be of interest.

Determination of CMRO₂ using MRI is usually based on the measurements of indirect hemodynamic signals (e.g. BOLD, CBF and CBV) (Chiarelli et al., 2007b; Hoge et al., 1999), although it can be measured more directly using exogenous tracers such as ¹⁷O₂ (Zhu et al., 2002). It should be noted that these hemodynamic signals are thought to have different weightings based on differential vascular components. For instance, perfusion signal from PASL targets capillary beds where water exchange occurs between blood and brain tissue, while BOLD originates primarily from venous sites. Caution is needed when interpreting CMRO₂ data determined from hemodynamic signals, due to their potential mismatched origins (Fukunaga et al., 2008). However, since the majority of human fMRI studies use low spatial resolution (3–5 mm), most voxels may contain multiple vascular components and the concern on the mismatched signal origins may be limited. In addition, CMRO₂ values determined from BOLD and CBF generally agree with those from other modalities (Boas et al., 2003; Shidahara et al., 2002) and a recent study also showed that CMRO₂ is tightly coupled with CBF in time (Lin et al., 2009), suggesting that the approach to derive CMRO₂ using hemodynamic signals may be reasonable.

Recently Lin et al. evaluated the influence of MRI models (and parameters in the models) on the determination of CMRO₂ (Lin et al., 2008), and concluded that CMRO₂ measurement from MRI agreed well with PET results (commonly considered as a reasonable standard) if proper models and parameters were used. In this study we used a single-compartmental model, since we only measured BOLD and CBF (not CBV) for covering multiple networks in the brain. The calculation of CMRO₂ was based on a biophysical model (Chiarelli et al., 2007b; Hoge et al., 1999), which was derived from several generally accepted biophysical and physiological concepts, such as Fick's principle of mass preservation, Grubb's relationship between CBF and CBV (Grubb et al., 1974) and the biophysical description of effective transverse relaxation (R_2^*) by Ogawa et al. (Ogawa et al., 1993). Three physiological parameters were presumed for calculating the CMRO₂ information in this model: M factor, α and β . Although Chiarelli et al. indicated that the determination of ' M ' in the model, representing the maximum percent change of BOLD signal, is of great importance to the quantification of CMRO₂ (Chiarelli et al., 2007a), variations of the M factor within the typical published range (0.05–0.25) had only minor effects on the CMRO₂ connectivity strength (Fig. 3a). Similarly, α , representing the elasticity of cerebral blood volume (CBV) relative to CBF (Grubb et al., 1974), had negatively influence on the connectivity of CMRO₂. The β value, representing the size composition of vasculature (between 1 and 2, $\beta=1$ for large vessels $>10\ \mu\text{m}$ and $\beta=2$ for small vessels $<10\ \mu\text{m}$ within the voxel) (Ogawa et al., 1993), produced slightly positive correlations with the CMRO₂ signal change and its functional connectivity, due to the increasing CMRO₂ sensitivity with decreasing vessel size. However, the influences of these parameters on the CMRO₂ functional connectivity was less than 3% within the ranges used in our study, which should be negligible for further analyses.

In summary, we demonstrated that functional connectivity of the brain based on synchronized spontaneous fluctuations can be detected not only in BOLD and perfusion contrast, but also in CMRO₂. This observation provides direct evidence supporting the hypothesis that spontaneous fMRI signal fluctuations have a metabolic origin. Since regional metabolism is closely coupled with local neuronal activity, these fluctuations are likely associated with ongoing neuronal activity. These results are in line with recent electrophysiological and fMRI resting state investigations (Leopold et al., 2003; Lu et al., 2007; Mantini et al., 2007), which show that, like fMRI signals,

electrophysiological signals between functionally-related brain regions are closely correlated.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institute on Drug Abuse (NIDA), National Institute of Health (NIH).

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