Distinction in Coherent Neural Network Between Resting and Working Brain States

Xiao Liu,^{1,2} Xiao-Hong Zhu,¹ and Wei Chen^{1,2}

Abstract

The resting brain is not silent; rather, it is characterized by organized resting-state networks showing spontaneous and coherent neuronal activities, which can be mapped using the spatiotemporal correlation of blood oxygenation level-dependent (BOLD) signal fluctuations measured by functional magnetic resonance imaging (fMRI). However, it remains elusive whether the similar fMRI approach is able to image the coherent network in a working brain, and if yes, whether there is a distinction between the resting- and working-state coherent networks. This study aimed to address these questions in the human visual cortex with a desired activation paradigm using continuous, sustained visual stimuli. It was found that the resting-state coherent network covering the human visual cortex was spatially reorganized during the stimulation into two coherent networks with distinct temporal characteristics of BOLD fluctuations: one covering the activated visual cortical region and the other covering the remaining (nonactivated) visual cortex. The stimulus-specific reorganization of the coherent network observed in the present fMRI study in human is consistent with previous electrophysiological findings from animal studies, and may suggest an essential mechanism for brain functioning. Finally, a similar fMRI experiment was also conducted under brief, short stimulation to examine how the stimulation paradigm can affect the observations.

Key words: coherent neural network; functional MRI (fMRI); reorganization of neural network; resting-state fMRI (rs-fMRI); resting-state network; working-state network

Introduction

DURING THE LAST two decades, synchronized neural activity has become an important topic in the neuroscience research field because it plays a variety of functional roles in large-scale integration, cell assembly binding, neuronal plasticity, and temporal coding (Buzsaki and Draguhn, 2004; Fries et al., 2007; Varela et al., 2001). The major approach to investigate the synchronized neural activity and associated neural network is to analyze the temporal correlations of electrophysiological signals collected from multiple neurons (or neuron groups) in different brain regions. However, electrophysiological recording techniques have limitations in studying the large populations of neurons covering the entire network of interest, and their invasive nature also limits their application in healthy humans. Comparatively, the functional magnetic resonance imaging (fMRI) technique based on the blood oxygenation level-dependent (BOLD) contrast (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1990, 1992) is able to noninvasively detect and image neural activity change by measuring the secondary hemody-

namic/metabolic responses with compromised spatial and temporal resolution.

It was recently found that spontaneous BOLD signals acquired under the resting state (RS) without any stimulation and/or task performance are characterized by slow (<0.1 Hz) and coherent fluctuation within anatomically connected and functionally specific brain systems—for example, the motor, visual, auditory, thalamus, hippocampus, language, and default mode systems (Biswal et al., 1995; Cordes et al., 2000; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998; Stein et al., 2000; Vincent et al., 2007). These findings suggest that the resting brain is not silent but rather highly active in an organized manner, and the spatiotemporal correlations of spontaneous BOLD signals could reflect the underlying functional connectivity under the RS (Biswal et al., 1995) and are useful for mapping a large number of resting-state coherent networks. Nevertheless, it is still elusive whether and how these resting-state networks would be modulated when the brain is activated by sensory stimulation and/or task performance.

¹Department of Radiology, Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, Minnesota.

²Department of Biomedical Engineering, University of Minnesota, Minneapolis, Minnesota.

Several studies have examined how the temporal correlation (i.e., BOLD coherence strength) within a resting-state coherent network changes during task performance or brain stimulation. However, uncertainty remains (Fox and Raichle, 2007) because of the contradictory findings. One possible reason for the discrepancy lies in the different tasks employed by those studies. The highly cognitive tasks, like the spatial working memory and the language task, used in some studies (Hampson et al., 2002; Lowe et al., 2000; Xu et al., 2006) may induce a slow and task-related BOLD fluctuation component within the activated networks and, thus, could result in an enhanced BOLD coherence strength, whereas others using tasks without such a slow component (Morgan and Price, 2004) did not observe an increase of coherence. Moreover, all of these early studies aimed to infer the neuronal interaction through the coherence strength of BOLD fluctuations under stimulation; therefore, they focused on the temporal correlations of BOLD signals within the defined regions of interest (ROI) in the activated brain regions without considering the spatial modulation of the resting-state coherent networks, which should be essential for understanding the relationship between the resting-state and activated coherent networks within the same sensory system.

The present study aimed to investigate the temporal and spatial modulation of coherent neural networks in the human visual system under continuous brain activation condition with desired visual stimuli. Our working hypothesis is that the resting-state coherent network could be dynamically reorganized upon stimulation, and such reorganization is stimulus specific. To avoid the confound effect from the stimulus-evoked and slow BOLD contribution, two sustained, continuous visual stimuli were used in this study: (i) the continuous hemi-field visual stimulus (CHVS); (ii) the continuous full-field visual stimulus (CFVS) with 8-Hz reversal, radial red-black checkerboards. The fMRI BOLD signals were collected from the occipital lobe of healthy subjects when the stimuli were presented continuously, as well as under the RS with the subjects' eyes closed. The coherent networks were mapped through the spatiotemporal correlation analysis of the acquired fMRI datasets. Conclusions were drawn through quantitative comparisons among the coherent network maps obtained under the three conditions, and between the coherent network maps and the conventional fMRI activation maps obtained by presenting the same hemi- or full-field visual stimuli using the block-design paradigm. Moreover, BOLD signals were also acquired and analyzed when the hemi-field visual stimulus was presented in an event-related paradigm to examine whether and how the BOLD correlations are different under such brief, short visual stimulation.

Materials and Methods

Subjects and visual stimuli

Two groups of human subjects participated in this study with written informed consent proved by the Institutional Review Board of the University of Minnesota. All of them were healthy and without history of neurological or psychiatric diseases. Ten subjects (Group I: 5 women and 5 men, 21–54 years old) performed the experiment with continu-

ous, sustained stimulation. One subject (male) participated twice, so there were 11 sets of data in total. One of the datasets suffering serious head motion was discarded; therefore, 10 datasets from 9 subjects were used for the final data analysis. Another group of seven subjects (Group II: four women and three men, 19–63 years old) took part in the experiment using brief, short visual stimulation. The hemi-field (100-degree sector on the right side of visual field) and full-field visual stimuli used in this study were 8-Hz reversal, radial red-black checkerboards with a white cross-mark at the display center for visual fixation. The hemi-field stimulus was always on the right side of visual field for all experiments.

MRI data acquisition

All MRI experiments were performed on a 4T/90-cm bore magnet (Oxford) interfaced with the Varian INOVA console (Varian Inc). A single-loop radiofrequency surface coil (10 cm in diameter) was applied to detect the brain MRI signal mainly from the occipital lobe with higher detection sensitivity. At the beginning of the experiments, anatomical images in transversal, sagittal, and coronal orientations were acquired with T₁-weighted TurboFLASH MRI method (Haase, 1990). The acquisition parameters used for the anatomical images were as follows: field of view (FOV)=20×20 cm²; repetition time (TR)=3 sec; 128×128 image matrix size; 5 mm thickness. For the fMRI experiment, the gradient-echo planar image (GE-EPI) method (Mansfield, 1977) was used to acquire three adjacent coronal image slices (FOV=20×20 cm²; TR=500; echo time [TE]=30 ms; 64×64 image matrix size; 5 mm thickness; nominal excitation pulse flip angle ~45°) covering the calcarine fissure in the human primary visual cortex, which was readily identified according to the anatomical images.

In the experiment with the continuous, sustained stimulation, to identify the brain areas activated by the hemi-field stimulus and full-field stimulus, the first two runs of the fMRI experiment were conducted with a block-design paradigm (two task blocks interleaved with three control blocks, 60 GE-EPI volumes per block) with the hemi- and full-field stimuli, respectively, for each subject. Other imaging runs were performed under three desired conditions: (i) the RS, (ii) the CHVS, and (iii) the CFVS conditions. During the RS condition, the subjects were instructed to close their eyes and refrain from cognitive, language, or motor tasks, but not to fall into sleep. The order of all steady-state runs was randomized. For the CHVS and CFVS conditions, the visual stimuli were presented 10 sec before the start of the fMRI acquisitions and kept on for the entire imaging run. The importance of the eye fixation to central white cross-mark, especially under the hemi-field stimulation, was repeatedly emphasized to the subjects before each fMRI run. The subjects' performance was judged based on the conventional fMRI maps obtained from the hemi-field visual stimulation for ensuring that the activation mainly occurred in the contralateral-hemispheric, rather than ipsilateral-hemispheric, visual cortical region. The fMRI measurement was repeated 2–4 runs for each condition, and each run acquired 300 or 550 GE-EPI volumes.

In the experiment with the brief, short stimulation, the resting-state fMRI runs and the block-design run with the hemi-field visual stimulus were also acquired first.

Then, the fMRI runs with an event-related paradigm were acquired repeatedly for five to six times. The hemi-field stimulus was presented to subjects every 30 sec for six times, and the duration of each visual stimulation was 250 msec. There were totally 240 trials data from the 7 subjects of Group II.

Data processing

Motion correction was first performed on all of the fMRI datasets using the 3D registration tool (3dvolreg) of AFNI (Cox, 1996). Then, the motion-corrected fMRI data acquired with the block-design paradigm were used to generate the functional activation maps for the hemi- and full-field stimuli, respectively, by using the period cross-correlation method: the fMRI time courses were cross-correlated with the block-design template (Bandettini et al., 1993).

For fMRI data acquired under the CHVS, CFVS, and RS conditions, the first 20 image volumes were discarded to ensure reaching a steady-state BOLD signal. The time courses of all image pixels were normalized by their means, and then band-pass filtered (0.005–0.1 Hz) in frequency domain to remove the DC component, linear drift, and reduce the possible fluctuations induced by cardiac and respiratory pulsations. Based on two fMRI activation maps, the most activated (the highest correlation with the block-design paradigm) 2×2 -pixel regions within the left- and right-hemisphere primary visual cortex were chosen as the left and right reference regions, respectively, and the image pixels near large vessels were carefully excluded from this selection. For each imaging run, the time courses from all image pixels were cross-correlated with the average time courses from the left and right reference regions to generate two correlation maps. A 3×3 Gaussian kernel was convoluted with the correlation maps to spatially smoothen them and reduce the salt-and-pepper phenomenon. These two correlation maps were then transformed to the left and right Z maps according to a well-established method (Vincent et al., 2007): the correlation coefficients were transformed to z score by Fisher's transformation, and then normalized by the theoretical standard deviation with the degree of freedom being adjusted according to Barlett's theory (Jenkins and Watts, 1968).

To perform statistical analysis and summarize the group results, two ROIs were defined to cover the activated visual cortical regions in the left and right hemispheres based on the fMRI activation maps. The left ROI includes the fMRI voxels showing the brain region with strong activation to the hemi-field stimulation (Pearson's correlation to block-design paradigm >0.6 , $p < 10^{-3}$ after Bonferroni correction), whereas the right ROI covers voxels showing strong activation to the full-field stimulation (Pearson's correlation to block-design paradigm >0.65 , $p < 10^{-3}$ after Bonferroni correction) but not overlapping with the left ROI. The threshold used for the fMRI activation map under the full-field visual stimulation (0.65) was slightly higher than that for the map under the hemi-field visual stimulation (0.6), because all subjects showed a little stronger response to the full-field visual stimulation. ROIs from all subjects were then visually inspected, and the left ROIs and right ROIs covered the left and right hemispheres, respectively, without seeing any significant overlapping.

For each imaging run, the Z scores of the left Z map (the correlation map with respect to the left-hemisphere reference region) were averaged within the left and right ROIs to obtain two statistics: $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$. The linear mixed model regarding the inter-subject variation as the random effect was used to combine the results from all subjects and to give the statistical inference. $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ were compared under each condition, and each was also compared across conditions. All of statistical analyses were performed using the "nlme" package in R (Jose Pinheiro et al., 2009; Team RDC, 2008).

For the data from the experiment with the brief, short stimulation, functional activation maps for the block-design fMRI runs and correlation maps for the resting-state runs were generated according to the identical pre-processing steps and strategies described above. Two ROIs were then defined as follows: the left visual cortex (LVC) covers the brain regions showing strong activation (Pearson's correlation to the block-design paradigm >0.6 , $p < 10^{-3}$ after Bonferroni correction) to the hemi-field stimulus, whereas the right visual cortex (RVC) covers brain regions having strong resting-state correlations (Pearson's correlation to the reference BOLD time course >0.75 , $p < 10^{-3}$ after Bonferroni correction) to the reference region in the LVC but not being covered by the LVC. Therefore, the LVC and RVC were defined based on the functional activation map and resting-state correlation map, respectively. The threshold used on the resting-state correlation map to define RVC (0.75) was higher than that used on the functional activation map (0.6), because BOLD correlations under the resting state (eyes-closed condition) was very strong and covered a wide range of visual cortex. The LVC and RVC defined in this way were then visually inspected for each subject, and they covered the left and RVC, respectively, without crossing hemispheres. Then, similar to the subtraction method used by the previous event-related functional study (Fox et al., 2006), the BOLD time courses were extracted from and averaged within the two ROIs defined above. Finally, the averaged BOLD time course of the RVC was subtracted from that of the LVC for all the 240 trials measured from the 7 subjects of Group II. At each time point, an *F*-test was performed to examine whether the trial-to-trial variation was significantly reduced after using subtraction method, and the results were Bonferroni-corrected for multiple comparison.

To have a more direct comparison between different experiments, the similar processing was also performed on the data from the experiment with the continuous, sustained stimulation. The BOLD time courses acquired under the CHVS, CFVS, and RS conditions were extracted and averaged over the left and right ROIs defined previously. After normalization, they were then cut into 35-sec segments and plotted in groups according to conditions and signal origins for examining the BOLD signal fluctuations and their coherence strengths. Similar to the above processing for the event-related experiment, the effect of subtraction method (the segment-to-segment variation before and after subtraction corresponding contra-hemispheric BOLD signals) was also tested with an *F*-test at each time point.

The amplitude of spontaneous BOLD signal fluctuation was also quantified and compared across different conditions. The standard deviation (over time) was first calculated for each segment to quantify the amplitude of BOLD fluctuation. Then, a two-sample *t*-test was used to examine whether

they are different between the left and right ROIs, or across different brain conditions.

Results

Distinct temporal behaviors of BOLD signals

Figure 1 summarizes the BOLD time courses measured under resting and activated conditions from one representative subject (Subject 1). As expected, both left and right reference regions responded to the full-field visual stimulus in the conventional block-design fMRI experiment (Fig. 1b), whereas only the left reference region showed the stimulus-evoked BOLD response when the hemi-field visual stimulus was presented in the contralateral (right-side) visual field (Fig. 1a). The BOLD signals measured under the CHVS, CFVS, and RS conditions (Fig. 1c–h) exhibited strong (up to $\pm 4\%$), low-frequency fluctuations in both activated and non-activated reference regions, and the fluctuation magnitudes are comparable to the stimulus-evoked BOLD changes (up to 8%) (Fig. 1a, b). Under the CHVS condition, the temporal behavior of BOLD signals from the left (activated) and the right (nonactivated) reference regions are not well correlated with each other (Fig. 1c, d). In contrast, under the CFVS (both reference regions were activated) and RS (both reference regions were not activated) conditions, the fluctuations of BOLD time courses from two reference regions showed strong temporal correlations (Fig. 1e–h). These observations were consistent among repeated measurements (e.g., run 1

versus run 2 in Fig. 1) in the same subject as well as across different subjects. The characteristics of the BOLD temporal behavior imply distinct neural networks under different conditions as investigated in this study.

Spatial modulation of coherent neural network under sustained stimulation

To further study the spatial relationship of BOLD signal fluctuations based on their temporal correlations, two correlation maps with respect to the left and right reference regions were computed and then converted to the left (i.e., tightly correlated to the left reference region) and right (i.e., tightly correlated to the right reference region) Z maps. These two maps represented the coherent neural networks containing these two reference regions, respectively.

The conventional fMRI activation maps and the Z maps from two representative subjects (Subject 1 and Subject 2) are illustrated in Figure 2. For each condition studied, the left and right Z maps were superimposed on anatomical images using red (the left Z map) and green (the right Z map) colors, with the yellow color denoting the overlapped brain regions between two.

There was almost no overlap (i.e., yellow areas) between the left and right Z maps measured under the CHVS condition (Fig. 2c), during which only the left-hemisphere visual cortex was continuously stimulated by the right-field visual stimulus. Moreover, the left Z map (red areas in Fig. 2c), which

FIG. 1. BOLD time courses from a representative subject under the resting and activated conditions. The BOLD time courses from the left- (red) and right-hemisphere (green) reference regions were normalized by the mean value without the use of the temporal filter. The first row is for the block-design functional magnetic resonance imaging runs with (a) hemi-field and (b) full-field visual stimuli; the second (c, d), third (e, f), and fourth (g, h) rows are for runs under the CHVS, CFVS, and RS conditions, respectively. Bars on horizontal axes indicate the periods during which the hemi-field (gray) and full-field (black) were presented. BOLD, blood oxygenation level dependent; RS, resting state; CHVS, continuous hemi-field visual stimulus; CFVS, continuous full-field visual stimulus.

FIG. 2. Functional activation maps and Z maps from two representative subjects. Left and right panel are for Subject 1 and Subject 2, respectively. The functional activation maps representing the evoked BOLD responses to the hemi-field visual stimulus (a) and the full-field visual stimulus (b) were superimposed on the anatomical images with a desired threshold; the left (red) and right (green) Z maps (with respect to left and right reference region, respectively) representing the spatial pattern of coherent BOLD fluctuations were combined and overlapped on the same anatomical images for the following conditions: (c) CHVS, (d) CFVS, and (e) RS. The locations of the left and right reference regions were marked by white and black cross-marks in (c), (d), and (e). White curve in (d) outlined the difference between Z maps under the CFVS and RS condition, which mainly covers *cuneus* region identified using the standard human brain atlas reference.

represents the activated coherent network, is similar to the conventional fMRI activation map obtained during the hemi-field visual stimulus (Fig. 2a), whereas the right Z map mainly covers the remaining nonactivated regions of the visual cortex. In contrast, the left and right Z maps are largely overlapped with each other under the CFVS and RS conditions (Fig. 2d, e), during which both of the left- and right-hemisphere visual cortices are either activated or not activated. Moreover, the Z maps for the CFVS condition (Fig. 2d) are similar to the conventional

fMRI activation map using the full-field visual stimulus (Fig. 2b); in contrast, the Z maps for the RS (Fig. 2e) cover much wider brain regions in the occipital lobe, especially the *cuneus* area (outlined by the white lines in Fig. 2d), than those Z maps obtained under the CFVS condition.

These observations indicate that the resting-state coherent network is spatially reorganized into two distinct coherent networks under continuous, sustained visual stimulation condition: one covers the activated brain region, and the

FIG. 3. An example of ROIs and the summary of statistics $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ from all subjects. (a) The left and right ROIs from Subject 1; (b) the summary of $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ from all subjects. $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ for CHVS and CFVS were compared with those for RS, and the statistically significant differences are marked by the star symbol (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, respectively; stars are vertically arranged in the plot). Note: the calculation of $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ were based only on left Z maps. ROI, regions of interest.

other covers the remaining, nonactivated visual cortex with distinct BOLD temporal behaviors.

Modulation of coherence strength

Z scores of the left Z map were averaged within the left and right ROIs to obtain two statistics $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$, which quantify the intra-hemisphere coherence within the left-hemisphere visual cortex and inter-hemisphere coherence between the left- and right-hemisphere visual cortices, respectively. Figure 3a illustrates an example of ROIs (for Subject 1), and Figure 3b summarizes the statistical results of $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ from all subjects (9 subjects and 10 datasets in total). When calculating the left Z map, the Z values within the left reference region were taken from the auto-correlation, which probably resulted in slightly higher $\bar{Z}_{LL}$ values than $\bar{Z}_{RL}$ values for the CFVS and RS conditions (Fig. 3b). Even with this possible bias, the inter-hemisphere coherence (quantified by $\bar{Z}_{RL}$) is almost as strong as the intra-hemisphere coherence (quantified by $\bar{Z}_{LL}$) for the CFVS and RS conditions without a statistically significant difference ($p = 0.589$ for CFVS; and $p = 0.471$ for RS). In contrast, for the CHVS condition, the $\bar{Z}_{RL}$ value is significantly lower than $\bar{Z}_{LL}$ ($p = 1.37 \times 10^{-9}$), indicating a much weaker inter-hemisphere coherence than intra-hemisphere coherence. These results are consistent with the observations shown in Figs. 1c, d, and 2c.

The $\bar{Z}_{LL}$ and $\bar{Z}_{RL}$ values were also statistically compared across different conditions. To reduce the possible bias caused by the ROI analysis method, a conservative significance level of $p < 0.01$ was used. The inter-hemisphere coherence ($\bar{Z}_{RL}$, the green columns in Fig. 3b) decreased significantly under the CHVS condition compared to the RS condition ($p = 1.77 \times 10^{-9}$), whereas no significant difference was found between the CFVS and RS conditions ($p = 0.381$). The cross-condition

comparison of $\bar{Z}_{LL}$ (the red columns in Fig. 3b) can quantify the coherence strength modulation of BOLD fluctuations in the left-hemisphere ROI. It decreased slightly, but not significantly, under the CHVS and CFVS conditions as compared to the RS condition ($p = 0.053$ for CHVS; and $p = 0.354$ for CFVS).

Another simple way to examine the inter-hemisphere coherence strength is by the method of subtraction (Fox et al., 2006). BOLD time courses (35-sec segments from all 10 subjects in Group I) from the left and right ROIs, as well as their difference, are shown in Figure 4a. After the BOLD time courses of the right ROI were subtracted from those of the left ROI, the fluctuation magnitude was significantly reduced under the CFVS and RS conditions ($p < 0.001$ for all time points, Fig. 4b), indicating a strong inter-hemisphere correlation between the BOLD signals from the left and right ROIs under these two conditions (either both activated or nonactivated). In contrast, no significant reduction was observed under the CHVS condition (only several time points with a p -value < 0.05 or 0.01 , Fig. 4b), and it, from a statistical perspective, suggests that the temporal coherence of low-frequency BOLD fluctuations was dissociated between the activated and nonactivated brain regions. Figure 4 shows also that the amplitude of BOLD fluctuation seems to be stronger under the RS condition than under the CHVS and CFVS conditions. The statistical testing confirmed the significant reduction of BOLD fluctuation amplitude under the CHVS ($p < 0.001$ for both the left and right ROI) and CFVS ($p < 0.001$ for both the left and right ROI) conditions compared with the RS condition, whereas there are no significant difference between the left and right ROIs under all three conditions ($p = 0.36$ for CHVS, $p = 0.77$ for CFVS, and $p = 0.41$ for RS).

BOLD correlations under the short stimulation

The subtraction method was also applied to the fMRI BOLD data acquired with the brief, short hemi-field stimulation, and the results from all seven subjects (Group II) are shown in Figure 5. It is clear that the BOLD signals from the LVC showed evoked response to the hemi-field visual stimulus though with significant variation across trials, whereas the BOLD signals from the RVC did not. After the BOLD trials from the RVC were subtracted from those from the LVC, the trial-to-trial variability was significantly reduced ($p < 0.001$ for all time points except the time point at 4 sec, which is the peak of stimulus-evoked response), and this result is distinct from those from the experiment with the continuous, sustained hemi-field stimulus (see the first row of Fig. 4). To further statistically examine whether the subtraction method has significantly less effect at the peak point, the effects of subtraction method (the reduction in variation after applying subtraction) at each time point were compared with those during the period without evoked BOLD response (defined as -5 to -1 sec and 11 to 20 sec); the effect of subtraction method was significantly reduced only at 4 sec ($p = 6.7 \times 10^{-3}$, after Bonferroni correction).

Discussion

Modulation of BOLD coherence strength

In the present study, the temporal correlation of BOLD signals from activated brain regions decreased slightly, but not

FIG. 4. Subtraction can reduce the segment-to-segment variations of the BOLD signals for the RS and CFVS conditions, but not for the CHVS condition. **(a)** The BOLD time courses extracted from left (red) and right (green) ROIs, as well as their difference, were intentionally cut into many 35-sec segments and plotted with corresponding colors. **(b)** The averaged BOLD segments with the error bars representing the standard deviations that quantify the segment-to-segment variations. There are totally 160, 174, and 170 segments from all 10 subjects for the CHVS, CFVS, and RS conditions, respectively. The star symbols above error bars in **(b)** represent the significance level of F test on the effect of subtraction method ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$, after Bonferroni correction; stars are vertically arranged in the plot).

to a statistically significant level, under continuous visual stimulation as compared to the resting-state condition (Fig. 3). This result is different from several early reports (Hampson et al., 2002; Lowe et al., 2000), which reported that the correlation of BOLD signals increased when the resting-state coherent network was activated and the enhanced coherence strength was explained as the result of increased neuronal interactions. It is, however, consistent with the previous observation in human motor cortex with continuous finger-tapping task (Morgan and Price, 2004).

There are at least two possible explanations for this discrepancy. The first is that the previous studies focused on the brain networks involving high-level cognitive functions, for instance, memory and language, whereas the present study focused on the early stage of visual sensory system. These networks may behave differently; for instance, the spontaneous BOLD correlations are much stronger at sensory systems than within networks involving high-level memory or language functions. The second possibility is that the tasks employed by previous studies, like working memory task and continuously listening task, could potentially introduce slow, task-related fluctuations in the brain activity, evoke slow BOLD fluctuations in the activated brain regions, and thus increase the BOLD correlations. In contrast, the carefully designed visual stimuli and experimental paradigm of the present study were able to exclude slow and stimulus-evoked BOLD fluctuations for the fol-

lowing three reasons. First, the combination of a 2-Hz image sampling rate and a narrow, low-frequency-band filter (0.005–0.1 Hz) applied in data processing should minimize the folding problem resulting from the 8-Hz reversal visual stimuli as well as the potential contributions from cardiac and respiratory pulsations on the observed BOLD fluctuations. Only the heart beating ranging from 1.9 to 2.1 Hz can possibly fold back to the passing band of the filter; nevertheless, this range is beyond normal heart beating rate of ~ 1 Hz. Second, the linear summation of 8 Hz canonical hemodynamic response function results only in a 0.001% oscillation in the task-evoked BOLD signals after the BOLD response approaches a steady-state level; such a small BOLD oscillation is not comparable with the large BOLD fluctuations ($\sim 4\%$) observed in this study (Fig. 1); thus, the contribution directly driven by the visual stimuli is negligible. Third, spectral analysis did not show any specific frequency component dominating the fluctuation of BOLD signals.

It is well known that a family of synchronized neuronal oscillations, which is called alpha family (8–12 Hz), exists in the primary visual system under the RS. Upon stimulation, activated neurons could shift their synchronized oscillations to a high-frequency gamma band (30–100 Hz) rather than simply increase their phase synchronization in all frequency bands (Aoki et al., 1999; Bressler et al., 1993; Buschman and Miller, 2007; Hoogenboom et al., 2006; Rodriguez et al.,

FIG. 5. BOLD trials (240 in total from all 7 subjects in Group II) acquired with an event-related paradigm and the hemi-field visual stimulus. **(a)** The left visual cortex (LVC, blue) and right visual cortex (RVC, magenta) were defined for a representative subject based on the functional activation map (the first row) and the resting-state BOLD correlation map (the second row), respectively. **(b)** The BOLD trials extracted and averaged from all subjects' LVC (blue) and RVC (magenta), as well as their difference, are plotted with the corresponding colors; the right plot shows the mean of BOLD trials, with error bars representing standard deviations that quantify the trial-to-trial variations. The star symbols above error bars represent the significance level of F test on the effect of subtraction method ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$, after Bonferroni correction; stars are vertically arranged in the plot).

1999). It means that the synchronization of activated neuron groups is not necessary to be stronger than that of nonactivated neuron groups. On the other hand, the BOLD signal does not measure fast electrical activity of neurons directly, but instead much slower hemodynamic response secondary to the neuronal activity change. Even if neurons increased their phase synchronization during activation, it is unlikely that the slow BOLD response is sensitive to such fast neuronal changes associated with phase synchronization. Therefore, it is possible that the resting-state coherent network measured by fMRI BOLD fluctuation could be modulated during brain activation with an insignificant change in the temporal coherence strength.

It has been reported previously that the amplitude of BOLD fluctuation could change between eyes-fixed and eyes-opened condition; however, the direction of modulation found in these studies are somehow conflict with each other (Bianciardi et al., 2009; Yang et al., 2007). The present study did not include the eyes-fixed condition, but $\sim 20\%$ reduction in BOLD fluctuation amplitude was indeed observed under

the continuously stimulated conditions compared to the RS condition.

Spatial reorganization of the resting-state coherent networks

A resting-state coherent network covering a large portion of the visual cortex was observed in this study, which is consistent with early studies (Lowe et al., 1998; Mantini et al., 2007). When the visual stimuli were continuously presented to subjects, it was found that the original resting-state coherent network was dynamically modulated according to the retinotopic attributes of the visual stimulation. Specifically, the visual cortex was reorganized spatially to two distinct coherent networks during the continuous, sustained visual stimulation: one covering the activated visual cortex and the other covering the nonactivated visual cortex as illustrated in Figure 2.

This finding is consistent with a previous study showing that BOLD signals from the MT/V5 brain region had stronger

correlations with itself than with other brain regions when continuously moving concentric circles were presented and thus formed a more limited coherent network than that observed under the resting-state (Hampson et al., 2004). The present study demonstrated that such reorganization can happen even at an earlier visual stage (V1). More importantly, it was found that the reorganization is retinotopic specific and nonactivated brain regions maintained their correlations as in the resting-state.

In conventional fMRI studies, stimulus is repeatedly presented several times interleaved with the control periods (e.g., block-design paradigm); then, brain regions consistently showing evoked BOLD responses to the stimulation are identified as activated regions for generating fMRI activation maps. Temporal correlations of the stimulus-evoked BOLD signals acquired with such a paradigm have also been proposed for evaluating the functional connectivity or effective connectivity (with the information about the direction of brain processing) among activated brain regions (Buchel and Friston, 1997; Friston, 1994; Horwitz, 2003). This approach is based on the temporal correlation analysis of the stimulus-evoked BOLD time courses.

In contrast, the correlation of slow BOLD signal fluctuations among different brain regions without the confounding effect from stimulus-evoked BOLD modulation was evaluated using the continuous stimuli. The activated coherent network, which was identified through the spatiotemporal correlations of slow BOLD signal fluctuations under continuous stimulation, matched well spatially with the activated brain regions showing the evoked BOLD responses to the same stimulus, as demonstrated in the conventional fMRI activation maps in Figure 2. More interestingly, this activated coherent network is spatially and temporally distinguishable from another coherent neural network covering the remaining nonactivated visual cortex.

This finding may suggest that activated brain regions shown in the conventional fMRI maps are probably not just activated regions in response to visual input but also an activated network, and it represents a dynamically organized neuron population with modified neuronal interactions (or synchronized neuronal activity) and is distinct from the resting-state coherent network.

Spatial coverage of the coherent networks

Compared with the activated coherent network under the CFVS condition, the coherent network under the RS condition extends into much larger brain regions, such as the *cuneus* area, which belongs to visual association areas involved in the dorsal pathway and high-level visual functions. The simple reversal checkerboard stimuli used in the present study were unable to activate these areas even with full coverage of the visual field. For the CHVS condition, the coherent network covering the nonactivated brain regions (the green map in Fig. 2c) included those associated visual areas. These observations suggest that the neurons in the nonactivated visual cortical regions remain similar neuronal interactions as under the RS condition.

The correlations of BOLD signals under the RS may reflect a type of intrinsic interactions between neurons from a large portion of the visual cortex in the occipital lobe. The external stimulation will only selectively affect neurons in certain

brain regions and switch their interactions to an activated state with much less profound effects on the intrinsic interactions in other nonactivated brain regions. Therefore, the activated coherent network will be a subset of the resting-state coherent network as demonstrated in Figure 2. Moreover, such transition from intrinsic to activated neuronal interaction mode may not need a large amount of the brain energy budget compared with the total energy consumed under the RS (Raichle, 2006), and this could provide an efficient working mechanism for brain function.

Since the stimuli with various retinotopic attributes were used in the present study, the eyes' fixation is important for the outcome and interpretation of our results. In this study, subjects were requested repeatedly to focus on the central cross (fixation point) before every fMRI run with stimuli, and both the functional activation maps and Z maps partly confirmed the subjects' compliance with this requirement. First, the fMRI activation map acquired with the hemi-field stimulus (Fig. 2a) did not show activation in the ipsilateral-hemisphere visual cortex. Second, suppose the subject's focus was intermittently attracted by the hemi-field stimulus under the CHVS condition, which is most likely eye movement, the fovea area of the ipsilateral-hemisphere visual cortex would be intermittently activated and the peripheral area of the contralateral-hemisphere visual cortex would be intermittently de-activated correspondingly. Therefore, there would be two anti-correlated coherent networks on these two brain regions. However, none of Z maps under the CHVS condition (Fig. 2c) have shown such patterns. The formation of two coherent networks in the activated and nonactivated brain regions under continuous visual stimulation observed in the present study is hardly explained by visual focus deviation.

Possible neural basis of coherent network reorganization

It has been suggested (Leopold et al., 2003) that the band-limited power modulation of electroencephalogram (EEG) signals might have a significant contribution to spontaneous BOLD fluctuations. This notion has gained more supports from a series of studies. Correlations have been found between the spontaneous fluctuations of BOLD signals and EEG power modulation (Liu et al., 2011) or band-limited EEG power modulation of alpha-band (Feige et al., 2005; Goldman et al., 2002; Moosmann et al., 2003), delta-band (Lu et al., 2007) and gamma-band (Shmuel and Leopold, 2008) under the resting conditions without any stimulation. These studies provide important clues to the possible neural correlate of resting-state coherent networks. However, what happens when the brain is activated by external stimuli?

Many electrophysiological studies have demonstrated that the activated neurons would engage in synchronized oscillations at the gamma-band with decreased alpha-band activity (Aoki et al., 1999; Fries et al., 2007; Hoogenboom et al., 2006; Rodriguez et al., 1999). Two previous studies can potentially link such a frequency shift of synchronized neuronal oscillations to the change of hemodynamic signals. One magnetoencephalographic (MEG) study (Hoogenboom et al., 2006) having a similar experiment setup as ours not only observed that the dominant frequency of MEG signals shifted from alpha-band toward gamma-band oscillations, but also confirmed that this shift happened mainly in the activated visual

cortex regions identified by the conventional fMRI map. The other study (Niessing et al., 2005) observed a strong coupling between the gamma-band local field potential (LFP) oscillations and the evoked hemodynamic response recorded by optical imaging; more interestingly, this study also found that the strength of the hemodynamic responses to the same stimulus fluctuated over trials, and the range of such fluctuation is as large as that induced by the stimulus.

On the basis of these findings and current knowledge, the following conjecture regarding the neural basis for explaining our observations was formulated. Under the RS, neurons within the resting-state network maintain their intrinsic interactions through synchronized LFP oscillations that likely are contributed significantly by low-frequency bands (e.g., the alpha-band oscillations in the occipital lobe), and the slow power modulation of the synchronized oscillations results in low-frequency, coherent BOLD fluctuations within the resting-state coherent network owing to a tight neurovascular coupling relationship. When the brain is activated continuously by sustained stimuli, the activated neurons change their interactions into the activated state by shifting the dominant LFP oscillations to high-frequency bands (e.g., gamma band) and form a new synchronized network within the activated brain regions. The power modulation of the synchronized oscillations and thus the corresponding temporal behaviors of the BOLD signal fluctuations become distinct between the activated and nonactivated brain regions; thus, two distinct coherent neural networks can be differentiated between the activated and nonactivated brain regions using slow-frequency BOLD signals and spatiotemporal correlation analysis.

Brief versus sustained visual stimulation

In the experiment with the brief, short hemi-field visual stimulation using single-event paradigm, it was found that after the BOLD signals from the nonactivated visual cortex were subtracted from those of the activated visual cortex, the trial-to-trial variability of event-related BOLD response in the activated region was reduced significantly. This observation confirmed the finding of a previous single-event fMRI study in the somatomotor cortex (Fox et al., 2006) using the right-handed button-press task, which led to a superposition hypothesis (Fox and Raichle, 2007) that the spontaneous neural activity reflected by coherent BOLD fluctuations under the RS could remain unchanged and be linearly superimposed on the stimulus-evoked neural activity under stimulation. However, the present study also demonstrated that under continuous, sustained hemi-field visual stimulation (i.e., CHVS), the BOLD time courses subtraction between the LVC (activated, left ROI) and the RVC (nonactivated, right ROI) did not result in the reduction of the segment-to-segment variability (Fig. 4). Therefore, the reorganization hypothesis would be a better explanation for the dissociation of the working-state coherent network during sustained and steady-state activation condition from the resting-state coherent network as observed in the present study.

An explanation for the discrepant results when using the different (i.e., brief versus sustained) stimulus paradigm is as follows. As aforementioned, the hemodynamic response measured by the BOLD signal is secondary to and much slower than the electrical activity of neurons, and it can last tens of seconds following the change of brain activity evoked by brain

stimulation. Such a neurovascular coupling relationship is usually modeled as a linear time-invariant system with the slow hemodynamic response function as the impulse function. When a single, brief stimulus is applied, the activated neurons would temporarily change their activity within a short time window (e.g., tens to hundreds of milliseconds) after the onset of the stimulus and then recover to the pre-stimulus state (spontaneous activity) rapidly (Pfurtscheller et al., 2000), whereas the BOLD signals will sustain for a much longer time (typically ~20 sec) after neural activity changes. Therefore, BOLD signals from the activated brain region would actually include three components: the component induced by the stimulus-evoked neural activity and those induced by the spontaneous neuronal activities before and after the stimulus. Since the magnitudes of the spontaneous BOLD fluctuation are comparable to the stimulus-evoked BOLD change, the latter two components could have a significant contribution to the total BOLD signals observed after the onset of the stimulus, and therefore may result in a certain degree of coherence between the activated and the nonactivated (control) brain regions within the resting-state coherent network. This notion would explain the coherent BOLD fluctuations between the activated and nonactivated cortical regions in response to the single-event motor task (Fox et al., 2006), as well as to the short, brief hemi-field visual stimulation in the present study. In contrast, the continuous, sustained stimuli used in the present study could continuously modulate the activity of activated neurons. Therefore, the slow BOLD signal fluctuations obtained during the sustained, steady-state stimulation condition can mainly reflect the coherent neural activity of activated neurons without the confounding effects from the pre- and post-stimulus BOLD contributions. Under this circumstance, reorganization of the resting-state coherent network can be observed in the human visual cortex during activation. This explanation is partially supported by the results from the event-related study (Fig. 5b), which shows that the reduction of the trial-to-trial variability is much smaller at the peak of the evoked response (~4 sec) compared with other time points.

Acknowledgments

The authors thank Dr. Kamil Ugurbil for support. This work was partially supported by NIH Grants NS41262, NS057560, NS070839, EB006433, P41 RR08079, and P30NS057091; the Keck Foundation; and a grant from the Institute for Engineering in Medicine of the University of Minnesota.

Author Disclosure Statement

No competing financial interests exist.

References

- Aoki F, Fetz EE, Shupe L, Lettich E, Ojemann GA. 1999. Increased gamma-range activity in human sensorimotor cortex during performance of visuomotor tasks. *Clin Neurophysiol* 110:524–537.
- Bandettini PA, Jesmanowicz A, Wong EC, Hyde JS. 1993. Processing strategies for time-course data sets in functional MRI of the human brain. *Magn Reson Med* 30:161–173.
- Bandettini PA, Wong EC, Hinks RS, Tikofsky RS, Hyde JS. 1992. Time course EPI of human brain function during task activation. *Magn Reson Med* 25:390–397.

- Bianciardi M, Fukunaga M, van Gelderen P, Horovitz SG, de Zwart JA, Duyn JH. 2009. Modulation of spontaneous fMRI activity in human visual cortex by behavioral state. *Neuroimage* 45:160–168.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 34:537–541.
- Bressler SL, Coppola R, Nakamura R. 1993. Episodic multiregional cortical coherence at multiple frequencies during visual task performance. *Nature* 366:153–156.
- Buchel C, Friston KJ. 1997. Modulation of connectivity in visual pathways by attention: cortical interactions evaluated with structural equation modelling and fMRI. *Cereb Cortex* 7:768–778.
- Buschman TJ, Miller EK. 2007. Top-down versus bottom-up control of attention in the prefrontal and posterior parietal cortices. *Science* 315:1860–1862.
- Buzsaki G, Draguhn A. 2004. Neuronal oscillations in cortical networks. *Science* 304:1926–1929.
- Cordes D, Haughton VM, Arfanakis K, Wendt GJ, Turski PA, Moritz CH, et al. 2000. Mapping functionally related regions of brain with functional connectivity MR imaging. *AJNR Am J Neuroradiol* 21:1636–1644.
- Cox RW. 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29:162–173.
- Feige B, Scheffler K, Esposito F, Di Salle F, Hennig J, Seifritz E. 2005. Cortical and subcortical correlates of electroencephalographic alpha rhythm modulation. *J Neurophysiol* 93:2864–2872.
- Fox MD, Raichle ME. 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 8:700–711.
- Fox MD, Snyder AZ, Zacks JM, Raichle ME. 2006. Coherent spontaneous activity accounts for trial-to-trial variability in human evoked brain responses. *Nat Neurosci* 9:23–25.
- Fries P, Nikolic D, Singer W. 2007. The gamma cycle. *Trends Neurosci* 30:309–316.
- Friston KJ. 1994. Functional and effective connectivity in neuroimaging: a synthesis. *Hum Brain Mapp* 2:56–78.
- Goldman RI, Stern JM, Engel J, Jr., Cohen MS. 2002. Simultaneous EEG and fMRI of the alpha rhythm. *Neuroreport* 13:2487–2492.
- Greicius MD, Krasnow B, Reiss AL, Menon V. 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A* 100:253–258.
- Haase A. 1990. Snapshot FLASH MRI. Applications to T1, T2, and chemical-shift imaging. *Magn Reson Med* 13:77–89.
- Hampson M, Olson IR, Leung HC, Skudlarski P, Gore JC. 2004. Changes in functional connectivity of human MT/V5 with visual motion input. *Neuroreport* 15:1315–1319.
- Hampson M, Peterson BS, Skudlarski P, Gatenby JC, Gore JC. 2002. Detection of functional connectivity using temporal correlations in MR images. *Hum Brain Mapp* 15:247–262.
- Hoogenboom N, Schoffelen JM, Oostenveld R, Parkes LM, Fries P. 2006. Localizing human visual gamma-band activity in frequency, time and space. *Neuroimage* 29:764–773.
- Horowitz B. 2003. The elusive concept of brain connectivity. *Neuroimage* 19:466–470.
- Jenkins GM, Watts DG. 1968. *Spectral Analysis and Its Applications*. Boca Raton: Emerson-Adams Press.
- Jose Pinheiro DB, DeBruin S, Sarkar D; the R Core team. 2009. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–92. <http://cran.r-project.org/web/packages/nlme/index.html>
- Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, et al. 1992. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A* 89:5675–5679.
- Leopold DA, Murayama Y, Logothetis NK. 2003. Very slow activity fluctuations in monkey visual cortex: implications for functional brain imaging. *Cereb Cortex* 13:422–433.
- Liu X, Zhu XH, Zhang Y, Chen W. 2011. Neural origin of spontaneous hemodynamic fluctuations in rats under burst-suppression anesthesia condition. *Cereb Cortex* 21:374–384.
- Lowe MJ, Dzemidzic M, Lurito JT, Mathews VP, Phillips MD. 2000. Correlations in low-frequency BOLD fluctuations reflect cortico-cortical connections. *Neuroimage* 12:582–587.
- Lowe MJ, Mock BJ, Sorenson JA. 1998. Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage* 7:119–132.
- Lu H, Zuo Y, Gu H, Waltz JA, Zhan W, Scholl CA, et al. 2007. Synchronized delta oscillations correlate with the resting-state functional MRI signal. *Proc Natl Acad Sci U S A* 104:18265–18269.
- Mansfield P. 1977. Multi-planar image formation using NMR spin-echos. *J Phys C Solid State Phys* 10:L55–L58.
- Mantini D, Perrucci MG, Del Gratta C, Romani GL, Corbetta M. 2007. Electrophysiological signatures of resting state networks in the human brain. *Proc Natl Acad Sci U S A* 104:13170–13175.
- Moosmann M, Ritter P, Krastel I, Brink A, Thees S, Blankenburg F, et al. 2003. Correlates of alpha rhythm in functional magnetic resonance imaging and near infrared spectroscopy. *Neuroimage* 20:145–158.
- Morgan VL, Price RR. 2004. The effect of sensorimotor activation on functional connectivity mapping with MRI. *Magn Reson Imaging* 22:1069–1075.
- Niessing J, Ebisch B, Schmidt KE, Niessing M, Singer W, Galuske RA. 2005. Hemodynamic signals correlate tightly with synchronized gamma oscillations. *Science* 309:948–951.
- Ogawa S, Lee T-M, Kay AR, Tank DW. 1990. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci U S A* 87:9868–9872.
- Ogawa S, Tank DW, Menon R, Ellermann JM, Kim S-G, Merkle H, et al. 1992. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A* 89:5951–5955.
- Pfurtscheller G, Neuper C, Krausz G. 2000. Functional dissociation of lower and upper frequency mu rhythms in relation to voluntary limb movement. *Clin Neurophysiol* 111:1873–1879.
- Raichle ME. 2006. Neuroscience. The brain's dark energy. *Science* 314:1249–1250.
- Rodriguez E, George N, Lachaux JP, Martinerie J, Renault B, Varela FJ. 1999. Perception's shadow: long-distance synchronization of human brain activity. *Nature* 397:430–433.
- Shmuel A, Leopold DA. 2008. Neuronal correlates of spontaneous fluctuations in fMRI signals in monkey visual cortex: implications for functional connectivity at rest. *Hum Brain Mapp* 29:751–761.
- Stein T, Moritz C, Quigley M, Cordes D, Haughton V, Meyerand E. 2000. Functional connectivity in the thalamus and hippocampus studied with functional MR imaging. *AJNR Am J Neuroradiol* 21:1397–1401.
- Team RDC. 2008. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

- Varela F, Lachaux JP, Rodriguez E, Martinerie J. 2001. The brain-web: phase synchronization and large-scale integration. *Nat Rev Neurosci* 2:229–239.
- Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, Van Essen DC, et al. 2007. Intrinsic functional architecture in the anaesthetized monkey brain. *Nature* 447:83–86.
- Xu G, Xu Y, Wu G, Antuono PG, Hammeke TA, Li SJ. 2006. Task-modulation of functional synchrony between spontaneous low-frequency oscillations in the human brain detected by fMRI. *Magn Reson Med* 56:41–50.
- Yang H, Long XY, Yang Y, Yan H, Zhu CZ, Zhou XP, et al. 2007. Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. *Neuroimage* 36: 144–152.

Address correspondence to:

Wei Chen

Department of Radiology

Center for Magnetic Resonance Research

University of Minnesota

2021 6th Street SE

Minneapolis, MN 55455

E-mail: wei@cmrr.umn.edu