Resting States Affect Spontaneous BOLD Oscillations in Sensory and Paralimbic Cortex

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INTRODUCTION

Neural activity is continuously modulated even during rest. For example, scalp recorded EEGs provided initial evidence that during wakeful rest the brain exhibits a posterior predominant, continuously modulated, 8- to 12-Hz activity, i.e., the alpha rhythm (Adrian and Matthews 1934; Berger 1929, 1930). Although the EEG correlates are well characterized, no comparable description exists for blood oxygenation level-dependent (BOLD) correlates of resting states. On the one hand, studies of simultaneously recorded EEG and BOLD signals have yielded somewhat contradictory results regarding the temporal and spatial relation between BOLD amplitude and alpha power. Although some have reported that BOLD activity in the occipital cortices is positively correlated with ongoing modulations of the EEG alpha power (Laufs et al. 2003), others have reported a negative correlation (Feige et al. 2005; Goldman et al. 2002; Moosmann et al. 2003). Recent observations have indicated that increases in alpha power are indeed associated with decreases in the BOLD signal in occipital and parietal regions (de Munck et al. 2007), although this relation shows very significant individual differences (Goncalves et al. 2006). Furthermore, BOLD correlates of spontaneous variations of alpha rhythm power have been found both in frontal and parietal regions (Goncalves et al. 2006; Laufs et al. 2006), despite the predominance of alpha rhythms over the posterior occipital-parietal lobe.

On the other hand, functional connectivity studies (Biswal et al. 1995; Cordes et al. 2001; Fransson 2005; Greicius et al. 2003; Hampson et al. 2002; Lowe et al. 1998) have provided strong support for the notion that spontaneous BOLD oscillations reflect, at least in part, spontaneous oscillations in neural activity. However, functional connectivity methods are also sensitive to non-neural signals and therefore do not provide an unbiased estimate of the value of neural correlations.

In this paper we took a different approach. Rather than pursuing the BOLD correlates of EEG modulations or the pattern of BOLD–BOLD correlations, we simply asked whether different resting states are associated with changes in the power of the ongoing BOLD signal. We characterized the BOLD signal in terms of its frequency spectrum to detect regions where the spectral density of the BOLD signal was affected by the resting state type. Analysis in the frequency domain is ideally suited to quantify the properties of the BOLD signal under steady-state conditions and in the absence of externally paced events (Damoiseaux et al. 2006). Finally, we present estimates of the correlation between components of the BOLD signal that were specifically modulated by resting state type and therefore were likely to reflect purely neural effects. These data were used to establish the temporal coherence of resting state effects across regions.

METHODS

Subjects and paradigm

Ten healthy right-handed subjects (6 females; average age, 23.6 yr) participated in a simultaneous BOLD functional MRI (fMRI)/EEG experiment. The BOLD data were obtained from a public database available at brainscape.org (study BS003). The experiment comprised nine scans, each 5.5 min long. Before each scan, subjects were instructed to either keep their eyes open, eyes closed, or maintain fixation on a foveal crosshair for the duration of the scan. The order in which the three conditions were run was randomized.

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RESTING STATES AFFECT SPONTANEOUS BOLD OSCILLATIONS

923

EEGs

In 9 of 10 subjects, EEG data were acquired simultaneously with fMRI (DC-3,500 Hz, 20-kHz sampling rate) using the MagLink system and the synamps/2 amplifier (Compumedics Neuroscan) with 32 electrodes (modified 10/20), including bipolar, vertical eye, and cardiac leads. The reference electrode was located between Fz and Cz and the ground electrode between Fz and FPz. Gradient artifact was reduced using Scan 4.3 software (Compumedics Neuroscan) with ballistocardiogram artifacts reduced using in-house software (Vincent et al. 2007). These procedures also allowed the detection of the EKG epochs and the estimation of the duration of the interbeat interval. EEG was inspected both visually and using spectral information in the alpha, theta, and delta bands to ensure that subjects were not asleep.

The effects of resting state on the mean and SD of the interbeat interval were assessed statistically using repeated-measures ANOVAs that included subjects as the random factor and resting state type as the fixed factor.

Imaging methods and preprocessing

BOLD data were downloaded from an electronic database publicly available at brainscape.org (study BS003). The imaging data were available in a preprocessed format. The acquisition parameters and preprocessing steps are briefly summarized here. fMRI scans were acquired with a Siemens 3 Tesla Allegra system (Erlagen, Germany). An asymmetric spin-echo-echo-planar-imaging (EPI) sequence was used [repetition time (TR) = 3.013 s, which included a 1-s gap, echo time (TE) = 25 ms, flip angle = 90°]. Each run comprised of 110 volumes of 32 contiguous 4-mm axial slices (4 mm isotropic in plane) with whole brain coverage. Structural images included one sagittal MP-RAGE T1-weighted image (TR = 2.1 s, TE = 3.93 ms, flip angle = 7°, 1 × 1 × 1.25 mm) and a T2-weighted fast spin-echo image.

Image preprocessing included the following steps: 1) compensation for slice-dependent time shifts, 2) elimination of odd/even slice intensity differences caused by interpolated acquisition, 3) realignment of all data acquired in each subject within and across runs to compensate for rigid body motion, and 4) normalization to a whole brain mode value of 1,000 (Ojemann et al. 1997). The functional data were transformed into atlas space (Talairach and Tournoux 1988) by computing a sequence of affine transformations (1st frame EPI sequence to T2-weighted fast spin-echo to MP-RAGE to atlas representative target), which were combined by matrix multiplication. Reslicing the functional data to 3-mm isotropic voxels in conformity with the atlas than involved only one interpolation. For cross-modal (i.e., functional to structural) image registration, a locally developed algorithm was used (Rowland et al. 2005). Whole brain images after preprocessing included 48 × 64 × 48 3-mm isotropic voxels.

Analysis: power spectral density and statistical maps

The analysis comprised three steps: detrending, estimation of spectral density, and statistical inference at the group level. Subject specific general linear models (GLMs) (Friston et al. 1995) were used to estimate the amplitude of the power spectrum (i.e., spectral density) from each scan’s detrended data. To remove the linear drift from the BOLD time series, each scan was detrended by fitting a constant and linear trend to the acquired time series by ordinary least squares using the following regression model

\[
\text{BOLD}(t) = c_0 + \sum_{k=1}^{42} [a_k \cos(2\pi f_k t) + b_k \sin(2\pi f_k t)]
\]

where \(c_0\) is the constant term and \(a_k\) and \(b_k\) are the coefficients of the 42 sine/cosine pairs (i.e., full Fourier basis set) modeling frequencies from one full cycle \(f_k = 0.0031\) Hz to 42 full cycles \(f_k = 0.132\) Hz determined by a practical Nyquist limit [i.e., \(1/(2.5 \times TR)\)]. Spectral density, the amplitude of modulation at each frequency, was computed from the Fourier coefficients \(a_k\) and \(b_k\) using the following formula

\[
A(f_k) = \sqrt{\frac{\sum_{\text{scans}} (a_k^2 + b_k^2)}{\#\text{scans}}} , \quad k = 1, \ldots, 42
\]

The spectral density of the BOLD signal was computed separately for scans in which subjects kept their eyes closed, eyes open, or maintained fixation. The spectral density was normalized by the mean of the nine constant terms \(c_0\) (1 for each scan) and multiplied by 100 to express it in percent BOLD signal change.

The spectral density maps were smoothed with a 6-mm full-width-at-half-maximum three-dimensional Gaussian kernel to blur individual differences in brain anatomy. The aim of the statistical analysis was to detect regions in which the spectral density of ongoing BOLD oscillations was modulated by the resting state type. For this purpose, we used a group level statistic: a two-factor, repeated-measures, voxel-wise ANOVA. The random factor was subjects, and the two fixed factors were resting state type, i.e., eyes open, eyes closed, and fixation, and frequency, which contained 42 levels. The ANOVA yielded three statistical maps: 1) the main effect of resting state type \((F_{2,18})\), which reflects the effect of behavioral condition on the overall variance of the ongoing BOLD signal; 2) the resting state type by frequency interaction map \((F_{2,13,738})\), which highlights those regions in which the behavioral state affects the amplitude of the ongoing BOLD signal in a frequency dependent fashion; and 3) the main effect of frequency \((F_{2,13,738})\), which reflects the deviation of the spectral density of the ongoing BOLD signal from that of white noise whose spectral density is constant across the frequency spectrum. The voxel-wise statistical maps (main effect of resting state type, interaction of resting state type by frequency, and the main effect of frequency) were gaussianized and corrected for multiple comparisons (\(\alpha = 3.0\), minimum 45 face connected voxels, \(P < 0.05\) corrected) with a Monte Carlo based method (Forman et al. 1995; McAvoy et al. 2001).

Regional analyses

Fifteen regions of interest (ROIs) were defined on the basis of the multiple comparisons-corrected resting state type by frequency interaction map (Fig. 1; Table 1). ROIs were drawn by hand. All regional statistics including variances and covariances were based on the BOLD time series averaged across all voxels within a region. Repeated-measures ANOVAs and Student’s t-test were used to determine the significance of differences in pairwise regional BOLD–BOLD correlations among resting state types. For this purpose, the correlation coefficients were first z-transformed using a standard procedure (Fisher 1921).

Principal component analysis

The estimated neural correlations were entered into a 15 × 15 matrix, i.e., one element for each possible regional pairing. To examine how many components accounted for the neural effects, the eigenvectors and eigenvalues of the correlation matrix were computed. This is simply a principal components analysis. The decom-
position of the correlation matrix yields a set of eigenvalues that represent the proportion of the overall variance accounted by the corresponding eigenvector and a set of eigenvectors whose scalar elements represent the weighted contribution of each regional BOLD response to that component. The product of the scalar elements of the eigenvector and its eigenvalue gives the proportion of the BOLD variance accounted by the eigenvector for each region.

Measurement of end-tidal CO2

Three male subjects performed 5.5 min of eyes closed and eyes open rest (randomized order between subjects) while lying supine in a mock MR scanner. Continuous end-tidal CO2 was collected via nasal cannula and measured using the InVivo Magnitude (InVivo, Orlando, FL) patient monitoring system. Data were recorded at 100 Hz using PowerLab Chart Software (ADInstruments, Colorado Springs, CO). The protocol was approved by the Washington University Institutional Review Board.

To facilitate comparison with the BOLD signal, the computation of spectral density was carried similarly to the analysis of the BOLD data. First, the end-tidal CO2 measurements were interpolated by means of cubic splines (interp1, Matlab v5.2, The MathWorks, Natick, MA) to the BOLD data TR of 3.013 s. The interpolated time series was detrended and fit with the full Fourier basis set, which exactly replicated the model used to analyze the BOLD data (see Eqs. 1 and 2). To statistically assess changes in end-tidal CO2 across resting state types, a repeated-measures ANOVA was performed on the spectral density (see Eq. 3). Subjects were the random factor and resting state type and frequency were the fixed factors.

**RESULTS**

Spontaneous BOLD activity is affected by resting state type

The main effect of resting state type highlighted a subset of regions also found within the interaction of resting state type by frequency map; hence we present only the latter. Figure 1A shows representative axial slices obtained from the resting state type by frequency interaction map. Displayed values are z-transformed F statistics. Several regions in the occipital lobe, including primary visual cortex, the lateral occipital and MT complexes, fusiform gyri, and dorsal occipital-parietal regions were highlighted. Furthermore, regions outside visual striate and extrastriate cortex were found, whose ongoing BOLD activity modulated as a function of resting state type. These included left and right retrosplenial cortex, left auditory cortex, and bilateral sensory-motor cortices. Peak coordinates, center of mass, and region sizes are listed in Table 1. A notable feature of these data are that the effect of resting state type in visual areas seems to replicate the anatomical pattern of activation evoked by a visual stimulus, with clear boundaries between separate visual regions, such as primary visual cortex, lateral occipital, and MT as shown in Fig. 1B.
effects of behavioral state cannot be trivially attributed to visually evoked responses being added to the ongoing activity for two reasons. First, our data clearly indicate that areas beyond primary and extrastriate visual cortex (e.g., sensory-motor cortex and auditory cortex) modulated their ongoing, spontaneous BOLD signal according to resting state type. These areas are not known to respond to passive visual stimulation. Second, although intermittent visual stimulation and hence phasic BOLD responses could have been introduced by spontaneous eye movements and eye blinks, this should result in greater modulation of spontaneous BOLD activity during the eyes open condition than the eyes closed condition. This was not the case. Figure 2C shows the spectral density of the BOLD signal in the left occipital pole, which probably corresponds to the foveal representation of primary visual cortex. In this region, the comparison of the spectral densities indicates that the amplitude of spontaneous modulation of the BOLD signal was greater when subjects kept their eyes closed than during eyes open or fixation. Table 2 reports the values of the temporal variance of the BOLD signal in right visual cortex in the three different resting state types for all 10 subjects. Variance is a simpler measure of variability than the spectral

<table>
<thead>
<tr>
<th>Region</th>
<th>Abbreviation</th>
<th>Peak Coordinates</th>
<th>Center of Mass</th>
<th>Volume, mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left sensory-motor</td>
<td>LSM</td>
<td>−35</td>
<td>−42</td>
<td>54</td>
</tr>
<tr>
<td>Right sensory-motor</td>
<td>RSM</td>
<td>35</td>
<td>−39</td>
<td>63</td>
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<tr>
<td>Left ventral intraparietal sulcus/visual area 3a</td>
<td>LVpV3a</td>
<td>−23</td>
<td>−81</td>
<td>24</td>
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<td>Right ventral intraparietal sulcus/visual area 3a</td>
<td>RVpV3a</td>
<td>26</td>
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<td>27</td>
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<td>Left visual cortex</td>
<td>LV1</td>
<td>−5</td>
<td>−90</td>
<td>6</td>
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<td>Right visual cortex</td>
<td>RV1</td>
<td>11</td>
<td>−84</td>
<td>9</td>
</tr>
<tr>
<td>Left lateral occipital</td>
<td>LLO</td>
<td>−20</td>
<td>−90</td>
<td>12</td>
</tr>
<tr>
<td>Right lateral occipital</td>
<td>RLO</td>
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<td>−90</td>
<td>0</td>
</tr>
<tr>
<td>Left medial temporal</td>
<td>LMT</td>
<td>−47</td>
<td>−78</td>
<td>9</td>
</tr>
<tr>
<td>Right medial temporal</td>
<td>RMT</td>
<td>38</td>
<td>−69</td>
<td>6</td>
</tr>
<tr>
<td>Left retrosplenial cortex</td>
<td>LrSplen</td>
<td>−14</td>
<td>−63</td>
<td>−6</td>
</tr>
<tr>
<td>Right retrosplenial cortex</td>
<td>RrSpen</td>
<td>20</td>
<td>−51</td>
<td>−3</td>
</tr>
<tr>
<td>Left fusiform gyrus</td>
<td>LFus</td>
<td>−14</td>
<td>−63</td>
<td>−9</td>
</tr>
<tr>
<td>Right fusiform gyrus</td>
<td>RFus</td>
<td>20</td>
<td>−51</td>
<td>−9</td>
</tr>
<tr>
<td>Left auditory cortex</td>
<td>LAud</td>
<td>−50</td>
<td>−24</td>
<td>18</td>
</tr>
</tbody>
</table>

Peak coordinates and center of mass are given in mm according to the atlas of Talairach and Tournoux (1988).

FIG. 2. Spectral density of regional ongoing BOLD signals for 3 different resting state types averaged over 10 subjects. Regions include right sensory-motor (A), left auditory (B), left visual (C), and right retrosplenial (D) cortex, selected from the map shown in Fig. 1. Across these and all the other regions, the eyes closed condition was associated with greater modulation of the ongoing BOLD signal than the eyes open and fixation conditions. These effects were mostly limited to 3 frequency bands centered at 0.01, 0.03, and 0.04 Hz. Error bars are standard error computed across the 10 subjects.
density, which breaks the signal in frequency-dependent components, and it shows that, in this particular region, the BOLD signal power almost doubled in the eyes closed condition compared with the fixation condition. Moreover, greater amplitude of spontaneous BOLD oscillations in the eyes closed condition was not limited to primary visual cortex but generalized to all areas that showed a significant interaction of resting state type by frequency, without exceptions. To illustrate this point, spectral densities from regions in right sensory-motor cortex, left auditory cortex, and right retrosplenial cortex are shown in Fig. 2, A, B, and D. We conclude that visual stimulation introduced by eye movements and blinks could hardly account for the spectral differences, because one would expect greater BOLD modulations during eyes open than eyes closed, at least in visual areas. On the contrary, our data showed consistent increases in BOLD power across a set of sensory areas for several modalities in the fixation and eyes open conditions compared with the eyes closed condition. Therefore BOLD modulations by resting state type are most likely driven by endogenous signals rather than slavishly reflecting the sensory input.

Interestingly, the spectral density plots shown in Fig. 2, A–D, also illustrate two other points. First, the spectral density of the BOLD signal is significantly nonuniform, in agreement with previous observations (Purdon and Weisskoff 1998; Weisskoff 1996; Zarahn et al. 1997). We also found that the power distribution of the BOLD signal had a 1/f distribution not only in gray matter but across the entire brain (data not shown). Second, the modulation of the ongoing BOLD activity by resting state type has a complex, band-pass behavior; the effects were mainly restricted to at least three bands with center frequencies at 0.01, 0.03, and 0.04 Hz, respectively.

**Effects of resting state type are by two spatially overlapping, but temporally uncorrelated, oscillatory modes**

To further characterize the effects of resting state on the ongoing BOLD signal, we developed a procedure to estimate the correlation between neural components of the BOLD response in regions showing an interaction of resting state type by frequency. The raw correlation coefficients measure the similarity between simultaneous BOLD signals recorded from separate brain regions. A difficulty in interpreting these BOLD–BOLD correlations is that the BOLD time series not only reflects local neural activity but also other physiological and nonphysiological signal sources. Hence correlations based on the BOLD time series provide a biased estimate of neural correlations. To overcome this limitation, we used a simple model of the regional BOLD variances and pairwise covariances to remove the effects of non-neural signals (see APPENDIX). This model assumes that 1) there exist both neural and non-neural sources of BOLD variability, 2) sources of variability unrelated to local neural activity are not affected by resting state type, and 3) non-neural sources of variability are uncorrelated with neural sources of BOLD variability. To show the effect of resting state on raw BOLD–BOLD correlations, we present the correlations between left retrosplenial cortex and right occipital cortex with the remaining regions, because these two regions show somewhat different results.

The values of the pairwise regional BOLD–BOLD correlations between the left retrosplenial region and all the other regions in the eyes closed and fixation conditions as well as the estimated neural correlations are shown in Fig. 3A. BOLD–BOLD correlations with left retrosplenial cortex were found to vary significantly over regions ($F_{13,117} = 20.26, P = 3.79e^{-24}$) and to differ significantly between resting state types ($F_{1,9} = 6.61, P = 0.030$). A significant interaction of region by resting state type ($F_{13,117} = 6.06, P = 1.58e^{-8}$) also indicated that the effects of resting state type on the correlation with the left retrosplenial region strongly depended on the region. For each regional pairing, BOLD–BOLD correlations in the eyes closed and fixation conditions were statistically compared using a two-way, paired Student’s $t$-test. A single asterisk indicates a significant difference at the 0.05 level and a double asterisk at the 0.01 level. The black bars report the correlation between the components of the BOLD signal specifically modulated by resting state type.

**TABLE 2. Subject variance for RV1 and LrSplen**

<table>
<thead>
<tr>
<th>Subject</th>
<th>RV1</th>
<th></th>
<th></th>
<th>LrSplen</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>Fixation</td>
<td>Eyes Open</td>
<td>Eyes Closed</td>
<td>Fixation</td>
<td>Eyes Open</td>
<td>Eyes Closed</td>
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<td>1</td>
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<td>0.3203</td>
<td>0.4805</td>
<td>0.1551</td>
<td>0.1324</td>
<td>0.2839</td>
</tr>
<tr>
<td>2</td>
<td>0.3984</td>
<td>0.8841</td>
<td>1.3643</td>
<td>0.2289</td>
<td>0.4749</td>
<td>0.4765</td>
</tr>
<tr>
<td>3</td>
<td>0.3479</td>
<td>0.3032</td>
<td>0.3256</td>
<td>0.5943</td>
<td>0.5322</td>
<td>0.6945</td>
</tr>
<tr>
<td>4</td>
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<td>0.5547</td>
<td>1.1225</td>
<td>0.2013</td>
<td>0.1909</td>
<td>0.4274</td>
</tr>
<tr>
<td>5</td>
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<td>0.2638</td>
<td>0.3177</td>
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<td>0.2293</td>
<td>0.3834</td>
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<td>6</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
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<tr>
<td>Mean</td>
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<td>0.4273</td>
<td>0.6615</td>
<td>0.2994</td>
<td>0.3117</td>
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<tr>
<td>SE</td>
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<td>0.0710</td>
<td>0.1498</td>
<td>0.0786</td>
<td>0.0479</td>
<td>0.1072</td>
</tr>
</tbody>
</table>

All values are calculated from the BOLD time series in units of percent change of baseline. RV1, right visual cortex; LrSplen, left retrosplenial cortex.
conditions ($F_{1.9} = 0.023$, $P = 0.88$). We conclude that the effect of resting state type on the correlation between right visual cortex and other regions was small and of uncertain significance.

The median value and its 95% CI for the regional correlations of the component of the BOLD signal that was modulated by resting state type were 0.67 and 0.64–0.70, respectively. These values are higher than those reported in previous studies of regional BOLD correlations, based on the same data (Fox et al. 2005), suggesting that the procedure successfully isolated signal components related to changes in neural activity across resting state types. The finding that spontaneous local field potentials also exhibit equally high temporal coherence in nonhuman primates (Leopold et al. 2003) is consistent with this viewpoint.

To determine whether there is a dominant component driving neural activity in the eyes closed condition relative to the fixation condition, the eigenvectors and eigenvalues of the matrix of estimated neural correlations were computed. This procedure assessed the regional coherence of simultaneous changes in BOLD signal. Figure 4 shows the proportion of regional variance accounted for by each of the 15 eigenvectors for each of the 15 regions. For all but two of the regions, the first eigenvector accounted for 55% or more of the overall neural modulation. The first and second eigenvectors accounted for a comparable proportion of the overall variance (30–50% each) in left and right visual cortex regions. We conclude that, in the eyes closed condition, most of the increase in activity was caused by two oscillatory modes: one that was synchronous across all regions and a second that was synchronized between left and right occipital cortex but did not generalize across the entire network.

Non-neuronal sources of noise are not affected by resting state type

A potential confound is the effect of physiological variables on cerebral blood flow and therefore BOLD. If physiological noise changes across resting state types, contrary to our assumption, the observed BOLD effects may not be ascribed to changes in neural activity, contrary to the viewpoint expressed here. To address this point, we assessed whether heart rate, a source of physiological noise, was affected by resting state type, by comparing the mean interbeat interval and variability of the interbeat interval across resting state types. The ballistocardiogram was recovered from the scanner record using a set of procedures detailed in a previous publication (Vincent et al. 2007). The mean of the duration of the interbeat interval, averaged across subjects, was 0.960, 0.938, and 0.938 s in the eyes closed, eyes open, and fixation conditions, respectively. Likewise, the SD of the duration of the interbeat interval averaged across subjects was 0.0858, 0.0896, and 0.0797 s in the eyes closed, eyes open, and fixation conditions, respectively. Repeated-measures ANOVAs showed that neither the mean interbeat interval or the SD of the interbeat interval was affected by resting state type ($F_{2,16} = 2.68$, $P = 0.0993$ and
interaction of resting state type by frequency (the cardiac rhythm (Anrep et al. 1936). Since if it did, this should have had, in turn, consequences on the cardiac rhythm. No consistent difference between eyes open and eyes closed. The latter two conditions differed little, with fixation showing somewhat lower amplitude modulation of the ongoing BOLD signal than eyes open (see Fig. 2, A–D). These results clearly rule out the possibility that the effects of resting state type may arise spuriously as a consequence of intermittent visually evoked responses caused by eye movements or blinks, since this should have resulted in greater BOLD modulation during eyes open and fixation than during eyes closed, contrary to what we found. Could changes in the amplitude of neural and BOLD modulation simply result from changes in the mean level of activity? It is known that visually responsive neurons exhibit a positive relation between average spike counts and spike count variance (reviewed in Koch 1999). Therefore the finding that modulations of the BOLD signal are increased in the eyes closed condition compared with the eyes open and fixation conditions may simply reflect that neural activity and its modulation is greater in the former than the latter conditions. This hypothesis is not supported by a previous fMRI study in which resting state conditions were alternated within scans rather than between scans. In fact, Feige et al. (2005) have shown that the average BOLD signal increases during eyes open compared with eyes closed in visually responsive areas. Hence it is quite unlikely that differences in the baseline level of neural activity accounted for differences in the amplitude of modulation around the baseline. Finally, a potential concern is that the effects of resting state type on the BOLD signal could be caused by changes in physiological noise rather than neural activity. Previous work has shown that the effects of physiological sources of noise on the spontaneous BOLD signal are widespread and have been suggested to closely match the anatomy of the default system (Birn et al. 2006). However, estimates of the contribution of physiological noise to the spontaneous BOLD oscillations in gray matter have indicated that it accounts for a small proportion of the overall signal. For example, Wise et al. (2004) measured the effect of variation of end-tidal CO₂ on BOLD signal variability. They found that end-tidal CO₂ accounted for 6.5% of the overall voxel-wise BOLD variance in gray matter voxels and 25% of the overall BOLD variance for the signal computed averaging over the entire brain. Similar estimates have been obtained by others

Effects of resting state type are endogenously driven

The first noteworthy result is that resting state type affects the ongoing BOLD activity in several sensory and paralimbic regions in the posterior portion of the brain. Prior studies of resting state effects on spontaneous BOLD oscillations have not reported any difference between eyes open and eyes closed wakeful rest. Recently, it has been noted that light sleep is associated with increased BOLD oscillations in visual cortex and across the entire brain compared with resting with eyes open (Fukunaga et al. 2006). Horovitz et al. (2008) have found a more focal anatomical pattern involving visual cortex, cingulate gyrus, superior temporal gyrus, lateral and medial sensory-motor regions, and inferior parietal and frontal cortex. Our data suggest a similar, but even more restricted, anatomical pattern for the effects of resting state type during wakefulness. In fact, we found that only sensory-motor, auditory, primary, and extra-striate visual cortices, as well as retrosplenial cortex (see Fig. 1), showed significant increases of their ongoing BOLD activity during the eyes closed condition compared with the eyes open or fixation conditions. The latter two conditions differed little, with fixation showing somewhat lower amplitude modulation of the ongoing BOLD signal than eyes open (see Fig. 2, A–D). These results clearly rule out the possibility that the effects of resting state type may arise spuriously as a consequence of intermittent visually evoked responses caused by eye movements or blinks, since this should have resulted in greater BOLD modulation during eyes open and fixation than during eyes closed, contrary to what we found. Could changes in the amplitude of neural and BOLD modulation simply result from changes in the mean level of activity? It is known that visually responsive neurons exhibit a positive relation between average spike counts and spike count variance (reviewed in Koch 1999). Therefore the finding that modulations of the BOLD signal are increased in the eyes closed condition compared with the eyes open and fixation conditions may simply reflect that neural activity and its modulation is greater in the former than the latter conditions. This hypothesis is not supported by a previous fMRI study in which resting state conditions were alternated within scans rather than between scans. In fact, Feige et al. (2005) have shown that the average BOLD signal increases during eyes open compared with eyes closed in visually responsive areas. Hence it is quite unlikely that differences in the baseline level of neural activity accounted for differences in the amplitude of modulation around the baseline. Finally, a potential concern is that the effects of resting state type on the BOLD signal could be caused by changes in physiological noise rather than neural activity. Previous work has shown that the effects of physiological sources of noise on the spontaneous BOLD signal are widespread and have been suggested to closely match the anatomy of the default system (Birn et al. 2006). However, estimates of the contribution of physiological noise to the spontaneous BOLD oscillations in gray matter have indicated that it accounts for a small proportion of the overall signal. For example, Wise et al. (2004) measured the effect of variation of end-tidal CO₂ on BOLD signal variability. They found that end-tidal CO₂ accounted for 6.5% of the overall voxel-wise BOLD variance in gray matter voxels and 25% of the overall BOLD variance for the signal computed averaging over the entire brain. Similar estimates have been obtained by others

FIG. 5. Spectral density of the group averaged end-tidal CO₂. End-tidal CO₂ data were obtained in 3 subjects lying in a mock scanner while resting with their eyes open or closed. The ordinate is the amplitude of CO₂ oscillations, the abscissa the frequency. No consistent difference between the amplitude of end-tidal CO₂ oscillations were observed in the frequency range <0.04 Hz, where most of the effects of resting state type on the BOLD signal were found.
Relation between ongoing BOLD and EEG alpha power

We cannot conclusively attribute the BOLD effects described here to a specific electrophysiological phenomenon. However, we were struck by several observations indicating that modulations of spontaneous BOLD oscillations and alpha rhythms by resting state type share a number of commonalities. First, the effects were found in sensory but not regions of the default system (Gusnard and Raichle 2001), with the possible exception of retrosplenial cortex. This suggests that they may not be mediated by top down mechanisms but rather by bottom up mechanisms, possibly involving subcortical structures (Hughes and Crunelli 2005). Second, the cortical distribution of the BOLD effects of resting state type match quite closely the distribution of alpha rhythms, which arise not only from visual regions, but also from other sensory regions, including sensory-motor cortex (Pineda 2005) and auditory cortex (Niedermeyer 1990). The modulation of the spectral density of the ongoing BOLD signal resembles those of the alpha power frequency spectra obtained under the same behavioral conditions (see Fig. 4, A and B, in Linkenkaer-Hansen et al. 2001). Recent data have confirmed this, having shown spontaneous BOLD oscillations in primary visual cortex were most coherent with power modulations in the alpha band (Mantini et al. 2007).

Two partially independent cortical networks support vigilance

Regions affected by resting state type show prominent pairwise correlations. An unbiased estimate of the correlations between regional neural activities was obtained from the differences between BOLD data in the eyes closed and fixation conditions, based on the assumption that the covariance structure of non-neural signals did not change with resting state type. Although this assumption is not completely supported by our physiological recordings, which indicated some effects of resting state type on end-tidal CO₂ variability, nevertheless, these effects did not match the effects of resting state type on spontaneous BOLD oscillations. The estimated correlation coefficients varied between 0.29 and 1.0, which is in the same range or higher than those reported for correlations between local field potentials recorded from adjacent regions of visual cortex in nonhuman primates (Leopold et al. 2003). Interestingly, we found that BOLD fluctuations were mostly synchronous within the set of regions highlighted in Fig. 1, suggesting a common, shared origin for these signals. There were nevertheless departures from complete synchronization. BOLD oscillations in left and right primary visual cortex were partly independent from those in other regions, including regions in extrastriate visual cortex. While surprising, the finding that resting state signals may differentiate between visual striate and extrastriate regions is not entirely novel. For example, Damoiseaux et al. (2006) reported a decomposition of resting state data into independent networks. These included two visual networks, one centered on primary visual cortex and the medial occipital-parietal lobe and the second on extrastriate visual cortex along the lateral aspect of the posterior occipital-temporal and parietal lobe. Similarly, resting state networks, obtained during light sleep in infants, have highlighted a network centered on the medial aspect of the occipital-parietal lobe but failed to show an equivalent network in lateral regions (Fransson et al. 2007). Therefore medial occipital structures may hold a specific status developmentally, which is maintained in the adult. More generally, our correlation analysis indicates that the effects of resting state type, rather than representing amplitude modulations of local, independent neuronal activities, instead represent the activity of two oscillatory networks that are temporally asynchronous but partly overlapping.

Functional significance of modulation of spontaneous BOLD activity

The focality of the modulations of the ongoing BOLD signal also raises the question of their behavioral significance. Patients with strokes involving the medial portion of the occipital and temporal lobe, where the strongest effects of resting state type on the BOLD signal were found, show remarkable disturbances of their vigilance (Caplan 1980; Medina et al. 1974, 1977). This clinical picture is specific for strokes affecting this particular location or the right temporal parietal junction (Mesulam and Weintraub 1992), the latter being thought to mediate reorienting to novel sensory stimuli (Corbetta and Shulman 2002). These clinical observations could be used to speculatively advance the hypothesis that the cerebral system, here highlighted by the comparison of resting states, supports basic aspects of vigilance through the gating of sensory processing.
\[ F = N_F + N_O \]  
(\text{A1})

Since the increased power measured in the eyes closed condition must correspond to an increase in variance, which must coincide with an incremental increase in neural activity, the observed BOLD signal in the eyes closed condition \( C \) is equal to the increase in neural activity caused by maintaining the state of eyes closed \( N_c \), added to that already present during fixation \( N_f \) and all other non-neural sources \( N_o \)

\[ C = N_c + N_f + N_o \]  
(\text{A2})

**Simulation methods**

Our goal is to compare correlations in neural activity between pairs of regions. We will have six observed variances/covariances computed from the BOLD data for each region pair:

\[\text{Var}(F_1) \] observed variance in the fixation state of region 1 \n\[\text{Var}(F_2) \] observed variance in the fixation state of region 2 \n\[\text{Var}(C_1) \] observed variance in the eyes closed state of region 1 \n\[\text{Var}(C_2) \] observed variance in the eyes closed state of region 2 \n\[\text{Cov}(F_1,F_2) \] observed covariance between regions 1 and 2 in the fixation state \n\[\text{Cov}(C_1,C_2) \] observed covariance between regions 1 and 2 in the eyes closed state

Note \[\text{Var}(x) = \text{Cov}(x,x)\]. Substituting Eqs. A1 and A2 into the six observed values and solving the algebra yields the following six relations

\[\text{Var}(F_1) = \sigma^2_{N_0} + \sigma^2_{N_f} + 2\rho_{N_f,N_0}\sigma_{N_f}\sigma_{N_0} \]

\[\text{Var}(F_2) = \sigma^2_{N_0} + \sigma^2_{N_f} + 2\rho_{N_f,N_0}\sigma_{N_f}\sigma_{N_0} \]

\[\text{Var}(C_1) = \sigma^2_{N_0} + \sigma^2_{N_f} + 2\rho_{N_f,N_0}\sigma_{N_f}\sigma_{N_0} + 2\rho_{N_c,N_0}\sigma_{N_c}\sigma_{N_0} \]

\[\text{Var}(C_2) = \sigma^2_{N_0} + \sigma^2_{N_f} + 2\rho_{N_f,N_0}\sigma_{N_f}\sigma_{N_0} + 2\rho_{N_c,N_0}\sigma_{N_c}\sigma_{N_0} + 2\rho_{N_c,N_c}\sigma_{N_c}\sigma_{N_c} \]

\[\text{Cov}(F_1,F_2) = \rho_{N_0,N_0}\sigma_{N_0}\sigma_{N_0} + \rho_{N_0,N_f}\sigma_{N_0}\sigma_{N_f} + \rho_{N_0,N_c}\sigma_{N_0}\sigma_{N_c} \]

\[\text{Cov}(C_1,C_2) = \rho_{N_0,N_0}\sigma_{N_0}\sigma_{N_0} + \rho_{N_0,N_f}\sigma_{N_0}\sigma_{N_f} + \rho_{N_0,N_c}\sigma_{N_0}\sigma_{N_c} + \rho_{N_c,N_0}\sigma_{N_c}\sigma_{N_0} \]

\[\rho_{N_c,N_c}\sigma_{N_c}\sigma_{N_c} + \rho_{N_c,N_f}\sigma_{N_f}\sigma_{N_c} + \rho_{N_c,N_c}\sigma_{N_c}\sigma_{N_c} \]

Assuming the neural activity is independent and therefore uncorrelated with non-neural sources of variance, with a bit of algebra, the correlation between the neural activity of regions 1 and 2 in the eyes closed state \( \rho_{N_c,N_c} \) can be shown to be

\[\text{Cov}(C_1,C_2) - \text{Cov}(F_1,F_2) - \rho_{N_c,N_c}\sigma_{N_c}\sigma_{N_c} = \rho_{N_c,N_c}\sigma_{N_c}\sigma_{N_c} \]

where

\[\sigma^2_{N_f} = \text{Var}(F_1) - \sigma^2_{N_0} \]

\[\sigma^2_{N_c} = \text{Var}(F_2) - \sigma^2_{N_0} \]

\[\sigma^2_{N_c} = -\rho_{N_c,N_c}\sigma_{N_c}\sigma_{N_c} \pm \sqrt{\rho_{N_c,N_c}^2\sigma^2_{N_c} + \text{Var}(C_1) - \text{Var}(F_1)} \]

The expected value of \( \rho_{N_c,N_c} \) was estimated by Monte Carlo integration over the remaining parameters (i.e., \( \sigma^2_{N_f}, \sigma^2_{N_c}, \rho_{N_c,N_f}, \rho_{N_c,N_f}, \rho_{N_c,N_c}, \) and \( \rho_{N_c,N_c} \)) which were assumed to have a uniform distribution over their domains.

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**References**

- Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 8: 700–711, 2007.

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