Dissociated mean and functional connectivity BOLD signals in visual cortex during eyes closed and fixation

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McAvoy M, Larson-Prior L, Ludwikow M, Zhang D, Snyder AZ, Gusnard DL, Raichle ME, d’Avossa G. Dissociated mean and functional connectivity BOLD signals in visual cortex during eyes closed and fixation. J Neurophysiol 108: 2363–2372, 2012. First published August 8, 2012; doi:10.1152/jn.00900.2011.—We investigated the effects of resting state type on blood oxygen level-dependent (BOLD) signal and functional connectivity in two paradigms: participants either alternated between fixation and eyes closed or maintained fixation or eyes closed throughout each scan. The BOLD signal and functional connectivity of lower and higher tiers of the visual cortical hierarchy were found to be differentially modulated during eyes closed versus fixation. Fixation was associated with greater mean BOLD signals in primary visual cortex and lower mean BOLD signals in extrastriate visual areas than periods of eyes closed. In addition, analysis of thalamocortical functional connectivity during scans in which participants maintained fixation showed synchronized BOLD fluctuations between those thalamic nuclei whose mean BOLD signal was systematically modulated during alternating epochs of eyes closed and fixation, primary visual cortex and the attention network, while during eyes closed negatively correlated fluctuations were seen between the same thalamic nuclei and extrastriate visual areas. Finally, in all visual areas the amplitude of spontaneous BOLD fluctuations was greater during eyes closed than during fixation. The dissociation between early and late tiers of visual cortex, which characterizes both mean and functionally connected components of the BOLD signal, may depend on the reorganization of thalamocortical networks. Since dissociated changes in local blood flow also characterize transitions between different stages of sleep and wakefulness (Braun AR, Balkin TJ, Wesenten NJ, Gwadry F, Carson RE, Varga M, Baldwin P, Belenkyn G, Horschvitch P, Science 279: 91–95, 1998), our results suggest that dissociated endogenous neural activity in primary and extrastriate cortex may represent a general aspect of brain function.

fMRI; oscillatory signals; resting state; spontaneous activity; thalamocortical networks

The first recordings of electroencephalographic (EEG) activity demonstrated that simply keeping one’s eyes open or closed changes the ongoing cerebral activity at rest (Berger 1929, 1930). With the advent of functional imaging techniques, the correlates of physiological states have been further characterized. Two main results stand out. First, the cortical distribution of basal cerebral blood flow (CBF) has been shown to differ between sleep and wakefulness (e.g., Dang-Vu et al. 2010). In particular, PET studies in humans indicated that during rapid eye movement (REM) sleep posterior regions of the brain show a dissociated pattern of blood flow changes compared with slow-wave sleep (SWS) and wakefulness. Whereas medial regions, roughly corresponding to primary visual cortex, show decreased basal CBF during REM sleep (Braun et al. 1998), lateral (Braun et al. 1998) and dorsal medial (Madsen et al. 1991) regions corresponding to extrastriate visual areas show increased CBF. This dissociated pattern of CBF changes in visual regions, along with frontal and temporal regions, pons, thalamus, and limbic areas (Braun et al. 1997; Dang-Vu et al. 2010; Madsen et al. 1991), has been suggested to be specifically associated with REM sleep and to potentially account for the phenomenological qualities of oniric activity (Braun et al. 1998; Hobson 2009). The second notable finding is that during wakefulness certain brain regions belonging to the default mode network (DMN) show systematic decrements in CBF whenever subjects engage in an active task compared with when they maintain a state of quiet wakefulness (Raichle et al. 2001; Shulman et al. 1997). Thus baseline functional signals change across different stages of sleep and between wakeful rest and behaviorally active states.

The availability of objective, physiological markers of sleep and wakefulness has been particularly important for studies mapping the neural circuitry responsible for regulating the sleep-wake cycle and alertness (Bremer 1935; Moruzzi and Magoun 1949). Thalamus plays a major role in shaping ongoing cortical rhythms (Hughes and Cruenlli 2005), and both cortical and thalamic structures are endowed with the ability to maintain rhythms in the alpha frequency range (Kristiansen and Courtois 1949; Lopes da Silva and Storm van Leeuwen 1977; Lopes da Silva et al. 1980), which characterizes quiet wakefulness with eyes closed. A number of imaging studies have indicated that blood oxygen level-dependent (BOLD) signals in mediiodorsal thalamus (MD) are strongly modulated by alpha power (Difrancesco et al. 2008; Feige et al. 2005; Goldman et al. 2002; Goncalves et al. 2006; Mantini et al. 2007; Moosmann et al. 2003), even though this thalamic region is not known to have direct connections to cortical visual areas (Jones 2007). In addition, opening and closing the eyes has been found to modulate the BOLD signal in mediiodorsal and lateral geniculate thalamic regions (Marx et al. 2003, 2004).
In the present work, we examined modulations of the mean BOLD signal and spontaneous BOLD fluctuations across fixation and eyes-closed epochs. This was motivated in part by our previous finding that the amplitude of spontaneous BOLD fluctuations, in primary and extrastriate visual regions as well as other sensory and paralimbic cortical regions, is increased during periods of eyes closed compared with eyes open and fixation (McAvoy et al. 2008). Whether this effect could be related to changes in the underlying mean level of the BOLD signal was not determined. Earlier work has provided somewhat puzzling results regarding the effects of opening and closing one’s eyes in visual cortical regions. While some have reported increased BOLD signals in primary visual cortex during eyes open compared with closed (Marx et al. 2004; Uludag et al. 2004), others have reported increased BOLD signals in extrastriate visual cortex (e.g., Marx et al. 2003) during eyes closed compared with open. In view of the crucial role of thalamus in establishing and maintaining cortical rhythms as noted above, we also examined thalamocortical functional connectivity during fixation and eyes closed and the pattern of temporal dependencies between spontaneous activity in thalamic and cortical regions across resting states.

Overall our findings indicate that the dissociated pattern of CBF, first observed in sleep (Braun et al. 1998) between primary and extrastriate visual regions, generalizes to transitions between opening and closing the eyes as measured with the BOLD signal, and may therefore be better understood in relation to still elusive factors that change among behavioral states rather than as a hallmark of specific stages of sleep. Moreover, thalamocortical functional connectivity data suggest that these changes may be related to a reconfiguration of the transfer of information between thalamus and cortex.

MATERIALS AND METHODS

Paradigms and subjects. Two protocols were used to investigate the relation between opening and closing the eyes and the BOLD signal: 1) the Berger paradigm, in which subjects alternated between fixation and eyes-closed conditions repeatedly within a scan, and 2) a resting-state paradigm in which subjects either maintained fixation or kept their eyes closed for the duration of the scan. Data from both paradigms have been published previously (Fox et al. 2005a, 2005b; McAvoy et al. 2008).

The Washington University Institutional Review Board approved both experimental protocols, and subjects gave written consent prior to participation. In the Berger paradigm, 11 healthy right-handed subjects (7 women, 4 men; average age 28.1 yr) participated in the fMRI experiment. The bore and scanning room were completely darkened through the use of special draperies. Participants were instructed to fixate on a dim light presented through a fiber-optic cable within the scanner bore or to maintain a state of wakeful rest with their eyes closed. Verbal instructions to open and close the eyes were given every 40 s. The imaging session included six scans, each lasting 5.3 min. The starting state, fixation or eyes closed, was counterbalanced across subjects and scans.

In the resting-state paradigm, 10 healthy right-handed subjects (6 women, 4 men; average age 23.6 yr) participated in a simultaneous fMRI/EEG experiment. The experiment consisted of nine scans, each 5.5 min long. Before each scan, subjects were instructed to either keep their eyes open or closed or maintain fixation on a foveal cross hair for the duration of the scan. The order in which the three conditions were run was randomized. Imaging data were obtained from a publicly available database at brainscape.org (study BS003). In 9 of 10 subjects, EEG data were acquired simultaneously with fMRI (DC-3500 Hz, 20-kHz sampling rate) using the MagLink system and the synamps2 amplifier (Compumedics Neuroscan) with 32 electrodes (modified 10/20), including bipolar, vertical eye, and cardiac leads. The reference electrode was located between Pz and Cz and the ground electrode between Fz and FPz. Electrodes to record the electrooculogram (EOG) were placed in a bipolar configuration ~3 cm above and below the outer canthus of the left eye. Gradient artifact was reduced with Scan 4.3 software (Compumedics Neuroscan), with ballistocardiogram artifacts reduced with in-house software (Vincent et al. 2007). EEG was inspected both visually and with spectral information in the alpha, theta, and delta bands to ensure that subjects were not asleep. Only the EOG, not the EEG, data were used to generate predictors of the BOLD signal.

Imaging methods and preprocessing. Images were acquired with a Siemens 3-T Allegra system (Erlangen, Germany). In the Berger paradigm, an asymmetric gradient-echo planar imaging sequence was used (repetition time = 2.5 s, echo time = 25 ms, flip angle = 90°). Each scan comprised 128 volumes of 32 contiguous 4-mm axial slices (4 mm isotropic in plane) providing whole brain coverage. In the resting-state paradigm, an asymmetric gradient-echo planar imaging sequence was used (repetition time = 3,013 s, which included a 1-s gap, echo time = 25 ms, flip angle = 90°). Each scan included 110 volumes of 32 contiguous 4-mm axial slices (4 mm isotropic in plane) providing whole brain coverage. Structural images included one sagittal MP-RAGE T1-weighted image (repetition time = 2.1 s, echo time = 3.93 ms, flip angle = 7°, 1 × 1 × 1.25 mm) and a T2-weighted fast spin-echo image.

Image preprocessing was carried out with the same procedure for both data sets and included the following steps: 1) compensation for slice-dependent time shifts, 2) elimination of odd/even slice intensity differences due to 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 Tournois 1988) by computing a sequence of affine transformations (first 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 then involved only one interpolation. For cross-modal (i.e., functional to structural) image registration, a locally developed algorithm was used (Rowland et al. 2005).

Mean BOLD signals. For the Berger data, subject-specific general linear models (GLMs) (Friston et al. 1995) were used to estimate, frame by frame, the mean BOLD signal time locked to the verbal instruction to open or close the eyes. The GLM regressors included two sets of delta functions, each modeling the BOLD response over 16 time points. Additional regressors included a constant term and linear trend for each scan to account for slow drifts in the BOLD time series. The first 40-s epoch of each scan was discarded because it included a period of non-steady-state magnetization. The estimated mean signals were normalized by the value of the constant term averaged over scans and then spatially smoothed with a 6-mm full width at half-maximum (FWHM) three-dimensional (3D) Gaussian kernel to blur interindividual differences in brain anatomy.

Statistical significance of the mean signal changes evoked by opening and closing eyes was assessed with a group-level repeated-measures analysis of variance (ANOVA) that included subjects as the random factor and two fixed factors, resting state type (eyes closed and fixation) and time (16 time points). The resulting statistical maps, which included the resting state type × time interaction (FSC(T15,150)), were z-transformed and corrected for multiple comparisons (FWE corrected, P < 0.05 corrected) with a Monte Carlo-based method (Forman et al. 1995; McAvoy et al. 2001).

Thalamocortical functional connectivity. The Berger and resting-state BOLD data were detrended to remove the effects of slow drifts. Since the Berger data included BOLD signals evoked by the alternating 40-s periods of fixation and eyes closed, these mean signals were...
subtracted. Both data sets were low-pass filtered at a cutoff frequency of 0.08 Hz (Biswal et al. 1995; Lowe et al. 1998). Subject-specific GLMs included, as independent variables, the time series of the seed region and a constant term for each run. The seed region estimates were normalized by the value of the constant term averaged over scans and then spatially smoothed with a 6-mm FWHM 3D Gaussian kernel. Statistical significance was assessed with a group-level two-tailed, one-sample Student’s t-test. The statistical maps were z-transformed and corrected for multiple comparisons (\( t \geq 3.0, \text{minimum } 17 \text{ face-connected voxels, } P < 0.05 \) corrected).

Since functional connectivity of the thalamus was dominated by the DMN and in order to uncover secondary patterns of thalamocortical functional connectivity, a second GLM was computed that included an additional regressor: the BOLD time series in a predefined mask of the DMN. The mask was obtained from the statistical map published in the first study, which documented the existence of the DMN (Raichle et al. 2001; Shulman et al. 1997).

The functional connectivity analysis is limited to assessing simultaneous signal changes between a given seed region and the rest of the brain, and thus provides only a limited view of the relation between thalamic and cortical spontaneous activity. A temporally extended picture of this relation can be obtained by computing the cross-covariance, which allows one to examine the nature of functional connectivity in detail. For example, if the difference in functional connectivity between eyes closed and fixation is due to changes in the relative timing between cortical and thalamic signals, then the cross-covariance will be shifted along the time axis without a change in shape. On the other hand, if changes in functional connectivity are due to a common signal being added to thalamic and cortical regions, then the cross-covariance between these regions will change by an additive, symmetric function.

Prior to computation of the cross-covariance, the resting-state BOLD time series was detrended and low-pass filtered at a cutoff frequency of 0.08 Hz, and the DMN time series and constant term were regressed out. Finally, the resulting residual time series was normalized by the value of the constant term averaged over scans. The cross-covariance was calculated by systematically shifting the seed region and voxel time series with respect to one another along the time axis and computing the covariance between temporally realigned BOLD values. The time shift, or lag, included both negative and positive delays. Each subject contributed four cross-covariances to the analysis, one for each combination of resting state type (eyes closed and fixation) and thalamic seed, that is, MD and lateral geniculate nucleus (LGN), which were spatially smoothed with a 6-mm FWHM 3D Gaussian kernel. The significance of the effects of opening and closing the eyes on the cross-covariance was evaluated with a group-level repeated-measures ANOVA that included subjects as the random factor and fixed factors of resting state type (eyes closed and fixation), lag (81 time steps spanning 2 min in both leading and lagging directions), and seed region (MD and LGN). The resulting statistical maps, which included the resting state type \( \times \) lag interaction \( (F_{15,1350}) \), were z-transformed and corrected for multiple comparisons (\( t \geq 3.0, \text{minimum } 13 \text{ face-connected voxels, } P < 0.05 \) corrected).

Oscillatory BOLD signals. The Berger data were also used to estimate the amplitude of spontaneous BOLD fluctuations during fixation and eyes closed. For each subject the constant term and linear trend were subtracted from the BOLD time series. The residual time series was then normalized by the value of the constant term averaged over scans. The standard deviation was computed separately for fixation and eyes-closed epochs, yielding two time courses of the amplitude of the average deviation of the BOLD signal from its mean value, each 16 time points. These estimates were spatially smoothed with a 6-mm FWHM 3D Gaussian kernel. Statistical significance was assessed with a group-level repeated-measures ANOVA that included subjects as the random factor and two fixed factors, time (16 time points) and resting state (eyes closed and fixation). The resulting statistical maps, which included the resting state type \( \times \) time interaction \( (F_{15,1350}) \), were z-transformed and corrected for multiple comparisons (\( t \geq 3.0, \text{minimum } 13 \text{ face-connected voxels, } P < 0.05 \) corrected).

**RESULTS**

**Mean BOLD signals in visual cortices dissociate between fixation and eyes closed.** Figure 1 shows axial slices highlighting subcortical and cortical regions where the BOLD signal was modulated during alternating 40-s epochs of fixation and eyes closed. In two thalamic regions the BOLD signal increased during fixation. The first region was located along the medial surface of dorsal thalamus (MD); the second, more ventral, lateral, and posterior was identified as the lateral geniculate (LGN) (Marx et al. 2003, 2004). Primary and secondary visual cortex (V1), along the medial posterior occipital pole, also showed increased BOLD signals during fixation. However, extrastriate visual regions, including the lateral MT complex, showed decreased BOLD signals during periods of fixation compared with eyes closed (see Fig. 1). Dorsal and ventral extrastriate visual regions showed similar time courses (not shown). The Talairach coordinates of each region are reported in Table 1.

**Functional thalamocortical networks are reconfigured by fixation and eyes closed.** We measured thalamocortical functional connectivity in the Berger paradigm. Thalamic seed regions (i.e., MD and LGN) were defined functionally from the map shown in Fig. 1. The axial slices in Fig. 2A show regions that exhibited significant synchronization with the MD seed. These comprised mostly the DMN, including the supplementary motor area (SMA), prefrontal, superior lateral prefrontal cortex (SLPFC), and retrosplenial cortex (rSplen). We also compared the correlation between the two thalamic seeds, the DMN (Raichle et al. 2001), and the attention network (Corbetta et al. 2008). The difference in correlation between spontaneous BOLD signals in the two thalamic seed regions and the DMN versus the attention network was highly significant (for MD \( r_{15} = 3.51, P < 0.01 \); for LGN \( r_{15} = 5.37, P < 0.001 \)). Moreover, there was no significant correlation between MD and the attention network \( (r_{15} = 0.25, P > 0.05) \), while the correlation of LGN with the attention

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network was significant ($t_{10} = 3.00, P < 0.05$). These data clearly indicate that the DMN signal dominated thalamic connectivity and that correlation with the attention network was either small or nonsignificant.

To uncover patterns of connectivity that did not include signals shared with the DMN, we added the DMN signal as a regressor in a second GLM. This procedure revealed a network of extrastriate visual regions including VipV3a, MT, and lingual gyrus (LG), shown in Fig. 2B, whose spontaneous BOLD fluctuations were negatively correlated with those of the MD seed, while those in the SMA, anterior cingulate (ACC), medial frontal gyrus (MFG), and basal ganglia (e.g., caudate) were positively correlated.

We also assessed thalamocortical functional connectivity of the MD seed using resting-state data, where subjects maintained either eyes closed or fixation throughout each scan. These data allowed functional connectivity to be investigated separately for fixation and eyes-closed periods. Since we wished to examine patterns of connectivity beyond those that encompassed the DMN, the DMN signal was included as a regressor in the GLM. Figure 2C shows that during eyes closed thalamic signals were negatively correlated with regions in extrastriate visual and sensory-motor (SM) cortex, while no correlation was evident with primary visual cortex and the attention network. However, during fixation thalamic signals were positively correlated with those in primary visual cortex.

Table 1. Peak coordinates of regions identified in Figure 1

<table>
<thead>
<tr>
<th>Region</th>
<th>Abbreviation</th>
<th>Peak Coordinates</th>
<th>Center of Mass</th>
</tr>
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<tbody>
<tr>
<td>Left sensory-motor</td>
<td>LSM</td>
<td>$-26$ $-36$ $57$</td>
<td>$-25$ $-35$ $59$</td>
</tr>
<tr>
<td>Right sensory-motor</td>
<td>RSM</td>
<td>$38$ $-24$ $60$</td>
<td>$31$ $-28$ $59$</td>
</tr>
<tr>
<td>Left frontal eye field</td>
<td>LFEF</td>
<td>$-35$ $-9$ $45$</td>
<td>$-32$ $-9$ $48$</td>
</tr>
<tr>
<td>Right frontal eye field</td>
<td>RFEF</td>
<td>$38$ $-9$ $48$</td>
<td>$35$ $-13$ $48$</td>
</tr>
<tr>
<td>Left mediiodorsal thalamus</td>
<td>LMD</td>
<td>$-11$ $-18$ $9$</td>
<td>$-10$ $-18$ $10$</td>
</tr>
<tr>
<td>Right mediiodorsal thalamus</td>
<td>RMD</td>
<td>$11$ $-15$ $9$</td>
<td>$11$ $-16$ $10$</td>
</tr>
<tr>
<td>Left visual cortex</td>
<td>LV1</td>
<td>$-2$ $-84$ $3$</td>
<td>$-5$ $-82$ $4$</td>
</tr>
<tr>
<td>Right visual cortex</td>
<td>RV1</td>
<td>$8$ $-87$ $6$</td>
<td>$8$ $-86$ $5$</td>
</tr>
<tr>
<td>Left medial temporal</td>
<td>LMT</td>
<td>$-47$ $-75$ $6$</td>
<td>$-46$ $-74$ $4$</td>
</tr>
<tr>
<td>Right medial temporal</td>
<td>RMT</td>
<td>$50$ $-72$ $3$</td>
<td>$49$ $-73$ $4$</td>
</tr>
<tr>
<td>Left lateral geniculate nucleus</td>
<td>LLGN</td>
<td>$-26$ $-24$ $-3$</td>
<td>$-25$ $-27$ $-5$</td>
</tr>
<tr>
<td>Right lateral geniculate nucleus</td>
<td>RLGN</td>
<td>$26$ $-27$ $-6$</td>
<td>$25$ $-26$ $-4$</td>
</tr>
</tbody>
</table>

Peak coordinates and center of mass are given in millimeters according to the atlas of Talairach and Tournoux (1988) All regions have a volume of 567 mm$^3$. 

Fig. 1. Mean blood oxygen level-dependent (BOLD) signals during alternating periods of fixation and eyes closed. Axial slices show the group-level statistical map of the interaction of resting state type $\times$ time, highlighting regions whose mean signal was modulated over time by eyes closed and fixation. Displayed statistics are gaussianized $F$ statistics corrected for multiple comparisons ($P < 0.05$). Regions include sensory-motor cortex (SM), frontal eye fields (FEF), mediiodorsal thalamus (MD), primary visual cortex (V1), medial temporal cortex (MT), and the lateral geniculate nucleus (LGN). While V1 shows a positive deflection in mean signal during fixation and a negative deflection during eyes closed, MT shows the opposite pattern. Peak coordinates are listed in Table 1.
and the attention network and negatively correlated with ventral medial prefrontal cortex (VMPFC) (see Fig. 2D).

**BOLD correlates of spontaneous blinks and eye movements.** EOG data were available for nine participants, allowing us to examine the BOLD correlates of spontaneous oculomotor signals and their contribution to functional connectivity. Figure 3A shows a sample of one participant’s EOG record, containing large signal changes due to blinks during fixation and smaller signal changes.
due to slow eye movements during eyes closed. Figure 3B highlights regions where the BOLD signal was modulated by blinks during fixation, and Fig. 3C highlights regions where the BOLD signal was modulated by eye movements during eyes closed. Not surprisingly, no statistically significant effects were found for blinks during eyes closed and for eye movements during fixation. The map of the BOLD correlates of blinks highlighted an extensive cortical and subcortical network, including the right superior parietal lobule (SPL), a region slightly anterior to the left frontal eye field (FEF; see Fig. 3B; slice $Z = 57$), regions medial to the ventral extension of the intraparietal sulcus (VipV3a), pulvinar, primary visual cortex, and parahippocampal gyrus. In contrast, the map of eye movement effects during eyes closed showed a network of subcortical regions including dorsal caudate, globus pallidus, posterior putamen, thalamus, substantia nigra, and superior colliculus. Moreover, we recomputed thalamocortical functional connectivity before and after removing the oculomotor signals. With a group-level repeated-measures ANOVA used to examine the effects of oculomotor signals on thalamocortical functional connectivity, no voxel survived multiple comparison correction in the map of the interaction of resting state by a factor coding whether the oculomotor signals had been removed, suggesting that oculomotor signals play a minor role, if any, in shaping thalamocortical functional connectivity.

Resting states affect timing between thalamic and cortical BOLD fluctuations. To examine the relation between thalamic and cortical BOLD fluctuations over time, we calculated the cross-covariance between MD and LGN and the brain, using the resting-state data. Shown in Fig. 4 is the group-level statistical map of the interaction of resting state type $\times$ lag, highlighting cortical regions in which the thalamocortical cross-covariance changed between eyes closed and fixation. Also shown are the regional cross-covariances with the MD thalamic seed for V1, MT, SM, and FEF. During fixation, thalamic and cortical signals were synchronized as indexed by a fairly narrow peak at 0.0-s lag. However, during eyes closed there was a dramatic reorganization of the temporal dependencies between thalamic and cortical signals. For some regions, such as MT, SM, and FEF, the cross-covariances became antisymmetric, with thalamic signals showing negative covariances with leading cortical signals and positive covariances with lagging cortical signals. In contrast, V1 showed a complex, bimodal relation. These results indicate that differences in functional connectivity of primary and extrastriate visual cortex, during fixation and eyes closed, extend to time lags $>0.0$ s and include not only a change in the location of the peak cross-covariance value but also a change in the shape of the temporal dependencies between thalamic and cortical fluctuations. Interestingly, the cross-covariances do not clearly suggest that thalamic signals preceded cortical signals or vice versa.

Amplitude of spontaneous BOLD signals is modulated by opening and closing the eyes. We examined the standard deviation of the BOLD signal for the Berger data. Figure 5 shows the group-level statistical map of the resting state type $\times$ time interaction. A number of posterior cortical regions, mostly within visual areas, showed modulations of epoch-to-epoch variability between fixation and eyes closed. Also shown in

![Fig. 4. Group-level statistical map of the interaction of resting state type $\times$ lag, highlighting regions whose BOLD signals showed time-dependent covariations with those in the thalamic seeds between fixation and eyes closed. Displayed statistics are gaussianized $F$ statistics corrected for multiple comparisons ($P < 0.05$). The regional cross-covariances are computed from time series extracted from the MD seed and visual cortex (V1), MT, SM, and FEF during fixation and eyes closed. For negative lags, the regional time courses lead MD. Cross-covariances show marked effects of resting state type, being larger, broader, and more complex during eyes closed than during fixation.](http://jn.physiology.org/)

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Fig. 5 is the time course of the standard deviation of the BOLD signal for V1 and MT regions. In both regions the variability of the BOLD signal increased during eyes closed and decreased during fixation. Previous reports have indicated that the amplitude of spontaneous BOLD fluctuations is greater during eyes closed than during eyes open or fixation (Bianciardi et al. 2009; McAvoy et al. 2008), while others have found the opposite result (Yang et al. 2007). A straightforward account, consistent with the former findings, is that during eyes closed the within-epoch amplitude of spontaneous oscillations increased, leading to the observed increase in between-epoch variability, compared with the fixation condition. Furthermore, increased BOLD variability was observed both in primary and extrastriate visual regions during eyes closed, suggesting that BOLD signal variability, and by extension the amplitude of spontaneous BOLD fluctuations, do not demonstrate the dissociated pattern observed for mean and thalamocortical functional connectivity signals. Whether factors affecting mean and functional connectivity signals, on the one hand, and those affecting the amplitude of spontaneous BOLD oscillations, on the other, differ remains to be established.

DISCUSSION

Large-scale reorganization of ongoing neural activity during eyes open and closed has been studied in human and animal models and is thought to be associated with changes in both thalamic visual nuclei as well as posterior cortical regions (Hughes and Crunelli 2005). In this work we examined BOLD changes associated with opening and closing one’s eyes across the entire brain. We found changes in 1) mean signals of both thalamic and cortical regions, 2) thalamocortical functional connectivity, and 3) the amplitude of spontaneous fluctuations of the BOLD signal. Crucially, eyes closed and fixation produced changes in mean signals and functional connectivity that differed between primary and higher tiers of visual cortex.

Dissociated functional signals between primary and extrastriate visual cortex. The dissociated mean BOLD signal changes between primary and extrastriate visual regions (see Fig. 1) broadly replicate the dissociated pattern of CBF changes reported in previous PET studies where CBF was observed to increase in extrastriate visual cortex during REM sleep compared with wakefulness (Braun et al. 1998; Madsen et al. 1991) and SWS (Braun et al. 1998) while CBF in primary visual cortex decreased (Braun et al. 1998) during REM sleep compared with SWS. This was a somewhat puzzling result since dissociated signals in primary and extrastriate visual cortex have not been observed for visually evoked responses. Interestingly, in somatosensory discrimination tasks, positive BOLD responses, time locked to the presentation of a tactile stimulus, were found in primary visual cortex while negative responses were found in extrastriate visual cortex of healthy sighted individuals but not blind participants (Burton et al. 2004, 2006). These findings suggest that dissociated signals in visual regions may not reflect sensory processing per se, but more likely endogenous activity, either triggered by phasic changes in the external environment or tonically generated during the sleep-wake cycle.

Transcranial magnetic stimulation (TMS) of the FEF has been reported to increase the BOLD signal in primary visual cortex and decrease the BOLD signal in extrastriate visual cortex in both the absence and the presence of visual stimuli (Ruff et al. 2006). However, these findings could not be replicated in later studies from the same group (Ruff et al. 2008). While results from TMS experiments need to be interpreted cautiously, given the uncertainty regarding its physiological underpinnings, it is of some interest that the MD thalamic region we found to show prominent tonic changes in baseline BOLD signals (see Fig. 1) was primarily functionally connected with elements of the DMN (see Fig. 2A). Whether regions of the attention network, such as FEF, or the DMN may
be involved in resetting thalamocortical functional connectivity is a possibility raised by these observations.

On the other hand, there is good evidence of dissociated CBF responses in visual cortex with pharmacological challenges in healthy human participants (Furey et al. 2008; Mentis et al. 2001). In fact, decreased CBF in extrastriate and increased CBF in primary visual cortex are observed during cholinergic stimulations, suggesting a central role of subcortical cholinergic afferents in driving dissociated blood flow changes in visual regions. Moreover, augmentation of cholinergic transmission sharpens the spatial spread of visually evoked BOLD responses in early retinotopic visual cortex (Silver et al. 2008). Conversely, increased noradrenergic activity through stimulation of brain stem nuclei (Goadsby and Duckworth 1989), nonspecific amplification of aminergic transmission (Devous et al. 2001), and use of selective noradrenergic agonists (Swartz et al. 2000) is followed by decreases of CBF in both primary and extrastriate visual regions.

Oculomotor signals do not contribute to resting state effects on thalamocortical connectivity. We found that oculomotor signals were associated with significant BOLD modulations during fixation and eyes closed (see Fig. 3, B and C, respectively), raising the possibility that resting state differences in functional connectivity reflect low-level differences in motor behavior. Many of the regions that showed BOLD effects to spontaneous blinking have also been reported in previous fMRI studies (Chung et al. 2006; Tse et al. 2010; Yoon et al. 2005), suggesting that our EEG data were of comparable quality. Others have suggested that spontaneous fluctuations in the DMN (Ramot et al. 2011) are correlated with oculomotor signals during eyes closed. Instead, we observed prominent subcortical activations, in agreement with neurophysiological evidence suggesting a prominent role of basal ganglia in self-generated eye movements (Shires et al. 2010; Utter and Basso 2008). Furthermore, the study by Ramot et al. (2011) included BOLD effects that exceeded a low, uncorrected statistical threshold despite the study having a large number of participants, suggesting that the size of the eye movement effects on cortical BOLD signals was rather small. More importantly, we did not find oculomotor signals to significantly alter the connectivity between thalamic seeds and cortical spontaneous fluctuations.

Relation between thalamus and cortex. On the basis of comparisons of mean BOLD signal changes during eyes open and closed, other investigators have inferred an organization of cortical activity whereby eyes-open periods are characterized by increased activity in an exteroceptive network, encompassing the attention network, while eyes closed is characterized by increased activity in an interoceptive network, which includes extrastriate visual regions and somatosensory cortex (Brandt 2006; Marx et al. 2003, 2004). Although this proposal agrees with our functional connectivity results, a dissociation between primary and extrastriate visual regions was not found in a previous report that examined the functional connectivity of mediiodorsal and lateroventral thalamic structures during eyes open and closed (Zou et al. 2009). Nonetheless, a number of functional connectivity studies have found that primary and extrastriate visual cortices belong to separate resting state networks (Beckmann et al. 2005; Damoiseaux et al. 2006; Smith et al. 2009; Tyszka et al. 2011), indicating that spontaneous BOLD fluctuations in primary and extrastriate visual regions are not always coherent. An obvious, if speculative, interpretation of the changes we observed in mean and functional connectivity signals between fixation and eyes closed is that they both reflect changes in the thalamocortical transfer of information. Simpler explanations can probably be ruled out. For example, Schölvinck et al. (2010) found that the temporal phase between neural and BOLD signals can change with resting state type. One may then infer that resting state effects on functional connectivity reflect changes in the delay between thalamic and cortical BOLD signals. This hypothesis would predict a change in the location of the peak value but not the shape of the cross-covariance function, contrary to what we found (see Fig. 4).

Dissociated changes between primary and extrastriate visual cortex suggest that the relation between neural signals in thalamic and extrastriate visual regions may not require medication through primary visual cortex, either because there are direct thalamic projections to extrastriate visual regions (Schmid et al. 2010) or because BOLD changes in posterior regions reflect the effects of ascending modulatory, rather than sensory, projections. Alternatively, the flow of information from thalamus to higher tiers of visual cortex may be variably gated in primary visual cortex, according to physiological state (Damoiseaux and Greicius 2009; Schroeder et al. 2008; Watson et al. 2008).

Accounting for BOLD effects of physiological state. In trying to account for BOLD effects of eyes open and closed described here, and more generally those of physiological state on basal CBF and metabolism, it is obvious to consider concomitant changes in electrophysiological signals. Increased power in the alpha band is observed routinely during eyes closed compared with open (Berger 1929, 1930), and its modulation is considered the hallmark of that transition (Adrian and Matthews 1934). An extensive literature has addressed the BOLD correlates of the alpha rhythm and found that increased alpha activity is associated with increased BOLD signals in thalamic regions (de Munck et al. 2007; Difrancesco et al. 2008; Feige et al. 2005; Goldman et al. 2002; Goncalves et al. 2006; Larson-Prior et al. 2011; Martini et al. 2007; Moosmann et al. 2003) and decreased BOLD signals in primary and extrastriate visual cortex (de Munck et al. 2007; Difrancesco et al. 2008; Feige et al. 2005; Goldman et al. 2002; Goncalves et al. 2006; Laufs et al. 2006; Larson-Prior et al. 2011). Clearly, modulations of the alpha rhythm, by themselves, cannot account for the effects of eyes closed and fixation on the BOLD signal, since one would expect greater thalamic BOLD signals and smaller visual cortical BOLD signals during eyes closed than during fixation, contrary to what we found (see Fig. 1). Second, alpha power does not appear prominently during either SWS or REM sleep (Rechtschaffen and Kales 1968; Silber et al. 2007) and therefore could not account for the fact that transitions from eyes open to closed as well as from SWS to REM sleep show dissociated changes in functional signals between primary and extrastriate visual cortex.

An alternative possibility is that BOLD changes during eyes closed and fixation are driven by changes in cholinergic innervation of thalamic and cortical regions. Administration of cholinergic drugs is associated with dissociated CBF changes in primary and extrastriate visual cortex, as noted above (Furey et al. 2008; Mentis et al. 2001). However, the effects of cholinergic drugs on local BOLD signals and CBF are exactly

\[ \text{J Neurophysiol • doi:10.1152/jn.00900.2011 • www.jn.org} \]
opposite those measured during states of increased cholinergic tone, such as periods of increased alpha rhythm (Hughes and Crunelli 2005) and REM sleep (Hobson 2009). The lack of obvious, simple physiological or pharmacological correlates of the effects of eyes open and closed on BOLD signals and thalamocortical functional connectivity may therefore suggest that they stem from physiological and pharmacological interactions more complex than those considered in the imaging literature so far.

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AUTHOR CONTRIBUTIONS


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