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

Mark McAvoy¹, Linda Larson-Prior¹, Marek Ludwikow⁶, Dongyang Zhang¹, Abraham Z. Snyder¹,², Debra L. Gusnard¹,³, Marcus E. Raichle¹,²,⁴,⁵, Giovanni d'Avossa⁶

¹ Department of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA; ² Department of Neurology, Washington University School of Medicine, Saint Louis, Missouri, USA; ³ Department of Psychiatry, Washington University School of Medicine, Saint Louis, Missouri, USA, ⁴ Department of Anatomy and Neurobiology, Washington University School of Medicine, Saint Louis, Missouri, USA; ⁵ Department of Biomedical Engineering, Washington University School of Engineering, Saint Louis, Missouri, USA; ⁶ School of Psychology, Bangor University, Bangor, United Kingdom

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Corresponding author:
Mark McAvoy
Department of Radiology
Washington University School of Medicine
4525 Scott Ave, Campus Box 8225, Rm 2110
Saint Louis, MO 63110
Phone : 314 362 4409
Fax : 314 362 6110
Email: mcavoy@npg.wustl.edu

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We investigated the effects of resting state type on the 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 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 et al. 1998), our results suggest that dissociated endogenous neural activity in primary and extrastriate cortex may represent a general aspect of brain function.
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 to 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 (Madsen et al. 1991; Braun et al. 1997; Dang-Vu et al. 2010), has been suggested to be specifically associated with REM sleep and to potentially account for the phenomenological qualities of oneiric 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 to when they maintain a state of quiet wakefulness (Shulman et al. 1997; Raichle et al. 2001). 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 have 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 Crunelli 2005), and both cortical and thalamic structures are endowed with the ability to maintain rhythms in the alpha frequency range (Kristiansen and Courtois 1949; Lopez de Silva et al. 1977, 1980), which characterizes quiet wakefulness with eyes closed. A number of imaging studies have indicated that BOLD signals in mediodorsal thalamus are strongly modulated by alpha power (Goldman et al. 2002; Moosmann et al. 2003; Feige et al. 2005; Goncalves et al. 2006; Mantini et al. 2007; DiFrancesco et al. 2008) 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 mediodorsal 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 extra-striate visual regions as well as other sensory and paralimbic cortical regions, is increased during periods of eyes closed compared to 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 to closed (Marx et al. 2004; Uludağ et al. 2004), others have reported increased BOLD signals in extrastriate visual cortex (e.g. Marx et al. 2003) during eyes closed compared to 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.
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 has 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, eleven healthy right-handed subjects (7 females, average age 28.1) participated in the fMRI experiment. The bore and scanning room were completely darkened through the use of special draperies. Participants were instructed to either 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 40s. 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, ten healthy right-handed subjects (6 females, average age 23.6) 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, 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. Imaging data is archived in a public database available at brainscape.org (study BS003). In nine of ten subjects, EEG data were acquired simultaneously with fMRI (DC-3500 Hz, 20 kHz sampling rate) using the MagLink™ system and the synamps/2™ amplifier (Compumedics Neuroscan, TX) 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 approximately 3 cm above and below the outer canthus of the left eye. Gradient artifact was reduced using Scan 4.3™ software (Compumedics Neuroscan, TX) with ballistocardiogram artifacts reduced using in-house software (Vincent et al. 2007). EEG was inspected both visually and using spectral information in
the alpha, theta and delta bands to ensure that subjects were not asleep. Only the EOG, but not the EEG, data were used to generate predictors of the BOLD signal.

**Imaging methods and preprocessing**

Images were acquired with a Siemens 3 Tesla Allegra system (Erlagen, Germany). In the Berger paradigm, an asymmetric gradient-echo echo-planar-imaging sequence was used (repetition time = 2.5s, 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 echo-planar-imaging sequence was used (repetition time = 3.013s, which included a 1s 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 = 2.1s, echo time = 3.93 ms, flip angle = 7°, 1 x 1 x 1.25 mm) and a T2-weighted fast spin-echo image.

Image preprocessing was carried out using 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 1000 (Ojemann et al. 1997). The functional data were transformed into atlas space (Talairach and Tournoux 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 modelling the BOLD response over 16 timepoints. Additional regressors included a constant term and linear trend for each scan to account for slow drifts in the
BOLD time-series. The first 40s 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, then spatially smoothed with a 6 mm full width at half maximum (FWHM) three-dimensional (3D) Gaussian kernel to blur inter-individual 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) which included subjects as the random factor and two fixed factors: resting state type (eyes closed and fixation) and time (16 timepoints). The resulting statistical maps, which included the resting state type by time interaction ($F_{15,150}$), were $z$-transformed and corrected for multiple comparisons ($|z| \geq 3.0$, minimum 13 face connected voxels, $p < 0.05$ 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 40s periods of fixation and eyes closed, these mean signals were subtracted. Both data sets were low pass filtered at a cut-off 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, 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 ($|z| \geq 3.0$, minimum 17 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 which 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 (Shulman et al. 1997, Raichle et al. 2001).
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 because of 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 computing the cross-covariance, the resting state BOLD time-series was detrended, low pass filtered at a cut-off 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 mediodorsal thalamus (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 by lag interaction (F_{80,720}), were z-transformed and corrected for multiple comparisons (|z| ≥ 3.0, minimum 13 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
timecourses of the amplitude of the average deviation of the BOLD signal from its mean value, each 16
timepoints. These estimates were spatially smoothed with a 6 mm FWHM 3D Gaussian kernel. Statistical
significance was assessed with a group level repeated measures ANOVA which included subjects as the random
factor and two fixed factors: time (16 timepoints) and resting state (eyes closed and fixation). The resulting
statistical maps, which included the resting state type by time interaction ($F_{15,150}$), were z-transformed and
corrected for multiple comparisons ($|z| \geq 3.0$, minimum 13 face connected voxels, $p < 0.05$ corrected).

Blink and eye movement analysis

The electrooculogram (EOG) was used to recover the timing of spontaneous blinks and eye movements.
Blinks were defined as excursions of the EOG exceeding ±175 µV. The duration of a blink event was defined
conventionally to be 0.5s, and was centered on the peak value of the EOG. If the EOG signal had not returned to
within ±20 µV of the baseline by the end of interval, the event duration was extended until this criterion was
satisfied. The eye movement record was obtained from the EOG record after removing all blinks. The blink and
eye movement records were full wave rectified (Ramot et al. 2011) and convolved with a canonical
hemodynamic response function (Boynton et al. 1996). The contribution of blinks and eye movements to the
BOLD signal during the eyes closed and fixation scans was estimated using participant specific GLMs. The GLMs
included, beside the blink and eye movement regressors, a constant term and linear trend for each scan. The
estimates were normalized by the value of the constant term averaged over scans, 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 voxel-wise statistical maps were z-transformed and corrected for multiple
comparisons ($|z| \geq 3.0$, minimum 17 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 40s 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 to eyes closed (see Fig. 1). Dorsal and ventral extrastriate visual regions showed similar timecourses (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 which exhibited significant synchronization with the MD seed. These comprised mostly the DMN, including the supplementary motor area (SMA), precuneus, 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 $t_{10} = 3.51$, $p < 0.01$; and for LGN $t_{10} = 5.37$, $p < 0.001$). Moreover, there was no significant correlation between MD thalamus and the attention network ($t_{10} = 0.25$, $p > 0.05$), while the correlation of LGN with the attention 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 non significant.

To uncover patterns of connectivity which 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 supplementary motor area (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 which 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 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. Unsurprisingly, 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 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. Using a group level repeated measures ANOVA 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 the 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 by 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, sensory-motor cortex (SM), and the frontal eye field (FEF). During fixation thalamic and cortical signals were synchronized as indexed by a fairly narrow peak at 0.0s 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 greater than 0.0s 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 are 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 by 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. 5 is the timecourse 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 eyes open or fixation (McAvoy et al. 2008; Bianciardi et al. 2009), 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 epochs amplitude of spontaneous oscillations increased, leading to the observed increase in between epochs variability, as compared to 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.
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 to wakefulness (Madsen et al. 1991; Braun et al. 1998) and SWS (Braun et al. 1998), while CBF in primary visual cortex decreased (Braun et al. 1998) during REM sleep compared to 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 both in the absence and 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 using
pharmacological challenges in healthy human participants (Mentis et al. 2001; Furey et al. 2008). 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 brainstem nuclei (Goadsby and Duckworth 1989), non specific
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. 3BC, 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 (Yoon et al. 2005; Chung et al. 2006; Tse
et al. 2010), suggesting that our EOG 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 (Utter and Basso 2008; Shires et al. 2010).
Furthermore the study by Ramot et al. (2011) included BOLD effects, which 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 (Marx et al. 2003, 2004; Brandt 2006). Although this proposal agrees with our functional connectivity results, a dissociation between primary and extrastriate visual regions was not found in a previous report which examined the functional connectivity of mediodorsal 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 mediation 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 (Schroeder et al. 2008; Watson et al. 2008; Damoiseaux and Greicius 2009).
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 to 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 (Goldman et al. 2002; Moosmann et al. 2003; Feige et al. 2005; Goncalves et al. 2006; de Munck et al. 2007; Mantini et al. 2007; DiFrancesco et al. 2008; Larson-Prior et al. 2011) and decreased BOLD signals in primary and extrastriate visual cortex (Goldman et al. 2002; Feige et al. 2005; Goncalves et al. 2006; Laufs et al. 2006, de Munck et al. 2007; DiFrancesco et al. 2008; 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 fixation, contrary to what we found (see Fig. 1). Secondly, 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 both 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 (Mentis et al. 2001; Furey et al. 2008). However, the effects of cholinergic drugs on local BOLD signals and CBF are exactly 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 hence suggest that they stem from physiological and pharmacological interactions more complex than those considered in the imaging literature so far.
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Figure 1. Mean BOLD signals during alternating periods of fixation (red circles) and eyes closed (blue circles).
The axial slices show the group level statistical map of the interaction of resting state type by time, highlighting regions whose mean signal was modulated over time by eye 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), mediodorsal thalamus (MD), primary visual cortex (V1), medial temporal cortex (MT) and the lateral geniculate (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.

Figure 2. Group level statistical maps of thalamocortical functional connectivity between the MD seed and the brain. A. Berger data in which participants alternated between eyes closed and fixation. Highlighted regions encompass mostly the DMN including the supplemental motor area (SMA), precuneus, superior lateral prefrontal cortex (SLPFC), medial frontal gyrus (MFG), medial prefrontal cortex (MPFC), retrosplenial cortex (rSplen) and ventral medial prefrontal cortex (VMPFC). B. Berger data after partialing out the DMN signal. C. Eyes closed, resting state data after partialing out the DMN signal. A number of extrastriate regions are negatively correlated with the MD seed including VipV3a, MT and lingual gyrus (LG), along with sensory-motor cortex (SM). D. Fixation, resting state data after partialing out the DMN signal. Regions in the attention network (e.g. FEF and IPS) and V1 are positively correlated with the MD seed. Displayed values are Gaussianized T statistics corrected for multiple comparisons (p < 0.05).

Figure 3. Blink and eye movement analysis. A. 5.0 s long trace from one participant’s electrooculogram. Top trace was obtained from a fixation scan, while the bottom trace from an eyes closed scan. B. Group level statistical map of the main effect of blinks during fixation. C. Group level statistical map of the main effect of eye movements during eyes closed. Displayed statistics are Gaussianized T statistics corrected for multiple comparisons (p < 0.05).

Figure 4. Group level statistical map of the interaction of resting state type by 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, sensory-motor cortex (SM) and the frontal eye field (FEF) during fixation (red circles) and eyes closed (blue circles). For negative lags, the regional timecourses lead MD. Cross-covariances show marked effects of resting state type, being larger, broader and more complex during eyes closed than fixation.

Figure 5. Group level statistical map of the interaction of resting state type by time, highlighting regions in which the amplitude of spontaneous fluctuations was modulated over time by opening and closing the eyes. Displayed statistics are Gaussianized F statistics corrected for multiple comparisons (p < 0.05). In all regions the timecourse of the BOLD standard deviation increased in amplitude upon eye closure (blue circles) compared to fixation (red circles).
<table>
<thead>
<tr>
<th>Region</th>
<th>Abbreviation</th>
<th>Peak Coordinates (x, y, z)</th>
<th>Center of Mass (x, y, z)</th>
</tr>
</thead>
<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>31, -24, 60</td>
<td>31, -28, 59</td>
</tr>
<tr>
<td>Left frontal eye field</td>
<td>LFEF</td>
<td>-32, -9, 45</td>
<td>-32, -9, 48</td>
</tr>
<tr>
<td>Right frontal eye field</td>
<td>RFEF</td>
<td>35, -9, 48</td>
<td>35, -13, 48</td>
</tr>
<tr>
<td>Left mediodorsal thalamus</td>
<td>LMD</td>
<td>-10, -18, 9</td>
<td>-10, -18, 10</td>
</tr>
<tr>
<td>Right mediodorsal 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>-5, -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>-46, -75, 6</td>
<td>-46, -74, 4</td>
</tr>
<tr>
<td>Right medial temporal</td>
<td>RMT</td>
<td>49, -72, 3</td>
<td>49, -73, 4</td>
</tr>
<tr>
<td>Left lateral geniculate nucleus</td>
<td>LLGN</td>
<td>-25, -27, -3</td>
<td>-25, -27, -5</td>
</tr>
<tr>
<td>Right lateral geniculate nucleus</td>
<td>RLGN</td>
<td>25, -26, -4</td>
<td>25, -26, -4</td>
</tr>
</tbody>
</table>

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